THE ALKALOIDS

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ARNOLD BROSSI

VOLUME 32

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Chemistry and Pharmacology

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Arnold Brossi

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PREFACE

The forensic chemistry of habit-forming alkaloids, including opium, coca, ergot, and khat alkaloids, is much more sophisticated today than in 1970, when this topic was reviewed in Vol. 12 of this series. Interest in the field has recently been renewed, with the focus of attention being chromatographic separation techniques and sensitive methods for alkaloid detection. Presentation of many novel steroidal alkaloids isolated from Apocyanaceae and Buxaceae plants, together with partial synthesis of representative alkaloids, make this chapter a valuable supplement to those which appeared in Vols. 9 and 14 of this series. The medicinal plants still widely used in Chinese folk practices contain many different alkaloids. The structures and pharmacological properties of those alkaloids which have appeared in the literature since 1980 are presented here and may hopefully stimulate the appetite of those who are interested in total synthesis. Cyclization of *N*-acyliminium ion intermediates is an important method for the total synthesis of many types of alkaloids and is presented here with several examples. Quinoline alkaloids other than Cinchona alkaloids were reviewed in Vols. 3, 7, 9, and 17 of this series. Since the last report appeared 10 years ago, 80 new alkaloids were isolated and are presented here with their structures and pharmacological properties.

Arnold Brossi

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— CHAPTER 1 —

FORENSIC CHEMISTRY OF ALKALOIDS BY CHROMATOGRAPHIC ANALYSIS

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I. Introduction

There have been numerous psychoactive alkaloids found in the plant kingdom since morphine was first isolated by Setürner in 1832. Opium, which contains morphine and related alkaloids, was well known as an analgesic to the ancients. Opium and morphine are widely used to relieve pain and are frequently used as hypnotics, but they are addictive and narcotic. Heroin (diacetylmorphine) is pharmacologically similar in action to morphine but more narcotic. Both drugs are controlled by law and limited in their uses. Cocaine was found in coca leaves in 1860 and has been used in South America as a masticatory since early times. Cocaine and its salts are the oldest local anesthetics but their use is now entirely regulated because of their toxic and addictive properties. Ancient people used the so-called hallucinogenic plants ascribed for magical or mystical relevance in sacred and religious rites. Even now, some of them are used as snuffs or in drinks by South American Indians to induce psychotomimetic effects. *Peganum harmala* has been used as a spice as well as an intoxicant in India. Sacred mushrooms and Cactus used in Mexico have been introduced into Western countries. Mescaline from Cactus has psychotrophic activity and was chemically modified to prepare useful pharmacological compounds. Amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), and 2,5-dimethoxy-4-methylamphetamine (STP,DOM) were produced as stimulants. Chemistry has made it possible for psychoactive compounds from nature to be synthetically converted into more active and less toxic compounds, but some are now controlled by law. Every society has possessed drugs affecting mood, thought, and feeling. The use of both legal and illegal drugs is increasing, but the pattern of drugs used varies from time to time depending on their availability in illicit markets.

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Drugs and synthetic organic compounds are detoxified in the body and metabolized mainly in the liver, the intestines, and the kidneys. The chemical reactions of drugs and synthetic organic compounds in the body occur through oxidation, reduction, hydrolysis, or conjugation resulting in activation or inactivation. Most forensic compounds have been studied after elimination from the body as metabolites. Unchanged drugs are also excreted with metabolites, depending on dosage, frequency of drug use, concomitant use of other drugs, nutritional status, pathological state, age, body temperature, body weight, and so on.

Analytical data on forensic drugs, plants, and their extracts are of value in various fields such as law, toxicology, pharmacology, biochemistry, pharmacy, phytochemistry, ethnobotany, and analytical chemistry. It is always a problem to detect pico or nanogram quantities in biological samples such as blood or urine, and illicit samples which consist of only a fragment of a tablet, a scrap of blotting paper, a dusty capsule shell, sugar cubes, candy, cookies, fruit, hypodermic needles, leaves, seeds, teas. tobacco, and so on. Therefore, modern detection techniques such as various types of chromatography have been very useful for separation and identification of small amounts of drugs. Paper chromatography (PC) was originally used for detection of various kinds of alkaloids. Thin-layer chromatography (TLC) has been more common than PC because of rapid development, better resolution, and a wider choice of solvents and reagents used for detection. Gas chromatography (GC) is an adequate technique for qualitative and quantitative analysis. Electron capture (ECD), flame ionization (FID), and mass spectrometry (GC-MS) are useful detectors for GC. Polar drugs and their metabolites are often derivatized prior to GC analysis. Stationary phase columns have also been improved by using methyl-, methylphenyl-, trifluoropropylmethyl-, phenylcyano-, and propylmethyl-silicons. Glass capillary columns have been used more often recently than conventional packed columns. The temperature of the column has been increased by programming the system depending on the samples tested. High-pressure liquid chromatography (HPLC) is an excellent technique for nonvolatile, polar, and thermally degradable compounds not effectively analyzed by GC. Selection of normal and reversed-phase columns allows the use of both organic and inorganic solvents as the mobile phase. Ion-pair chromatographic analysis sometimes gives great improvements in resolution and speed of chromatograms. HPLC instruments are frequently equipped with ultra violet (UV), fluorescence, and other detectors. Compounds with no absorbance in UV, or no fluorescence, are often derivatized to be detectable by these techniques.

It is in this context that chromatographic techniques for identification of legal and illegal forensic and psychoactive compounds available in illicit markets and in biological samples, as well in plants used by primitive and civilized peoples, are reviewed.

II. Extraction, Preparation, and Derivatization

Drugs often contain a large amount of adulterants and impurities. It is often not clear what type of drug, or whether more than one drug, may be present. Thus, spot-test analysis with a proper color reagent on TLC is useful for screening (Table I). In these procedures, specific color reactions are very useful. Systematic identification methods for detecting drugs of abuse, many of which are alkaloids and related compounds, using spot tests (*1*) and TLC (*2*) have been devised. Spot tests by a specific color reagent are very useful for detection of drugs at the site where these drugs are found.

In forensic analysis by chromatography, substances of analytical interest are extracted by a suitable method. In the analysis of biological materials, including urine and blood, the content of drugs to be analyzed is usually low. It is often necessary to analyze metabolites whose content may be much smaller than that of

TABLE I
COLOR REAGENTS FOR ALKALOIDS AND RELATED COMPOUNDS OF FORENSIC INTEREST

Reagent	Specificity
Dragendorff's reagent	Alkaloids with the exception of aliphatic primary and secondary amines
Iodine–potassium iodide (Wagner's reagent)	Very sensitive to alkaloids, but not selective; various compounds also react.
Potassium iodoplatinate	Alkaloids, general, nondestructive; different colors appear for different alkaloids.
p-Dimethylaminobenzaldehyde (Ehrlich's reagent)	Selective to indole alkaloids, sensitive to LSD
Marquis's reagent (formaldehyde–H_2SO_4)	Specific to opium alkaloids, heroin, and amphetamines
Simon's reagent (sodium nitro prusside–acetaldehyde–Na_2CO_3)	Specific to aliphatic secondary amines such as methamphetamine
Cobalt thiocyanate	Very sensitive to cocaine, but not always specific
Ninhydrin	Amino acids and aliphatic primary amines such as amphetamine and cathine; several secondary amines such as ephedrine are also positive.
TCNQ(7,7,8,8-tetracyanoquinodimethane) and TCBI (*N*,2,6-trichloro-*p*-benzoquinone imine)	π-Acceptor reagent; various alkaloids and drugs. Different colors appear.
DADF(dihydrofluorescein diacetate)	Enhances TLC visualization of amines and alcohols; useful for colchinoids, mescaline, and codeine; also applicable to HPLC.

the original drugs. The presence of various coexisting biological materials often interferes with the analysis, and therefore cleanup procedures are necessary. Several extraction and cleanup procedures, such as solvent extraction, adsorption on the proper absorbent, and others may have to be devised.

Solvent extraction is the oldest and most versatile method. Basic compounds such as alkaloids are extractable from the aqueous into the organic layer under alkaline conditions. The extractability is better when polar solvents are used, but coextraction of various foreign substances may disturb the analysis: Amphetamine can be extracted with nonpolar hexane from urine and analyzed without interference with coexisting biological materials, but polar, hydroxylated metabolites such as 4-hydroxyamphetamine cannot be extracted with hexane. On the other hand, when a more polar solvent such as isoamyl alcohol is utilized, both the amphetamine and its metabolites are extractable, but coextraction of foreign biological materials seriously disturbs the analysis. In such cases, further cleanup procedures are necessary. In the extraction of ionizable substances, ion-pair extraction may permit a more selective separation. Alkaloid cations form ion-pairs with bulky anions, and the resulting, rather hydrophobic and totally neutral, ion-pairs may be extractable with organic solvents even in slightly acidic conditions. Furthermore, when colored ion-pair reagents such as Bromocresol Purple (*3*) and Methyl Orange (*4*) are utilized, ion-pairs in organic layer exhibit characteristic colors. In this connection the use of tetrabromophenolphthalein ethyl ester (TBPE) (as the potassium salt) is interesting as an ion-pair reagent, because its ability to ion-pair is very strong and the color of the formed ion-pairs differs markedly from that of the original reagent. Dichloromethane or chloroform solutions of TBPE exhibit yellow colors in non-ionized form. Most alkaloids form very stable ion-pairs with TBPE, which are extractable under weakly acidic, neutral, or basic conditions (*5*). The formed ion-pairs exhibit characteristic colors which range from green, dark blue, or purple to red.

When solvent extraction is ineffective, or formation of emulsions disturbs the extraction, the use of a proper adsorbent such as XAD_2 (styrene-divinylbenzene resin), Extrelut (diatomaceous earth), and charcoal is useful. In the usual treatment, the drugs to be analyzed are retained on the adsorbent and then eluted by a suitable solvent. This method, although slightly more complicated than solvent extraction, is now widely used, because selectivity of the extraction is good. Disposable cartridge adsorbents such as Sep-pak for the use of cleanup in HPLC are now commercially available. The use of preparative TLC, before GC and HPLC analysis falls under a similar category, although the procedures are more complicated.

Several other cleanup methods have been reported. Ion-exchange paper (*6,7*) such as SA_2 is useful for simple microdetection; the ion-exchange paper is easy to handle and can be sent by mail. Semipermeable membrane dialysis has also been applied. The procedure for amphetamines is as follows (*8*): Urine is basified

with aqueous ammonia and placed in a cellulose dialysis membrane tube (Visking Co., Ltd.). The tube is soaked in a double volume of extracting solvent, $CHCl_3$/MeOH (1 : 1) or isoamyl acetate/EtOH (1 : 1), and then dialyzed for 4–6 hours against the same solvent. Amphetamines pass through the membrane with water, but a larger part of the biological materials remains in the tube. Amphetamines are distributed mainly to an outer organic layer and, in part, to an outer aqueous layer. Amphetamines in organic solvent and in the water layer (the latter is extracted once more with organic solvent) are combined, condensed, and analyzed by chromatography. More than 90% of amphetamines and ephedrines can be recovered by this method. Similar procedures might be applicable for other drugs.

In the analysis of blood (or serum), deproteinization may be necessary. In forensic chemistry, analysis in other biological samples is also sometimes attempted. For example, the detection of amphetamines in the hair (*9*) and sweat (*10*) of drug addicts has been reported.

Derivatization is carried out to increase detectability or to improve separation. In most derivatization methods of alkaloids, either –OH or –NH groups are reacted with reagents. Reagents often react with both –OH and –NH groups, and various derivatives may be formed. The structure of the reaction products is not always fully described. Derivatization for chromatographic separation has been extensively reviewed (*11–15*): Table II indicates the most important derivatization reagents applicable to alkaloids. It should be noted that in derivatization procedures the extraction and cleanup of the sample is often attained simultaneously. Tertiary amines and quaternary ammonium compounds lacking such groups are difficult to derivatize.

For GC, nonvolatile alkaloids are converted into volatile derivatives, most often by acylation or silylation. Alkylation is usually less effective because the boiling points of these derivatives are too high. The acetyl derivative is the principal nonhalogenated acyl derivative employed in analysis. Higher acyl derivatives with no halogen atom are less useful because of their lower volatility. Although the volatility of acetyl compounds is also not high, acetylation is often favored because pure reagents can easily be obtained at low cost. Halogen-substituted especially fluoro-substituted acyl derivatives are often utilized because they are highly volatile. These are also suitable for microdetection, since detectability is improved with the use of an EC detector sensitive to halogen atoms. Silyl derivatives are also utilized, and their volatility is very high, quite frequently higher than that of the acyl derivatives. Silylating agents, which are usually trimethylsilylating reagents, as well as the silyl derivatives formed, are very sensitive to moisture and the procedures, therefore, should be carried out in a sealed vessel. Table II lists common trimethylsilylating reagents in the order of increasing reactivity, with BSTFA and BSA being the most commonly used reagents. The mixture BSA/TMSI/TMCS (1 : 1 : 1) is probably the most potent

TABLE II

DERIVATIZATION REAGENTS OF ALKALOIDS FOR GC AND HPLC

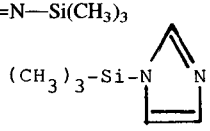
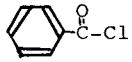
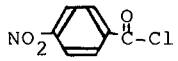
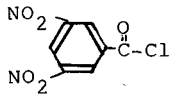
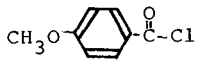
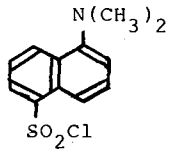
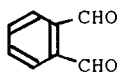
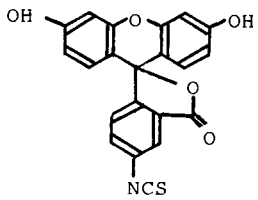
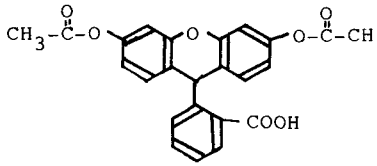
Reagent	Abbreviation	Structure
Acylating reagents		
acetic anhydride	Ac_2O	$CH_3-C(=O)-O-C(=O)-CH_3$
trifluoroacetic anhydride	TFAA	$CF_3-C(=O)-O-C(=O)-CF_3$
pentafluoropropionic anhydride	PFPA	$CF_3CF_2-C(=O)-O-C(=O)-CF_2CF_3$
heptafluorobutyric anhydride	HFBA	$CF_3CF_2CF_2-C(=O)-O-C(=O)-CF_2CF_2CF_3$
Silylating reagents		
hexamethyldisilazane	HMDS	$(CH_3)_3Si-N(H)-Si(CH_3)_3$
trimethylchlorosilane (with base catalyst)	TMCS	$(CH_3)_3Si-Cl$
N-methyl-*N*-trimethylsilyl-acetamide	MSA	$CH_3-C(=O)-N(CH_3)-Si(CH_3)_3$
N-trimethylsilyldienthylamine	TMSDEA	$(CH_3)_3Si-N(C_2H_5)_2$
N-trimethylsilyldimethylamine	TMSDMA	$(CH_3)_3Si-N(CH_3)_2$
N-methyl-*N*-trimethylsilyltri-fluoroacetamide	MSTFA	$CF_3-C(=O)-N(CH_3)-Si(CH_3)_3$
N,O-bis(trimethylsilyl)aceta-mide	BSA	$CH_3-C(=N-Si(CH_3)_3)-O-Si(CH_3)_3$
N,O-bis(trimethylsilyl)tri-fluoroacetamide	BSTFA	$CF_3-C(=N-Si(CH_3)_3)-O-Si(CH_3)_3$
N-trimethylsilylimidazole	TMSI	

TABLE II (*Continued*)

Reagent	Abbreviation	Structure
UV detection reagents		
benzoyl chloride		
4-nitrobenzoyl chloride		
3,5-dinitrobenzoyl chloride		
4-methoxybenzoyl chloride		
isothiocyanic acid esters		R–NCS
For fluorescence detection		
dansyl chloride	DANS-Cl	
o-phthalaldehyde	OPA	
fluorescein isothiocyanate		
dihydrofluorescein acetate	DADF	

silylating agent and even amides may be silylated. TMSI is the most potent silylating reagent for –OH groups, and usually does not react with –NH groups.

Derivatization for HPLC is carried out to increase the detectability when alkaloids show poor absorption in the UV or visible ranges. On the other hand, if alkaloids have strong absorption, derivatization is often unnecessary. When detection of very minute amounts of alkaloids is required, UV detection is ineffective. In such cases, fluoro-derivatization, such as by dihydrofluorescein diacetate (*16*), may give satisfactory results. Since alkaloids can usually be separated by reversed-phase ion-pair chromatography, derivatization is not always necessary. However, if alkaloids are converted into neutral derivatives, they will be separable by other modes of chromatography such as adsorption and reversed-phase partition chromatography. In HPLC, two types of derivatization, precolumn and postcolumn derivatization, are utilized, and of these, the former is more frequently used. The latter method is useful for automatic analysis of a large number of samples. Several important color reagents for UV and fluorescence detectors are listed in Table II. The detection limit by a UV detector amounts to a few nanograms or even less, when the absorption coefficients are high. The sensitivity with fluorescence detectors is so high that detection of a few picograms may be possible.

III. Analytical Techniques

A. GC AND GC-MS

Gas Chromatography, especially GC-MS, is now the most versatile analytical method used in forensic chemistry. The merit of the GC-MS analysis is its reliability in the identification of compounds, which is profoundly important in forensic chemistry. Thus, in spite of the high cost of the instrumentation required, GC-MS is now the standard method in forensic analysis. GC is, however, not always intrinsically suitable for the analysis of polar substances such as alkaloids because their volatility is usually not high. Some alkaloids and their derivatives, such as cocaine and heroin, which are acetylated compounds and therefore volatile can be gas chromatographed directly to give sharp peaks. Although earlier reports indicated that direct analysis of alkaloids is possible when a nonpolar stationary phase such as SE-30 (*17*) is used, this method is not considered satisfactory today. When polar and nonvolatile alkaloids are subjected to GC, they are strongly, and often irreversibly, adsorbed to the stationary phase. It is also probable that alkaloids are adsorbed to glass wool in the column

entrance and erroneous results might therefore be obtained. When such phenomena occur, very broad and tailing peaks are obtained. Retention times may be lengthened when the sample amount is low, and the calibration graphs are often not linear. The reproducibility is poor and repeated chromatography of the same sample might show different patterns. Elevated column temperatures may alleviate tailing, but alkaloids may decompose; degradation products may then be detected (*18*). These phenomena are particularly remarkable when alkaloids have hydroxyl groups. Hydroxyl groups and amino groups are strongly adsorbed by the active site of the stationary phase. Two methods have been devised to achieve satisfactory GC analysis of such alkaloids: One is to deactivate the "active sites" on the solid support by a suitable chemical treatment. The other, more widely applied method, is to convert alkaloids into neutral and volatile derivatives.

Several deactivation methods have been proposed, but these are rather tedious and therefore no longer used. Basic stationary phase treated with strong alkali such as potassium hydroxide, however, is commercially available and useful. The acidic active sites in a stationary phase are capped by base treatment, which may effectively suppress tailing. Thus, polar but volatile amphetamine and *N*-methylamphetamine (methamphetamine), which are related to ephedrines, can be gas chromatographed on a basic stationary phase without derivatization. In contrast, ephedra bases, which are polar and not volatile (since they contain hydroxyl groups) cannot be analyzed satisfactorily without derivatization, even on a basic stationary phase. The detectability of alkaloids and related compounds is improved by derivatization and is thus the preferred method, even when analysis without derivatization is possible.

In addition to conventional packed columns, capillary columns have been utilized in routine analysis in recent years. The glass capillary columns formerly used are very fragile and their manipulation difficult in routine analysis. Furthermore, due to a rather strong interaction with glass surfaces, alkaloids are often adsorbed and, consequently, a tailing of the peaks frequently observed. Recently, however, fused-silica capillary columns (developed in the last decade) have come into use. They are not easily broken and their surface is very inert. As a consequence, retention data obtained on glass capillary columns sometimes differ markedly from those obtained with fused-silica capillary columns. Since the separation achieved on capillary columns is much better than that on conventional packed columns, it is also possible to determine not only drugs but, simultaneously, adulterants and impurities. This "finger print" analysis may help to clarify the origin of drugs, which is of interest in forensic chemistry. The lifetime of the capillary columns is shorter than that of conventional columns, and shortened further if dirty samples are injected. For capillary GC analysis, it is recommended to purify the sample with great care.

B. HPLC

High-pressure liquid chromatography (HPLC) has been widely used for the analysis of various compounds and presumably is the best separation method available for nonvolatile compounds. Therefore, HPLC seems to be more suitable than GC for the analysis of alkaloids. The use of HPLC in forensic science, however, still remains the second-best method next to GC-MS. The reason is attributed to the fact that identification by HPLC is still tedious and unsatisfactory in comparison to GC-MS. For identification by HPLC, the peak fraction is collected and the solvent evaporated. The residue is then analyzed by a proper method such as MS. Improvements in LC-MS instrumentation are nearly completed, and LC-MS is quickly becoming one of the most important methods in forensic analysis today.

At present, various detectors are utilized for HPLC analysis, but identification is not always satisfactory. For the identification in HPLC, the use of a photodiode array UV detector is useful enabling the instantaneous measurement of UV and visible spectra of each peak. The detector is, however, rather expensive. Since most alkaloids have absorption bands in the UV region, and since UV spectra of alkaloids have been extensively reviewed (*19*), it is possible to detect many alkaloids and congeners with a UV detector, but the sensitivity is not always good. When detectability by UV detector is insufficient, specific and sensitive detectors such as electrochemical detectors are used. An alternative method is derivatization. The derivatization is carried out not only for UV and fluorescence detection but also for electrochemical analysis. Reproducibility by fluorescence and electrochemical detection is somewhat inferior to that measured by UV. Fluorescence intensity is very sensitive to the solvent used, and the presence of minute amounts of foreign substances in the eluent may cause quenching; oxygen dissolved in aqueous solvents may cause serious fluorescence quenching, which should be taken into consideration if a reversed-phase system is chosen.

Various modes of chromatography: adsorption, reversed-phase partition, ion-pair partition, and ion-exchange have been used in the analysis of alkaloids. Each method has its merit, but reversed-phase ion-pair chromatography is the most suitable method for ionizable and moderately bulky substances such as alkaloids.

Adsorption chromatography on silica gel packings has merit because the price of column packings is inexpensive and the lifetime of the columns long. The disadvantage is that alkaloids often exhibit tailing on silica gel. This restricts the use of silica gel and is attributed to the fact that basic alkaloids are adsorbed strongly to the acidic SiO_2 of silica gel. Addition of a slight amount of acid or base to the eluent sometimes gives better results. The presence of acid in the eluent promotes the desorption of the alkaloids from the SiO_2 by acid–base interaction. Alternatively, when base is added to the solvent, the base is adsorbed

by the SiO_2, preventing an adsorption of alkaloids. Thus, tailing may be alleviated by the addition of acid or base. It is still difficult, however, to operate silica gel columns under the best of conditions. Various silica gel packings with different adsorptive properties are available. In earlier reports, the use of more active packings was favored, but for separation of polar substances such as alkaloids the use of less active ones seems better. When alkaloids are converted into neutral and colored derivatives, the use of silica gel columns is very suitable. Alcohols chemically bonded to silica gel packings ("diol silica") have shown elution sequences similar to silica gel, but tailing is not so severe in the presence of neutral –OH groups. Other adsorbents such as aluminum oxide have been tried instead of silica gel, but results are inconsistent.

Reversed-phase chromatography is the most versatile method and 70–80% of all HPLC analyses are carried out by this technique, also utilized for the analysis of alkaloids. Octadecyl groups chemically bound to silica gel (C18,ODS) are often used, but sometimes other packings such as octyl(C8), phenyl, cyano, and short alkyl bonded packings are also utilized. The lifetime of C18 columns is, from our experience, shorter than that of silica gel, but longer than that of other chemically bonded packings. The mixed-solvent system of H_2O/MeOH or H_2O/MeCN with pH adjustment is often used. The later solvent is favored because better separability is obtained and the column pressure is low. Addition of other solvents such as THF, dioxane, acetone, and formamide may sometimes improve separability. Although reversed-phase chromatography is the most versatile mode in chromatography, several authors claim that it is not favorable for alkaloids because, as with silica gel columns, tailing is often observed (*20,21*). The tailing observed on C18 columns is caused by residual silanol groups. Many kinds of C18 packings with different properties are available. In several C18 packings, residual silanol groups are removed by treatment with chemicals (endcapping), and for the separation of polar substances such as alkaloids, such endcapped column packings should be used to minimize tailing. The separability of alkaloids is affected by pH as well as the ratios of organic solvents. Because alkaloids are ionizable, both ionized and nonionized species may coexist in aqueous solvents. When the eluent is basic, alkaloids exist as the free base, which is retained on nonpolar stationary phases (ion suppression chromatography), allowing better separation. Thus, basic solvents containing aqueous ammonia and ammonium carbonate are sometimes used. Such solvent systems, however, should be avoided for repeated analyses because C18 columns are chemically unstable in alkaline conditions (pH $>$ 7) and column life is shortened. In acidic eluents, alkaloids exist as ionic species and are poorly retained on the nonpolar stationary phase, resulting in poor separation. In such cases, reversed-phase ion-pair chromatography gives better results.

Ion-pair chromatography seems to give the best separation of alkaloids and is now widely applied. Normal-phase ion-pair chromatography was developed first

(*22,23*), and involves a tedious dynamic coating procedure, but is now only of theoretical interest as are other dynamic coating methods [*24*]. Reversed-phase ion-pair chromatography (*25,26*) is now the most versatile method for HPLC analysis of ionizable substances, in which some ion-pairing reagents are added to the eluent. The resulting rather hydrophobic ion-pair is retained on a hydrophobic stationary phase and separation is possible. As ion-pair reagents for cations, several bulky anions such as dodecyl sulfate, various alkylsulfonates, and others are used. Dodecyl sulfate is most often used as it is relatively inexpensive. From our experience, dodecyl sulfate often gives sufficient separation, although alkylsulfonates are frequently utilized in the literature. The use of optically active ion-pair reagents such as camphorsulfonate is interesting for separation of basic compounds into their optical isomers. Although ion-pair chromatography is a versatile method, a few alkaloids such as purine alkaloids and colchicine which have no basic character, and consequently do not form ion-pairs, cannot be separated by this technique. Stable ion-pairs are formed when alkaloids are bulky and have high basicity. Thus, ion-pair chromatography is especially suitable for alkaloids such as opium alkaloids, ergot alkaloids, and indole alkaloids. For the microdetection of alkaloids with low absorption coefficients, ion-pair chromatography is not useful because no color reaction can be carried out. Careful treatment in reversed-phase, and especially in ion-pair, chromatography is mandatory, or column life is very short. Thus, solvents and reagents should be pure and eluents filtered, degassed, and used immediately. Strongly acidic ($pH < 2$) or basic ($pH > 7$) eluents should be avoided. After analysis, columns should be washed carefully with the solvent mixed without salts (H_2O/ MeOH or H_2O/MeCN). Thus treated, the lifetime of a C18 column is rather long.

Ion-exchange chromatography, previously the most frequently used HPLC analysis, has now been widely replaced by reversed-phase ion-pair chromatography. This is largely due to the fact that the lifetime of ion-exchange columns is short and the column conditioning tedious. There exists little advantage in using ion-exchange chromatography except in the case of aminopropyl-bonded weak anion exchangers (amino-silica). The column packings are similar to those used in reversed-phase chromatography. Since the elution sequence is often opposite to that seen with C18 columns, and the separation good, a comparison of C18 with amino-silica techniques is advantageous. The column life of amino-silica is somewhat shorter than that of C18 columns.

C. TLC and Other Chromatographic Methods

Identification of alkaloids and related compounds of forensic interest is now seldom carried out by TLC alone, since other preferred methods such as GC and

HPLC have been developed. TLC, however, is still very important in forensic analysis as a simple screening method (*2,27–29*). Once prepared by investigators, TLC plates are now commercially available and precoated plates are widely used. Although various type of plates such as aluminum oxide, cellulose, polyamide, and others are available, silica gel is most frequently used. In the earlier literature on alkaloid analysis, silica gel or other adsorbents were used and prepared with buffer solution instead of pure water. This is, however, impossible if precoated plates are used. Instead, acidic or basic solvents are frequently used as eluents and their usefulness is described in the previous section. Another advantage of TLC is that color reactions can be carried out easily and extensively. TLC of alkaloids and related compounds has been reviewed (*30–33*).

With the development of HPLC, a new type of plates precoated with refined and sorted adsorbents became available. This new type of TLC is entitled high-performance thin-layer chromatography (HPTLC) and is patterned after HPLC; HPTLC yields better separation and further microanalysis. Various chemically bonded HPTLC plates, including C18 plates, are now available. Thus, TLC now includes adsorption, partition, ion-pair and ion-exchange, and is similar to HPLC.

Quantitative analysis by TLC was once carried out by the following tedious and rather inaccurate procedure: After the development by TLC the spot was scraped, extracted, and then analyzed by other methods such as UV. The improvement of densitometers, HPTLC plates, and microsampling, however, facilitates direct densitometric determination in TLC. It is now possible to determine microamounts of substances using TLC-densitometry, although the accuracy is still less, and treatment more tedious, than HPLC and GC.

Paper chromatography, once used for the analysis of a variety of compounds, is now substituted by TLC.

Electrophoresis can be applied to ionizable substances including alkaloids. Although electrophoresis is not used in forensic chemistry, this method may give excellent separation for alkaloids. Six ephedra alkaloids from *Ephedra herba* were separated dramatically by isotachophoresis (*34*). Such a separation would be difficult even with GC and HPLC.

Supercritical fluid chromatography (SFC) is the newest technique that may be applied to a variety of compounds. In this method, carbon dioxide is used as eluent, packed columns for HPLC and fused silica capillary columns for GC are suitable for SFC instruments. This technique differs from GC in that it is applicable to rather nonvolatile substances, and detectability and separation may be better than that provided with HPLC. The extractability of alkaloids by supercritical fluid gas from plants has been examined in combination with TLC (*35*). SFC instruments are now commercially available. It is also expected that SFC will interface with MS, and may be easier than LC-MS.

IV. Analysis of Alkaloids

A. Opium Alkaloids and Heroin

1. Purpose of Analysis

Analysis of opium alkaloids and related compounds is performed for the following purposes: (a) Analysis of alkaloids in plant materials and in opium. Papaveraceous plants, particularly *Papaver somniferum* and *P. setigerum* contain anesthetic alkaloids. *Papaver bracteatum* does not contain morphine but contains thebaine as a predominant alkaloid (Figure 1). In the analysis of opium, the major opioid alkaloids morphine, codeine, and thebaine are of special interest; but analysis of other nonopioid alkaloids, such as noscapine (narcotine), papaverine, narceine, and other, nonalkaloidal constituents is also carried out. Abuse of opium or morphine, once the most dangerous and widespread drugs of abuse, has now been overshadowed to a large extent by that of heroin. Heroin is

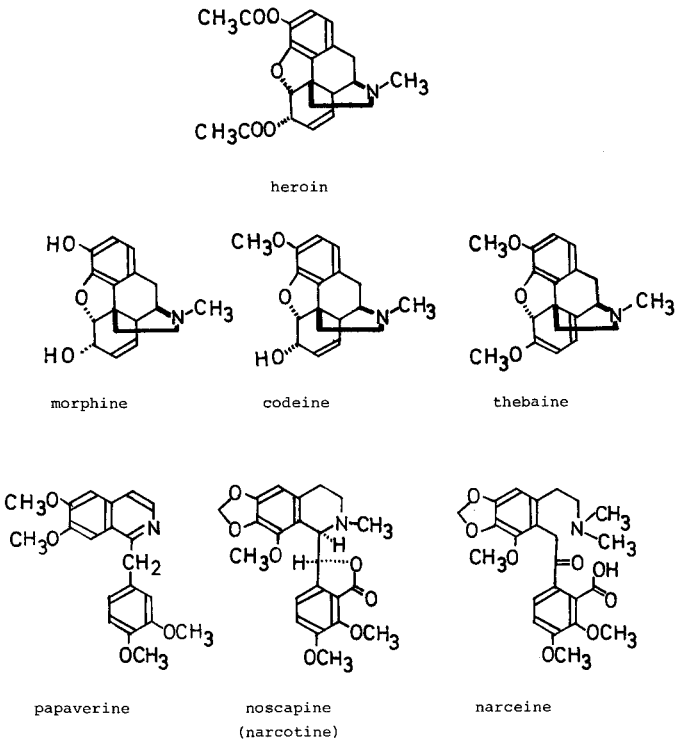


Fig. 1. Main alkaloids in opium and heroin.

more potent than morphine on a weight basis, but its action is similar to that of morphine. (b) Analysis of heroin obtained in drug seizures. Illicit heroin usually contains many impurities and adulterants such as morphine, codeine, papaverine, and other opium alkaloids. When unpurified morphine is used as the raw material, the synthesized heroin contains many opium alkaloids as impurities. Partially acetylated byproducts such as 6-acetylmorphine and, especially, 3-acetylmorphine, are often found. It is also often the case that confiscated drugs (''street drugs'') are mixtures of other drugs such as cocaine, amphetamine, and LSD. Many kinds of adulterants such as caffeine, quinine, strychnine, and especially barbiturate are added quite frequently. (c) Analysis of opium alkaloids and heroin in biological materials, usually for identification of drugs of abuse in addicts. In this situation, the metabolism of drugs must be considered. In man, heroin is enzymatically converted into 6-acetylmorphine and

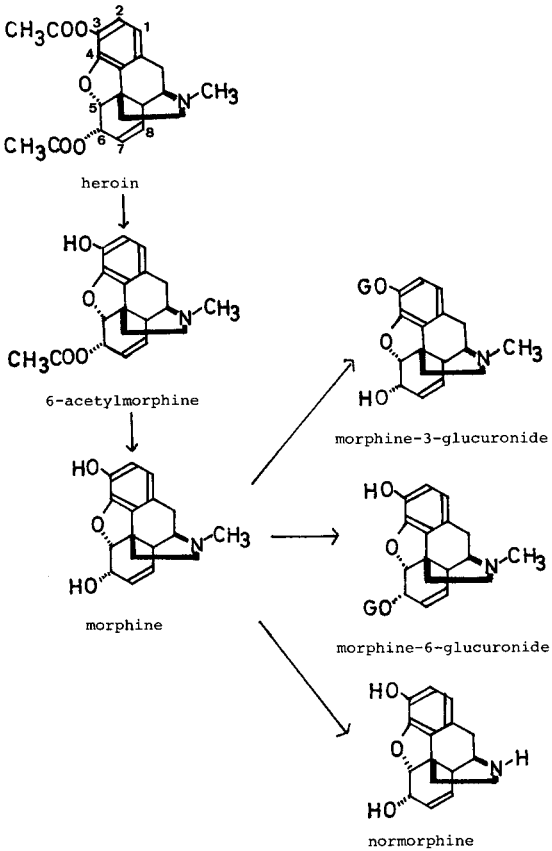


FIG. 2. Major metabolic pathways of heroin.

morphine (Figure 2). Thus, the urine and blood of heroin addicts usually contain these two biologically active metabolites along with heroin. It is possible that heroin itself is not detected in biological samples, and that only its metabolites, morphine and 6-acetylmorphine, may be detected when long periods of time elapse between administration and analysis. Morphine, in turn, is metabolized in man primarily through conjugation with glucuronic acid in the 3-position (morphine-3-glucuronide). The metabolic pathways of morphine are very complicated, and various minor metabolites such as morphine-6-glucuronide, normorphine, and others have been reported. It is often desirable to detect these metabolites as well as the originally administered drugs.

2. *GC and GC-MS Analysis*

Many attempts have been made to separate and measure opium alkaloids and heroin by GC and GC-MS. Early reports on the GC analysis of opium alkaloids (*17*) showed that it was possible to gas chromatograph these rather nonvolatile compounds without derivatization. Analysis without derivatization has usually been attempted on nonpolar or slightly polar stationary phases such as SE-30 (most widely used) (*17,36–39*), OV-17 (*40,41*), OV-1 (*42*), and others. The use of more polar stationary phases has also been applied in an attempt to increase selectivity (*43,44*), but retention times on polar stationary phases are often too long for practical analysis. For GC analysis of opium alkaloids with hydroxyl groups, derivatization gives better results.

Heroin (diacetylmorphine) can be gas chromatographed without derivatization to give a sharp peak, but morphine and other opium alkaloids quite often give tailing peaks, especially when they possess hydroxyl groups. Thus, for simultaneous determination of various opium alkaloids, derivatization is necessary.

Various derivatization methods (see Table II) including acylation by Ac_2O (*45*), TFAA (*46,47*), PFPA (*48*), HFBA (*49*) and pentafluorobenzoyl (PFB) bromide (*50,51*), and silylation by BSA (*52–55*), BSTFA (*56,57*), HMDS (*58–60*) and TMSI (*61*) have been reported. "On-column" derivatization is sometimes recommended (*62–66*) wherein sample and derivatization reagent are mixed, injected into the column and then reacted. The off-line derivatization method seems preferable, however, since the reaction may proceed only partially in on-column derivatizations. On-column acetylation of morphine by Ac_2O gives two reaction products, heroin (3,6-diacetylmorphine) and possibly 6-monoacetylmorphine (*62*), but the use of propionic anhydride gives only one peak, presumably dipropionylmorphine. When two different types of hydroxyl groups (for example, alcoholic and phenolic hydroxyl groups) exist in the molecule, different derivatives may be produced. The two primary narcotic alkaloids present in opium, morphine, and codeine, possess hydroxyl groups and can be easily derivatized, but derivatization of thebaine, a dimethyl ether-type analogue of morphine with no hydroxy group is difficult. GC analysis of thebaine at low

temperatures gives a very broad peak, yet, at higher temperatures, degradation takes place, making GC analysis of thebaine difficult (18). Other major alkaloids in opium (such as papaverine, noscapine, and narceine) lacking hydroxyl groups are not easily derivatized. A simultaneous GC detection of the various alkaloids from the poppy plant is difficult, and HPLC is more suitable for their separation.

To separate and identify picogram quantities of morphine and its congeners, the introduction of a halogen atom, especially fluorine (*67–69*) was conducted with the use of a very sensitive EC detector. To facilitate identification by GC, a combination of other techniques such as IR and other methods has been attempted; with the development of GC-MS (*70–72*) these methods have lost their importance for routine analysis.

For a decisive identification of drugs of abuse in biological materials, an analysis of the metabolites present (in addition to original drugs) is desirable. 6-Acetylmorphine and morphine present with heroin itself have been detected as major metabolites of heroin in the urine or blood of heroin addicts. Glucuronides of morphine may also be present in such samples, but a direct determination of morphine concomitantly with its glucuronides by GC or GC-MS is impossible. For such a purpose, free morphine is first analyzed and its glucuronide derivatives are then hydrolyzed for GC analysis to measure total morphine (*73*), a procedure which is both time-consuming and tedious. HPLC is advantageous for such a purpose because metabolites as well as heroin can be analyzed simultaneously.

Capillary GC, and capillary GC-MS analysis have also been used recently, as have conventionally packed columns. Capillary GC analysis of morphine has also been attempted after silylation (*74*) or acylation (*75,76*). Capillary GC analysis of heroin has been reported on a glass capillary column (*77*) and on a fused-silica capillary column (*78*). In capillary GC, heroin and a large number of impurities can be detected when coexisting alkaloids are derivatized. Thus, a "finger-print" analysis of various illicit heroin samples of different origin (*77*) is possible. With the development of fused-silica capillary columns with inert column surface, detection and separation of materials have been improved to a large extent. Picogram amounts of heroin have been analyzed without derivatization (*78*).

3. HPLC Analysis

A large number of reports on HPLC analysis of opium alkaloids and heroin have appeared. HPLC seems to be a very suitable method for the analysis of opium alkaloids (especially for the analysis of various alkaloids in the plant materials, or the detection of drug metabolites in biological materials) for the following reasons: (a) Opium alkaloids have strong absorption in the UV range, and tedious derivatization is unnecessary except when microdetection in picogram quantities, such as in biological samples, is required. (b) Separability of

opium alkaloids by reversed-phase ion-pair chromatography is excellent because these alkaloids are bulky and their ability to ion-pair is strong. (c) Simultaneous detection of drugs of abuse and their metabolites in biological samples can be achieved (i.e., glucuronide derivatives of morphine and morphine, 6- or 3-acetylmorphine and heroin in biological samples) which is impossible by means of GC. For such determinations, the use of fluorescence derivatization by dansyl chloride (DANS-Cl) (*79*) and other reagents (*80,81*) may be necessary when the content is very low. The use of an electrochemical detector (*82–87*) is also recommended. In spite of these advantages of HPLC over GC, forensic analysis of heroin and related compounds is still carried out by GC-MS, since peak identification by HPLC is still not easy. However, if an instrument for LC-MS were more widely available, LC-MS might become the standard method for forensic analysis of opium alkaloids and heroin.

In earlier works, specially devised dynamic coating techniques (*24,88,89*) were sometimes tried, but they are now of mere theoretical importance because various chemically bonded silica gel columns are now commercially available. Among the several methods of column chromatography used for the separation of opium alkaloids, heroin, and related compounds, reversed-phase ion-pair chromatography seems to give the best separations. Lurie (*90*) and Olieman *et al.* (*91*) demonstrated that opium alkaloids and heroin were definitively separated by reversed-phase ion-pair chromatography on C18 columns using heptanesulfonic acid as an ion-pairing reagent. Several reports then appeared for the separation of compounds for analysis in plant materials (*90*), confiscated seizures drugs (*92,93*), and biological samples (*94*). In these studies, the solvent systems used were H_2O/MeCN or H_2O/MeOH. C18 columns are frequently utilized, but the use of other packings such as C8, phenyl, or cyanopropyl columns is possible and better separation may be obtained (*95–97*). Aside from the usual ion-pair reagents such as alkylsulfonates and dodecyl sulfate, the use of other ion-pair reagents, such as camphor sulfonate (*98*) and dioctylsulfosuccinate (*99*) has been reported. Slightly acidic eluents buffered with acetate or phosphate are usually used. Since the degree of separation afforded by reversed-phase ion-pair chromatography is so excellent, the simultaneous detection of morphine and its glucuronide metabolites is possible (*94*).

When the ion-pair reagent is removed from the eluent, this system represents the usual reversed-phase chromatography system; analysis of opium alkaloids and heroin has also frequently made use of it (*18,100–103*), although as indicated earlier, reversed-phase chromatography tends to cause tailing of alkaloids (*20,21,104,105*).

Ion-exchange chromatography of opium alkaloids, heroin, and related compounds on cation-exchange (*104*) and anion-exchange columns has been reported (*106*). The use of ion-exchange chromatography, however, has now been replaced by reversed-phase ion-pair chromatography to a large extent. However,

the use of amino silica gel with the solvent system H_2O/MeOH or H_2O/MeCN buffered with phosphates (*107*) gives excellent separation comparable to that of reversed-phase ion-pair chromatography. Interestingly, the elution sequence of opium alkaloids on amino silica is often reversed to that observed with ion-pair chromatography on C18 columns. The elution sequence of ion-pair chromatography on C18 columns is as follows: morphine < codeine < thebaine < noscapine < papaverine when H_2O/MeOH/ AcOH/sodium heptane sulfonate (*90*) is used. On the other hand, the elution sequence on amino silica gel is as follows: papaverine < thebaine < codeine < morphine with MeCN/0.025 *M* KH_2PO_4 (*107*). Furthermore, on amino silica, retention times of opium alkaloids increase when the content of H_2O in the eluent is decreased, which is opposite the situation found with C18 columns. These results suggest that normal-phase partition characteristics, as well as weak anion-exchange properties, play significant roles in this system.

Adsorption chromatography of opium alkaloids tends to give broad and tailing peaks. Some authors, however, claim that silica gel gives satisfactory separation of opium alkaloids when a slight amount of base is added to the eluent (*108–110*). The elution sequence on silica gel is similar to that on amino silica: noscapine < papaverine < thebaine < codeine < morphine with hexane/CH_2Cl_2/EtOH/Et_2NH (*108*). Slight tailing was still observed even when bases were added to the eluent.

4. TLC Analysis

Since the first report by Borke and Kirsch (*111*), TLC of opium alkaloids has been extensively investigated, and the optimal conditions for analysis are well established. Stahl *et al.* compared 58 solvent systems for the analysis of opium alkaloids, and showed silica gel to be a suitable adsorbent (*31*). The most versatile systems are basic solvents on silica gel such as toluene/Me_2CO/95% EtOH/25% NH_4OH (40:40:6:2) (*112*) and toluene/AcOEt/Et_2NH (70 :20 :10). Major opium alkaloids can be readily separated under these conditions.

B. Ephedra Bases and Amphetamines

1. Purpose of Analysis

Many β-phenethylamine derivatives, which resemble catecholamines structurally, exhibit characteristic pharmacological actions. Amphetamine (AP), methylamphetamine (methamphetamine, MP), and their analogues are known as very strong stimulant drugs. Ring-substituted phenethylamines such as mescaline (the main hallucinogenic alkaloid in *Anhalonium williamsii*) and several synthetic phenethylamine derivatives have strong hallucinogenic properties. In Japan, MP addiction is now the most serious drug abuse problem. Abuse of stimulant

drugs is also a social problem in the United States and Europe, where AP is more frequently abused than MP. The stimulant effect of MP is somewhat stronger than that of AP. Amphetamines are closely related structurally to ephedra alkaloids. Indeed, AP and MP are reduction products of ephedrine (EP) and norephedrine (NE), respectively (Figure 3). *Ephedra herba* (*Ephedra spp.*) is one of the most common crude drugs in Chinese traditional medicine and is often used for the therapy of asthma. Its effective components are *l*-ephedrine (*l*-EP) and *d*-pseudoephedrine (*d*-ϕ-EP). Ephedra herba contains several other phenethylamines including *l*-norephedrine (*l*-NE), *d*-pseudonorephedrine (*d*-ϕ-NE), *l*-methylephedrine (*l*-NE), *d*-pseudomethylephedrine. Ephedra bases have some stimulating action and *d*-ϕ-NE is the main alkaloid in khat (*Catha edulis*) and partially responsible for its stimulating effect. Ephedrines contain two asymmetric carbon atoms, and each of these compounds exists as a pair of enantiomers. It is interesting to note that the pharmacological actions of isomers not present in plants such as *d*-EP and *l*-ϕ-EP are very weak. When the hydroxyl groups in ephedrine enantiomers are reduced, one asymmetric center disappears and *l*-EP and *d*-ϕ-EP, therefore, give the same reaction product, *d*-MP. Ephedra bases themselves are usually not abused, but they are of interest in forensic chemistry for the following reasons: (a) Confiscated stimulant drugs quite often contain ephedrines as the raw material used in their synthesis. (b) Ephedrines are added to the drug as an adulterant, and are an object in drug abuse examinations. (c) Ephedrines are stimulant drugs, as well as doping agents.

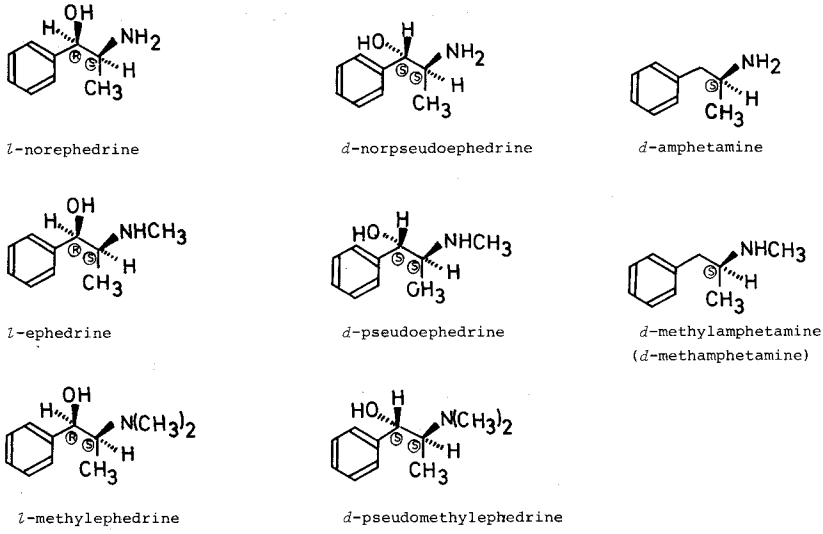


FIG. 3. Alkaloids in *Ephedra herba* and stimulant drugs.

Analysis of ephedra bases and stimulant drugs is performed for the following purposes: (a) The analysis of ephedra alkaloids in plant materials. (b) Analysis of confiscated illicit stimulant drugs (mainly AP and MP) seized in drug raids. Such drugs may contain a large amount of EP as an impurity or adulterant. Barbiturates are also sometimes found because they enhance the action of AP to a considerable extent. It is also desirable to distinguish the optical isomers of stimulant drugs. *d*-Methylamphetamine and *d*-amphetamine are strong stimulants, but the *l*-isomers are less potent. Consequently, the stimulating action of racemates is stronger than that of the *l*-isomers but weaker than that of the *d*-isomers. The use of *d*-isomers or racemates is prohibited in almost all countries, but laws concerning the *l*-isomers differ: In Japan the use of *l*-isomers is prohibited, but in the United States and in Europe, *l*-isomers are not always prohibited by law. The catalytic reduction of *l*-EP and *d*-ϕ-EP, which are naturally abundant, produces pure *d*-MP. In addition to ephedrines of natural origin, racemic ephedrines are also produced on an industrial scale. If synthesized and optically resolved, *l*-EP can be used as a raw material, however, contaminating *d*-EP is usually found. Sometimes illicit MP is found to be racemic and prepared from *dl*-ephedrine without optical resolution. Since NE is not abundantly present in plants, AP is not synthesized from the naturally occurring alkaloid, and thus optically pure *l*-AP is rarely found. (c) Detection and determination of stimulant drugs in biological samples obtained especially from drug addicts. The metabolic pathways of AP and MP are shown in Fig. 4. It is noteworthy that AP is one of the major metabolites of MP, and thus AP as well as MP is detected in urine or blood samples of MP addicts (Figure 4). It should also be noted that some other drugs which have a therapeutic use (listed in Table III) produce AP or MP as metabolites. Although it is rare that users of such drugs are misidentified as drug

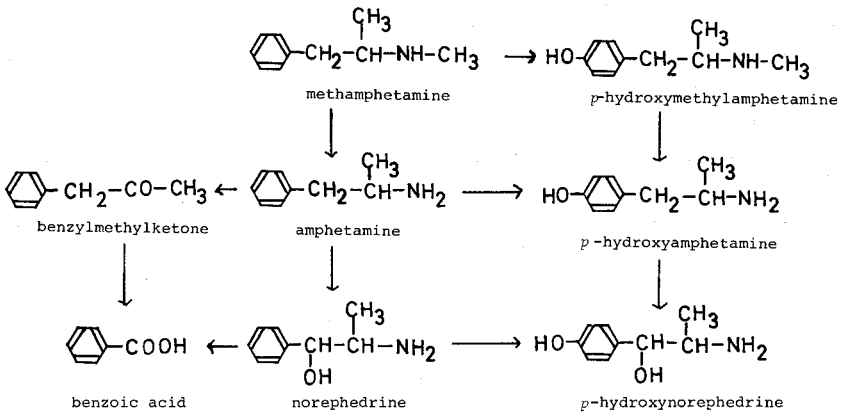
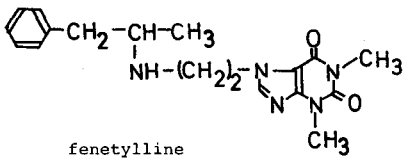
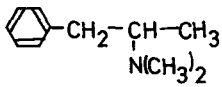
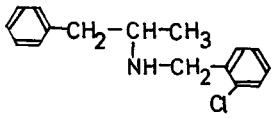
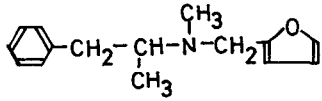
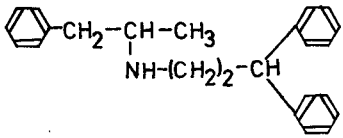
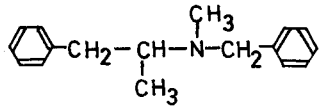


FIG. 4. Major metabolic pathways of *dl*-methamphetamine and *dl*-amphetamine.

TABLE III

DRUGS WHICH GIVE AMPHETAMINE(A) OR METHAMPHETAMINE(B) AS ONE OF METABOLITES

(A)	(B)
 fenetylline	 dimethylamphetamine
 clobenzorex(INN)	 frugalan(furfenorex)
 segontine(prenylamine)	 benzphetamine(didrex,inapety

addicts, it is desirable to analyze precisely not only the original drugs, but also their metabolites.

2. *GC and GC-MS*

The analysis of ephedra bases in plant materials (Ephedra herba) focuses on *l*-EP and *d*-ϕ-EP since the content of other alkaloids is very low. GC analysis of ephedra alkaloids, which contain hydroxyl groups and are therefore nonvolatile, requires conversion into volatile derivatives, otherwise broad and tailing peaks are obtained. Separation of *l*-EP and *d*-ϕ-EP has been done on SE-30 as the oxazolidine derivatives obtained by treatment with acetone (*113*). The use of usual acyl reagents such as Ac_2O, TFAA, PFBA (*114*), PFB chloride (*115*), and HFBA (*116*) has also been reported. Silyl reagents are applicable as well. Other methods of derivatization, such as the formation of oxazolidines as mentioned above and the use of *n*-butylboronate (*117*), have also been reported. It should be noted here that derivatization may yield several reaction products since ephedrines have both hydroxyl and amino groups. The structures of the reaction products are not always fully described. GC determination of the main alkaloids in *Ephedra herba* has been carried out by several workers (*118*), but simultaneous GC determination of the six ephedra bases in plant materials has not yet been carried out successfully.

GC determination of amphetamines is possible without derivatization when polar stationary phases (such as Apiezon, and polyethleneglycol (Carbowax) are treated with strong alkali (KOH) (*119*). Derivatization, however, is sometimes recommended in order to improve sensitivity. For the detection of hydroxylated metabolites, derivatization is necessary. Various derivatization methods including Schiff base formation (*120*) and reaction with isothiocyanates (*121*) have been used in addition to acylation (*122,123*) and silylation (*124*) methods. The use of fluoro-derivatives was found to be effective (*125*) with increased detectability by an ECD detector. These derivatives have been analyzed on nonpolar stationary phases such as SE-30, OV-17, and OV-1. For the analysis of hydroxylated metabolites of amphetamines, derivatization is necessary (*126*).

The differentiation of *d*- and *l*-isomers of ephedrines and amphetamines may be achived by the following two methods: (a) A racemic mixture (or an unknown single optical isomer) is reacted with a chiral reagent to yield a disastereomeric mixture that may usually be resolved on an achiral stationary phase. (b) The racemates may be directly resolved on a chiral stationary phase with or without derivatization. The former method is usually applied and several optically active reagents such as *N*-(*R*)-2-phenylbutyric anhydride (*127*), *N*-trifluoroacetyl-L-prolyl chloride (L-TPC) (*128*), and α-methylbenzyl isocyanate (*129*) have been utilized. The latter two reagents are commercially available and widely used. L-TPC reagent, however, often contains minute amounts of D-TPC, which might interfere in the determination and thus optical purity should be checked. Although the content of D-TPC is described on the vial, racemization of the reagent may progress on storage.

In contrast, the use of chiral stationary phases is free of this problem, but GC analysis of amphetamines on chiral stationary phase without prior derivatization remains unsuccessful, and derivatization is thus required. The use of achiral reagents, which gave good resolution of the enantiomers of amino acids, did not give satisfactory resolutions of amphetamines on chiral stationary phases. The combination of a chiral reagent such as TPC with a chiral stationary phase, however, gave good results (*130*) by which the four disastereomers could be separated.

GC-MS analysis of amphetamines is the most reliable identification method, and in Japan as well as in the United States and Europe, analysis of amphetamines of forensic interest is now carried out primarily by this technique (*131–135*). Although conventional packed columns are still widely utilized, the preferred use of fused-silica capillary columns will increase separation and detectability (*136*).

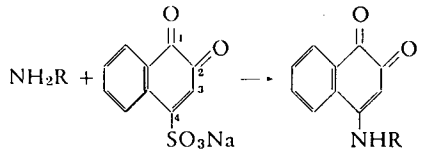
3. HPLC Analysis

The number of reports on the HPLC analysis of ephedrines and amphetamines is still modest. This is attributed to the fact that absorption coefficients (ε) of

phenethylamines are small (log $\epsilon < 2$), making HPLC microdetection difficult. Direct determination does not require tedious derivatization procedures but cannot be applied to forensic analysis except when the content of drugs is high. Determination of alkaloids in *Ephedra herba* by adsorption (silica gel) and reversed-phase (C18, ion suppression) chromatography was reported and ephedrine, pseudoephedrine, and methylephedrine, were separated (*137*). Reversed-phase ion-pair chromatography allowed the separation of ephedra alkaloids, including minor alkaloids (*138*). Rather high column temperatures and high concentrations of the ion-pair reagent (sodium dodecylsulfate), however, were required, suggesting that the ability to form ion-pairs is rather weak for these alkaloids. These conditions are not favored because the lifetime of a column under such conditions is not long. Furthermore, the level of detectability is reduced due to the low absorption coefficients of the ephedra alkaloids.

Adsorption chromatography on silica gel with basic solvents (*139*) and reversed-phase ion-pair chromatography using pentanesulfonate (*140*) or dodecyl sulfate (*141*) as ion-pair reagents have been reported for the separation of amphetamines from its metabolites. The detection limit, however, was large (100 ng) due to low absorption coefficients. Thus, for the microdetermination of ephedra bases as well as amphetamines, derivatization is indispensable. Several derivatization reagents such as OPA (Table II) (*142*), 4-chloro-7-nitrobenzo-2, 1,3-oxadizaole (NBD-Cl) (*141,143*), and naphthoquinone-4-sulfonate (NQS) were used (*144*).

We have reported (*144*) that detection of MP and AP in the urine of drug addicts could be achieved even when the amounts were very low. The method is as follows: Urine samples of drug addicts were made alkaline with NH_4OH and the free bases extracted with hexane. A small amount of HCl/EtOH was added, and the hexane distilled off. Solutions of NQS and $NaHCO_3$ were added to the residue. The mixture was heated to 70°C for 20 min and the red-colored reaction products extracted with $CHCl_3$, followed by HPLC analysis on silica gel with the solvent system hexane/$CHCl_3$/AcOEt/EtOH (50 : 25 : 10 : 1). When measured at 450 nm, 2 ng of AP and MP could be determined without interference from foreign substances (Figure 5). By this treatment, various other phenethylamines such as ephedrine, pseudoephedrine, norephedrine, mescaline, and STP were derivatized and analyzed. Farrell and Jefferies (*141*) compared five different methods for the HPLC analysis of amphetamines and found that NQS derivatization gave the best results and was applicable for the routine microanalysis of amphetamines in plasma and urine of drug addicts. We described the structure of the reaction products obtained from derivatization of amphetamines by NQS using IR, NMR, and MS (*145*). The color reaction for amphetamines proceeds quantitatively. We improved this color reaction in the following way: after extracting amphetamines from urine and subsequently distilling off the solvent of extraction, a 0.5% NQS solution, and an 8% $NaHCO_3$ solution were added



together with $CHCl_3$. The mixture was heated under stirring in a flask equipped with a reflux condenser. The red-colored reaction products were extracted into $CHCl_3$. Reaction and extraction were quantitatively complete within a few minutes, instead of 20 min. The $CHCl_3$ layer then was submitted to HPLC analysis. The extraction procedures were later improved by Farrell and Jefferies (*141*). Instead of the conventional solvent extraction, they adsorbed the amphetamines on XAD-2 resin and eluted with $CHCl_3$/isoPrOH (3 : 1). This treatment effectively removed foreign substances and permitted the extraction of hydroxylated metabolites in addition to the amphetamines. After derivatization with NQS, simultaneous HPLC analysis of amphetamines and metabolites was possible.

Separation of optical isomers of ephedrines and amphetamines by HPLC has been recently reported using several methods: (a) Enantiomers were converted into diastereomers by reaction with a chiral derivatizing agent and then analyzed

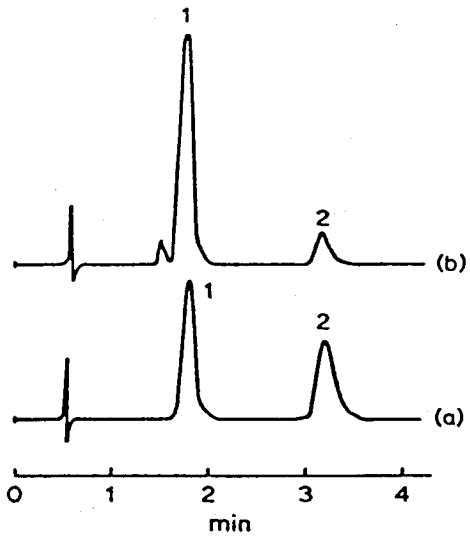


FIG. 5. Chromatograms of amphetamine and methamphetamine derivatives. (a) Standard sample (containing 1 μg of amphetamine and methamphetamine); (b) sample of urine of a methamphetamine addict. Peaks: 1 = methamphetamine; 2 = amphetamine. Column, Wakogel LC 5H (25 cm × 2 mm I.D.); eluent, chloroform-ethyl acetate-ethanol-*n*-hexane (25:10:1:50); flow-rate, 1.2 cm^3/min; detection, 450 nm; sample size, 100 μl.

on a conventional achiral stationary phase. This method is advantageous and sensitivity could be improved with a suitable chromophoric group attached to the chiral derivatizing agent. It is probable, however, that partial racemization occurs during the derivatizing reaction, and the optical purity of the derivatizing reagent is not always high. The use of several chiral reagents has been reported (*146–152*). (b) Enantiomers may be resolved directly on a chiral stationary phase. Several chiral stationary phases such as Sephadex, chiral crown ethers, microcrystalline cellulose triacetate, and chiral ligands bonded to silica gel have been reported, and among these the last one seems to be most promising. The chiral stationary phase is either physically adsorbed on the surface of a solid support (*153,154*), or chemically bonded to the support (*155*); both types of columns are now commercially available. The latter method seems to be advantageous since the lifetime of the column is much longer. With this approach, derivatization is (at least in principal) not always required, but direct separation of amines without derivatization is difficult. (c) We devised other methods for determining the optical purity of ephedrine by formation of Ni (II)dithiocarbamate chelates requiring neither chiral reagents nor chiral columns (*156,157*). This was accomplished in the following way: Aliphatic primary and secondary amines such as ephedrines and amphetamines were converted into dithiocarbamates with carbon disulfide under alkaline conditions and obtained quantitatively and almost instantaneously. The resulting dithiocarbamates formed stable metal chelates with various metal ions such as Ni(II). When both *l*- and *d*-isomers of ephedrine existed, the following mixed ligand complex was formed,

$$NiD_2 + NiL_2 \rightleftharpoons 2NiDL$$

where, D and L designate the dithiocarbamates derived from *d*- and *l*-isomers, respectively. Two binary complexes, NiD_2 and NiL_2, are not separated because they are enantiomers, but NiDL is the diastereomer of NiD_2 (and NiL_2), and could be separated. Separation was achieved on silica gel with hexane-isopropyl acetate (Figure 6). From the ratios of two peak areas, the optical purity of ephedrine was determined. Later, Low *et al.* extended this approach to a reversed-phase system and determined the optical purity of pseudoephedrine as well as that of ephedrine (*158*). They also attempted *in situ* formation of Ni (II)dithiocarbamates in the column in which Ni(II) ion was added to the eluent in the reversed-phase system. Both previous and on-column formation of Ni(II) chelates gave satisfactory results for the determination of optical purity of ephedrine and pseudoephedrine. Since the absorption coefficients of Ni(II) chelates are high ($\log \epsilon = 4.6$ at 325 nm), this derivatization method is suitable for the microdetection of ephedra bases and amphetamines. Ternary complex formation, however, which is useful for determination of optical purity, complicates the chromatographic separation (*159*). We devised a method to overcome this difficulty as follows: When a large excess of foreign amines (X_0) was added

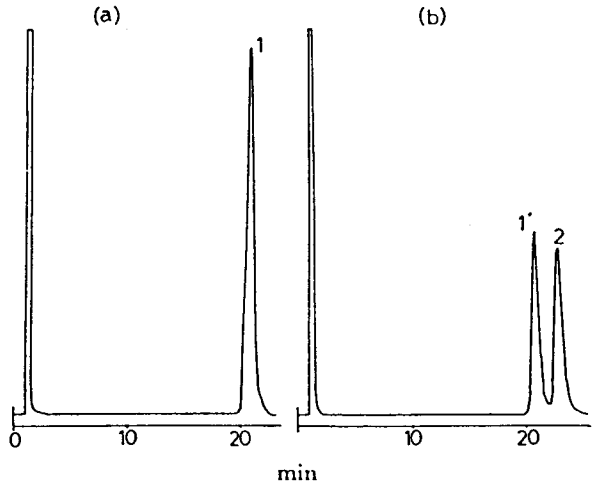


FIG. 6. Chromatograms of Ni(II) dithiocarbamate chelates derived from optically active and racemic ephedrines. Column: LiChrosorb SI 100 (4 mm × 25 cm). Eluent: hexane:isopropyl acetate = 100:11.5 (water saturated). Flow rate: 2.5 cm^3/min. Detector: 325 nm. Sample: (a) 0.2 m*M* *l*-ephedrine(*d*-ephedrine gave similar chromatograms), (b) 0.2 m*M* *dl*-ephedrine Sample size: 50 μl.

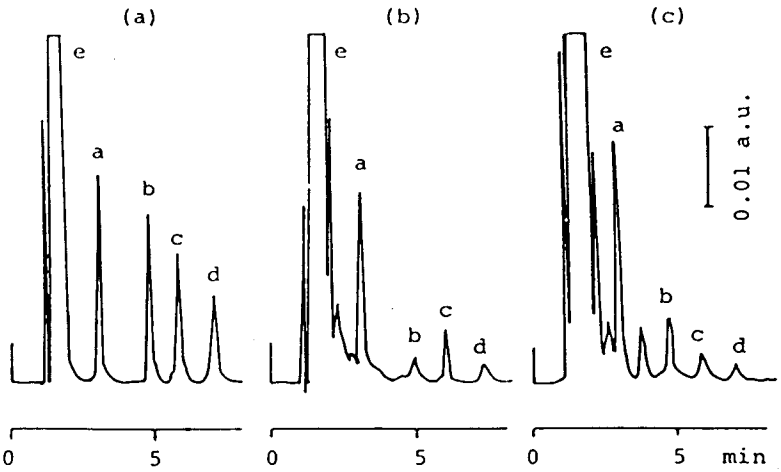


FIG. 7. Chromatograms of Ephedra Bases Column: Polygosil 60-5 (5 μm). 4 mm × 25 cm. Eluent: hexane-isopropyl acetate = 100:15 (water-saturated) at 2.0 ml/min. Detection: UV 325 nm (full scale 0.1 a.u.). (a) Standard sample (80 ng *l*-ephedrine, 80 ng *d*-pseudophedrine, 75 ng *dl*-norephedrine, and 75 ng *dl*-pseudonorephedrine in each injection); (b) *E. sinica;* (c) Pinelliae Tuber. Sample size: (a) 5 μl; (b) 50 μl; (c) 50 μl. a, *l*-ephedrine; b, *dl*-norephedrine, c, *d*-pseudoephedrine; d, *dl*-pseudonorephedrine; e, diethylamine (peak of excess binary complex MX_2).

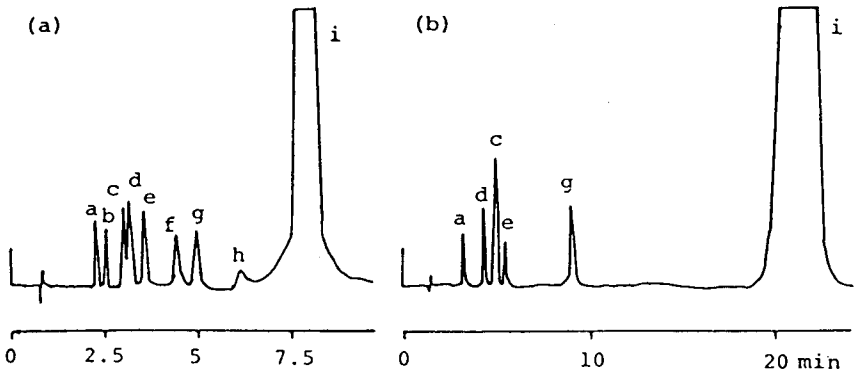


FIG. 8. Chromatogram of Psychotropic Amines on Reverse Phase Packings (a) Column: Hypersil ODS (5 μm). 4.6 mm × 15 cm. Eluent: acetonitrile-water = 100:75 at 2.0 ml min. Detection: UV 325 nm. (b) Column: Nucleosil 5C18 (5 μm). 4.5 mm × 15 cm. Eluent: acetonitrile-water = 100:45 at 2.0 ml min. a. *dl*-norephedrine; b. mescaline; c. *l*-ephedrine; d. *d*-pseudoephedrine; e. *d*-amphetamine; f. STP; g. *d*-methamphetamine; h. methylphenidate; i. dibutylamine (peak of excess binary complex MX_2).

together with the amines to be analyzed (A_0, B_0 etc.), most of A_0 and B_0 existed as ternary complexes (MAX, MBX) and the presence of binary complexes MA_2 and MB_2 was negligible. Here, X, A, and B designate dithiocarbamates derived from X_0, A_0, and B_0, respectively. Thus from the peak heights (or areas) of MAX and MBX, detection of individual amines at the nanogram level was possible. Analysis of ephedrines in the range of a few nanograms in plant materials was carried out using this method (*160*), and the method was so sensitive that very small amounts of ephedra bases could be detected in Pinelliae tuber (the tuber of *Pinellia ternata,* famous Chinese crude drugs, Figure 7) (*161*). This method was also used for the microdetection of AP and MP in urine of drug addicts (*162*) using normal-phase and reversed-phase chromatography (Figure 8). (d) Other recently developed methods are also applicable which combine an achiral stationary phase and a mobile phase containing a chiral eluent. Optically active ion-pair reagents such as camphor sulfonate, copper-amino acid chelates, and tartaric acids have been used successfully in the resolution of amino acids. This method has not been applied to resolve amphetamines but is worth investigating.

4. TLC Analysis

TLC of amphetamines has been extensively investigated in connection with the detection of various drugs of abuse, and a few reports have appeared on the separation of ephedra bases from plant materials. Kimura *et al.* separated four alkaloids, *l*-EP, *d*-ϕ-EP, *l*-ME, and *l*-NE from Ephedra herba on silica gel with

the solvent system 10% NH_4OH/isoPrOH (1 : 9) (*163*). Separation of *l*-EP from *d*-ϕ-EP was incomplete and the two spots partially overlapped. We reported the separation of *l*-EP and *d*-ϕ-EP on silica gel impregnated with 0.25*M* KOH (*164*). With the solvent system BuOH/EtOH/H_2O/acetaldehyde (24 : 12 : 4 : 0.02), the Rf values of *l*-EP and *d*-ϕ-EP were 0.14 and 0.31, respectively. Addition of a minute amount of acetaldehyde was essential for the separation. Densitometric determination was carried out after coloring with ninhydrin.

C. KHAT

The fresh leaves of khat (*Catha edulis*) are habitually chewed in certain parts of eastern Africa and southern Arabia. The fresh leaves have a very strong amphetamine-like stimulating action, but they rapidly loose their effect on drying. Therefore, the habit of chewing khat, which has been deeply linked with social and cultural tradition, has, until recently, remained endemic to the area where the plant grows. However, due to recent developments in transportation, the habit has been spread considerably, and shipments of khat to Europe and United States have been observed. Study of the active ingredients in khat has long been receiving the attention of chemists. Studies on chemical constituents and pharmacological action of khat have progressed markedly in recent years and are described in several reviews (*165–167*).

The presence of *d*-pseudonorephedrine (cathine) in khat was confirmed earlier (*168*). Since cathine induces some stimulating action, it has long been considered the only active constituent (*169–171*). Pharmacological study of cathine, however, has indicated that the stimulating action of fresh khat leaves cannot be explained in terms of cathine alone because its action is relatively weak (*172*). The presence of alkaloidlike substances has sometimes been observed, but attempts to isolate these alkaloids has not been successful for a long time. The fact that the action of khat is strong only when the leaves are fresh suggests that the stimulating action may be caused by rather labile compounds.

Chemical study of the constituents in khat was undertaken by the United Nations' Narcotics Laboratory (*173–175*) and it was this group that finally succeeded in the isolation of a second phenethylamine alkaloid, *S*-*l*-α-aminopropiophenone (*l*-cathinone), a ketonic congener of cathine with stimulating action comparable to that of amphetamine. In this study, the fresh leaves were acquired from plants and were immediately extracted or freeze-dried after collection. The crude extracts were concentrated under vacuum and then stored in a freezer after freeze-drying. Condensed khat extracts with methanol (or ethanol) were diluted with water and then partitioned by solvent extraction. The $CHCl_3$ fraction was rich in phenethylamines. In freshly prepared extracts in which cathine was only a minor constituent, several spots were seen on TLC ($CHCl_3$/MeOH = 9 : 1 or EtOAc/MeOH/NH_4OH = 85 : 10 : 5 on Kieselgel GF_{254}). A

major spot colored by ninhydrin developed an orange color which immediately turned pink after heating. Cathine, on the other hand, gave a stable pink color on heating. Attempts to isolate the new alkaloid by adsorption column chromatography or preparative GC coupled with GC-MS were fruitless because it rapidly decomposed. Conventional treatment of alkaloids by extracting the $CHCl_3$ fraction first with $0.25M$ H_2SO_4 and then back-extracting with $CHCl_3$ after basification of the sulfuric acid solution achieved a separation. The new alkaloid precipitated as an oxalate in ether. Its structure was determined to be *S-l*-α-aminopropiophenone on the basis of spectral data. The compound was named *l*-cathinone.

In addition to cathinone and cathine, the presence of several other alkaloids has now been confirmed. The presence of *l*-norephedrine (*176*) and another group of alkaloids called cathedulins (*177*) which have sesquiterpene cores has also been elucidated. Other phenethylamines called *d*-merucathine (*178*), *l*-pseudomerucathine (*167*), and *d*-merucathinone (*165, 178*) were also isolated from plants from Kenya (Figure 9). The structure of *d*-merucathine previously postulated as (3*S*,4*S*) (*178*) was revised by Wolf and Pfander as (3*R*,4*S*) on the basis of comparative synthetic studies and CD spectra (*179*).

It is believed that the stimulating action of khat can now be attributed to the presence of cathinone and cathine, although the possibility that some other constituents might be involved in producing the khat syndrome cannot be ruled

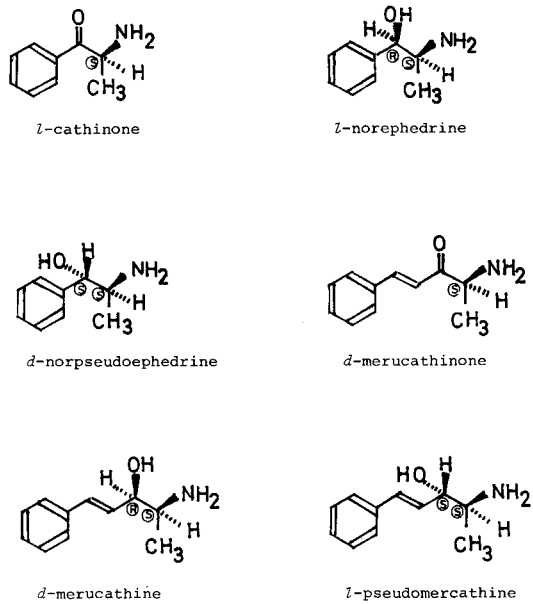


FIG. 9. β-Phenethylamine derivatives in khat.

out. The presence of *l*-ephedrine suggested previously by several workers has now been refuted (*180,181*). It seems surprising that such a strong stimulant as cathinone was not discovered for such a long time. The reasons are attributed to the fact that cathinone is chemically a rather unstable compound and a chemically labile precursor of cathine. Thus, during the drying of the leaves, cathinone is transformed into cathine and partially into *l*-norephedrine. Cathinone is easily oxidized *in vitro* to 1-phenyl-1,2-propanedione and benzoyl methanol (*167*). The cathinone dimers 3,6-dimethyl-2,5-diphenylpyrazine and its dehydro derivatives were also found as artifacts (*182*). Optically active *l*-cathinone also tends to racemize (*167*) especially in polar solvents due to the easy enolization of the keto-group. The instability of cathinone makes its extraction and quantification in khat very difficult. It is, therefore, probable that the abuse of khat evolved differently from that of other drugs of plant origin, such as coca, because of its instability and because its presence can only be checked in fresh leaves. The chewing of khat, however, has resulted in serious social problems in the area where the plant grows.

The number of reports on the analysis of khat alkaloids is limited at present. For the detection of cathinone, it is essential to treat fresh leaves under carefully controlled conditions (*173–175,183*). GC analysis by derivatization with TFAA (Table II) (*165,178*) was found to give excellent results because cathinone can now be separated from other structurally similar compounds such as cathine, norephedrine, ephedrine, and pseudoephedrine. GC-MS analysis (CI-MS) (*165*) was found to result in an excellent identification of cathinone. The attempts of other derivatization methods which are applicable to aliphatic primary amines such as acylation, silyation, Schiff-base formation, or reaction with isothiocyanate should be further examined. Derivatization reagents applicable to reactions with ketones might also be useful for the specific detection of cathinone. In such reactions, the formation of byproducts should be taken into account since cathinone is an unstable substance and possesses both an $-NH_2$ and a $-C{=}O$ group.

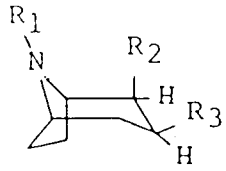
Normal-phase HPLC on silica gel packings was reported (*184*), and the contents of cathinone, cathine, and norephedrine in samples of different origins were determined, but the number of reports on HPLC analysis of cathinone and related compounds is still small. There exist several difficulties in the HPLC determination of khat alkaloids. First, UV-absorption coefficients of phenethylamines are low. The molar absorption coefficient of cathine is low ($\log \epsilon = 2.2$ at 257 nm). Introduction of a carbonyl group adjacent to the phenyl ring increases the absorption coefficient considerably. Thus, the absorption coefficient of cathinone is rather high ($\log \epsilon = 4.1$ at 247 nm (*165,167*). Direct UV detection of cathinone may give sufficient sensitivity unless analysis in the picogram range is required. For simultaneous microdetection of various khat alkaloids and related compounds, however, especially analysis in biological samples, direct UV detection

will not give sufficient sensitivity. Since the molar absorption coefficients and wavelengths of absorption maxima differ for each khat alkaloid, detection by photodiode-array UV detector facilitates the identification (*167*). In addition, since phenethylamines are rather small molecules, their ability to form ion-pairs is weak and reversed-phase ion-pair chromatography may not be suitable for their separation. The development of appropriate derivatizing methods is necessary to perform a microdetection by HPLC. In this connection, it seems that the analytical methods developed for Ephedra alkaloids may give useful information for the analysis of khat alkaloids because both alkaloids structually resemble the *d*-pseudonorephedrine and *l*-norephedrine present in both plant species. Biosynthetic and metabolic pathways of khat alkaloids have not been well examined. Mysterious is the fact that labile cathinone accumulated in khat is not promptly metabolized into cathine. The situation differs markedly from Ephedra herba. Brenneisen *et al.* recently examined the pharmacokinetics of *l*-, *d*-, and racemic cathinone in man (*185*). It was found that the metabolic pathways of *d*- and *l*-cathinone differed to a considerable extent. It seems that, after administration of optically active cathinone, partial racemization takes place. Further development of chromatographic analysis is required for forensic chemical, pharmacological, and metabolic studies of khat alkaloids. It is also desirable to distinguish optical isomers of various khat alkaloids especially for the study of metabolic pathways in the body.

D. Coca Alkaloids

Cocaine is isolated from the leaves of *Erythroxylum coca* in South America as a masticatory. Cocaine (Figure 10) is widespread as a stimulant in the illicit drug markets; its medical use is as a topical anesthetic. This has prompted the development of new rapid and sensitive methods for the determination of cocaine, its metabolites, and related compounds in samples from the illicit market and in biological materials.

The total alkaloid content in *Erythroxylum coca* leaves has been higher than that found in other species and varies with place of production. The cocaine content of Erythroxylum was examined by GC equipped with FID on a coiled glass column packed with 5% OV-101 on Gas Chrom Q(215°C) (*186*). Pulverized samples of nine Erythroxylum species obtained from herbarium specimens (collected during the years 1930–1968) weighing 19–200 mg were macerated with 10% ammonia chloroform, filtered, and concentrated. Suitable acetone solutions of the crude total alkaloids were prepared for GC and trace quantities of cocaine ranging from 0.00008 to 0.00882% were measured in the leaves of *E. campestre, E. deciduum, E. novogranatense, E. panamense, E. pelleterianum,* and *E. pulchrum.* No cocaine was detected in *E. citrifolium* and *E. rufum.* The concentration of cocaine from *E. coca* leaves analyzed within 2



	R_1	R_2	R_3
norecgonine	H	COOH	OH
benzoylnorecgonine	H	COOH	$OCOC_6H_5$
ecgonine	CH_3	COOH	OH
ecgoninemethylester	CH_3	$COOCH_3$	OH
benzoylecgonine	CH_3	COOH	$OCOC_6H_5$
cocaine	CH_3	$COOCH_3$	$OCOC_6H_5$

FIG. 10. Cocaine and related compounds.

days of harvest was 0.53% and that of a specimen which was 44 years old was 0.03% (*186*). Turner *et al.* analyzed (by GC) cocaine content in *E. coca* from three locations in Peru (*187*). Pulverized coca leaves were refluxed with 95% EtOH for 15 min, treated by acid–base partitioning with $CHCl_3$, and subjected to GC. The cocaine content in the three samples was 0.57–0.6%.

Cocaine, a lipophilic drug, is extensively biotransformed to the water-soluble metabolite, benzoylecgonine, in man. Cocaine concentration in plasma after administration of cocaine peaked after 5 min and then declined in a multiexponential fashion while the benzoylecgonine concentration in plasma increased rapidly within the first hour. The concentration of benzoylecgonine in urine consistently and greatly exceeded that of unchanged cocaine in the urine during an initial 8 hr period (*188,189*).

Koontz *et al.* used weakly basic anion-exchangers to extract and purify benzoylecgonine from urine (*190*). Bastos *et al.*, however, butylated metabolites and then detected them on TLC, since the original biotransformation products were water-soluble and not easily extracted with organic solvents or ion-exchange resins, or adsorbed on styrene-divinyl benzene copolymers (*191*). Kaistha and Tadrus extracted metabolites by three different procedures. (1) A single-step ion-exchange extraction of benzoylecgonine, (2) A two-step ion-exchange extraction of polydrugs and benzoylecgonine and (3) A single-step direct extraction of benzoylecgonine (*192*). In a simultaneous identification of cocaine and benzoylecgonine from biological samples, cocaine was extracted into $CHCl_3$ at pH 9.0 first, and then benzoylecgonine with $CHCl_3$/EtOH (4 : 1) or $CHCl_3$/PrOH (4 : 1) (*188,189,193,194*). Salting-out techniques were also used for the

extraction of benzoylecgonine (*190,191*). Extraction of cocaine from urine depends on pH and results in 94–100% recovery at pH 5.5–9.0, and 50–63% at pH 5.0–11.5, respectively (*195*). Almost 20% of the cocaine was lost in the first hour when cocaine was incubated *in vitro* in normal urine at 37°C; the loss was much greater when the pH of the urine was higher than 9.5. Cocaine loss was 40% after 5 days of freezing and more than 60% after a period of 1 month. Cocaine is relatively unstable with a half-life of 5 hr in buffered solution at pH 7.25 and 40°C (*196*). Hydrolysis of cocaine to benzoylecgonine occurs non-enzymatically (*195,197*). Cocaine is hydrolyzed to ecgonine methylester by cholinesterase in the blood or plasma, a process which might be inhibited by freezing or an addition of the cholinesterase inhibitor, sodium fluoride (*188,189,195*). Garrett and Seyda (*196*) studied the mechanisms of cocaine hydrolysis at different pH values and at various temperatures and found by HPLC that proton and base ion attack on cocaine proceeded via a benzolyecognine intermediate.

A number of methods were reported for quantitative analysis of cocaine and its metabolites in biological samples using chromatography. In some of them, the amounts of cocaine and benzoylecgonine obtained by TLC analysis were compared to those obtained by radioimmunoassays (RIA) and/or enzyme multiplied immunoassay techniques (EMIT) (*191,198*). A statistically significant concentration of urinary benzoylecogonine was shown to be 2 ng/ml and 1 μg/ml by RIA and EMIT, respecitvely (*191,198*). Cross-reactivity with cocaine and numerous other members of tropane alkaloids showed a high degree of specificity for benzoylecgonine. On the other hand, reported concentrations below 0.5 μg/ml of benzoylecgonine in urine measured by HPLC were not found by EMIT (*194*).

Separation of cocaine, its metabolites, and congeners on TLC/PC was examined using different thin-layer techniques, including glass fiber silica-gel impregnated sheets (*199*), charcoal-thin-layers (*200*), and ion-exchange resin-loaded papers (*190*). For a specific identification of cocaine and its metabolites, iodoplatinate (neutral or acidified) was used in addition to other reagents such as dilute sulfuric acid (*190,201*) and Dragendorff's reagent (*202,203*). Dragendorff's reagent followed by sulfuric acid and subsequent exposure to iodine vapors increased sensitivity for cocaine, and enhanced the sensitivity to benzoylecgonine 5–10 times over that achieved with Dragendorff's reagent alone (*204*).

Content of cocaine and its hydrolysis products on the illicit market or in biological materials were determined by GC with greater accuracy than TLC. Cocaine is directly detected by GC because of its lower polarity (*205–207*). On the other hand, cocaine, when hydrogenated to 2-hydroxymethyl tropine with $LiAlH_4$ and then derivatized with acyl or silyl reagents, could not easily be analyzed by GC (*193,208,209*). Hydrolysis products of cocaine, benzoylec-

gonine, ecgonine, and 2-hydroxymethyl tropine showed very poor chromatographic behavior on GC, this can be enhanced by two procedures to derivatize them to be detectable by GC. One reaction is alkylation of benzoylecgonine with CH_2N_2 or MeOH (EtOH) in the presence of H_2SO_4 to obtain cocaine (ethylester) after removing cocaine from extract (*187,191,210*). The other is acylation or silylation with acyl or silyl reagents, respectively. BSA (*205*), BSTFA (*205,206*), HFBA, and PFPA (Table II) (*193,208,209*), dimethylformamide dialkyl acetals (*206*) and PFB (pentafluorobenzyl bromide) (*188*) have been used for derivatization. Retention times for silyated benzoylecgonines (*205*) were larger (26 min) than those of the acylated analogs described above (3–16 min), although retention times vary with columns, flow rate, temperature, etc. The fluorinated analog (BSTFA) showed an increased resolution over benzoylecgonine derivatized with BSA (*205*). Benzoylecgonine esters with higher alkyls are formed with dimethylformamide dialkyl acetals (*206*). FID and ECD (Introduction section) are generally used as detectors for GC. Jatlow and Bailey used a selective nitrogen (alkali flame) detector to achieve an increase in sensitivity for cocaine content in plasma of as low as 5 ng/ml (*207*). There is no other procedure for the detection of benzoylecgonine by GC without derivatization which is easier and more sensitive. Jatlow and Bailey suggested that the detection limit may depend on biological background rather than detector sensitivity, even though they reduced the detection limit for cocaine on GC to 5 ng/ml (*194*). They also used HPLC for the separation of cocaine, benzoylecgonine, norbenzoylecgonine, and norcocaine. Only the first two were identified in humans but the others were found in animals after cocaine intake. Drug-free urines showed no peak with the same retention times as cocaine and benzoylecgonine when eluted with phosphate buffer/MeCN (83 : 17) on a Partisil-10 C18 column and detected with a UV detector at 200 and 235 nm. Local anesthetics and their metabolites with impurities known to occur in illicit cocaine samples of different polarities were analyzed by HPLC (*211*). The HPLC was equipped with a UV (230 nm) detector and operated with a C18-Hypersil column eluted with two solvents: solvent A, MeOH/H_2O/1% H_3PO_4/*n*-hexylamine (300 : 700 : 1000 : 14, pH 2.5); and solvent B, MeOH/1% H_3PO_4/*n*-hexylamine (1000 : 1000 : 14, pH 2.8). Solvent A was used for more polar compounds. The addition of the amine added to the mobile phase improved the poor peak shapes on chromatograms, these peak shapes resulted from the interaction of the basic compounds when a silica core was used for packing material. An illicit sample of cocaine hydrochloride of high purity was examined by HPLC using solvent system A, showing cocaine as a major peak and benzoylecgonine as a minor peak. Isomeric cocaines (Figure 11) exist as four diastereomeric structures with different configurations at C-2 and C-3 of the tropane ring. They are named cocaine, pseudococaine, allococaine, and allopseudococaine, with the two substituents oriented as 2-*exo*-3-*exo*, 2-*endo*-3-*exo*, 2- *exo*- 3-*endo*, and

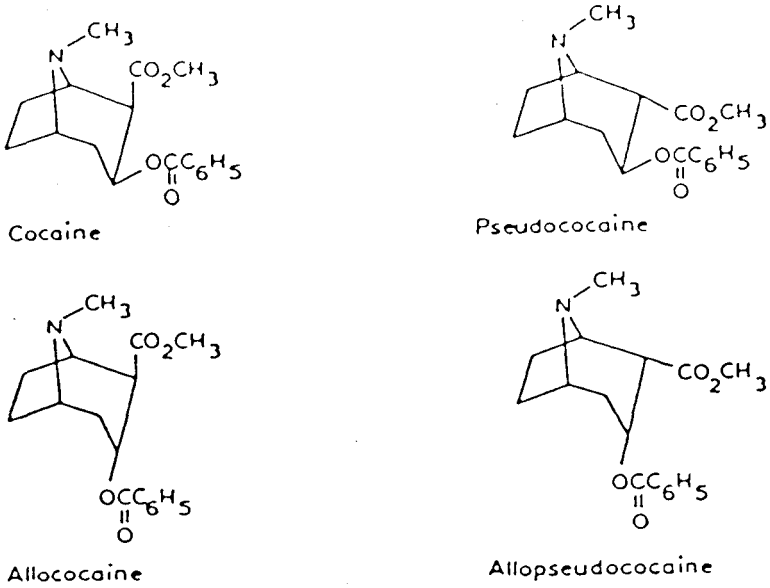
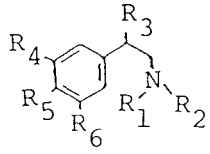


FIG. 11. Isomeric cocaines.

2-*endo*-3-*endo*, respectively. All four isomers were separated by HPLC and detected at 230 nm using a Partisil-10 PXS column, and heptane/ isoPrOH/ Et_2NH (75 : 25 : 0.2) as the eluent (*212*).

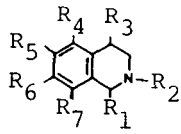
E. CACTUS ALKALOIDS

One of the most important native religions practiced by Mexican Indians involves ingestion of peyote (*Lophophora williamsii, Anhalonium lewinii)* for hallucinogenic purposes. The peyote cult has grown rapidly and tenaciously in the United States. It was previously legal to obtain supplies of peyote for rituals due to the lack of federal restraints (*213*). The San Pedro cactus, *Trichocereus pachanoi* is one of the most ancient of the magic plants of South America, where it is used to cure illness and promote witchcraft. The stems of San Pedro are easily obtained. There are a number of other cactus genera listed for hallucinogenic purposes used in North, Central, and South America and the West Indies, such as *Dolichothele, Pachycereus, Anhalonium, Carnegiea, Marginatocereus, Coryphantha, Lepidocoryphantha,* and others. Mescaline is well-known as one of the psychotomimetic cactus alkaloids. These alkaloids are either β-phenethylamines or the chemically related tetrahydroisoquinolines, with the exception of the imidazole alkaloid dolichotheline (Figures 12 and 13). Each alkaloid class is separated into phenolic and nonphenolic groups. An anion-



		R_1	R_2	R_3	R_4	R_5	R_6
I1	phenethylamine	H	H	H	H	H	H
I2	ubine	CH_3	CH_3	OH	H	H	H
I3	tyramine	H	H	H	H	OH	H
I4	N-methyltyramine	CH_3	H	H	H	H	H
I5	hordenine	CH_3	CH_3	H	H	OH	H
I6	octopamine	H	H	OH	H	OH	H
I7	oxedrine(synephrine)	CH_3	H	OH	H	OH	H
I8	β-O-methyloxedrine	CH_3	H	OCH_3	H	OH	H
I9	4-methoxyphenetylamine	H	H	H	H	OCH_3	H
I10	N-methyl-4-methoxy phenethylamine	CH_3	H	H	H	OCH_3	H
I11	longimammine	CH_3	H	OH	H	OCH_3	H
I12	phenylephrine	CH_3	H	OH	OH	H	H
I13	dopamine	H	H	H	OH	OH	H
I14	3-hydroxy-4-methoxy phenethylamine	H	H	H	OH	OCH_3	H
I15	3-methoxy-4-hydroxy phenethylamine	H	H	H	OCH_3	OH	H
I16	N-methyl-3-methoxy 4-hydroxyphenethylamine	CH_3	H	H	OCH_3	OH	H
I17	N,N-dimethyl-3-methoxy 4-hydroxyphenethylamine	CH_3	CH_3	H	OCH_3	OH	H
I18	3,4-dimethoxyphenethylamine	H	H	H	OCH_3	OCH_3	H
I19	N-methyl-3,4-dimethoxy phenethylamine	CH_3	H	H	OCH_3	OCH_3	H
I20	N,N-dimethyl-3,4-dimethoxy phenethylamine	CH_3	CH_3	H	OCH_3	OCH_3	H
I21	normacromerine	CH_3	H	OH	OCH_3	OCH_3	H
I22	macromerine	CH_3	CH_3	OH	OCH_3	OCH_3	H
I23	3,4-dimethoxy-5-hydroxy phenethylamine	H	H	H	OCH_3	OCH_3	OH
I24	3,5-dimethyoxy-4-hydroxy phanethylamine	H	H	H	OCH_3	OH	OCH_3
I25	mescaline	H	H	H	OCH_3	OCH_3	OCH_3
I26	N-methylmescaline	CH_3	H	H	OCH_3	OCH_3	OCH_3
I27	trichocerine	CH_3	CH_3	H	OCH_3	OCH_3	OCH_3

FIG. 12. β-Phenethylamine alkaloids in cactus.



		R_1	R_2	R_3	R_4	R_5	R_6	R_7
II1	longimammosine	H	CH_3	H	H	OH	H	H
II2	N-methyl-4,6-dihydroxy tetrahydroisoquinoline	H	CH_3	OH	H	OH	H	H
II3	longimammatine	H	CH_3	H	H	OCH_3	H	H
II4	salsoline	CH_3	H	H	H	OH	OCH_3	H
II5	isosalsoline	CH_3	H	H	H	OCH_3	OH	H
II6	N-methylsalsoline	CH_3	CH_3	H	H	OH	OCH_3	H
II7	N-methylisosalsoline	CH_3	CH_3	H	H	OCH_3	OCH_3	H
II8	lophocerine	$(CH_3)_2CHCH_2$	CH_3	H	H	OCH_3	OH	H
II9	6,7-dimethoxytetrahydro isoquinoline	H	H	H	H	OCH_3	OCH_3	H
II10	norcarnegine (salsolidine)	CH_3	H	H	H	OCH_3	OCH_3	H
II11	carnegine	CH_3	CH_3	H	H	OCH_3	OCH_3	H
II12	cis-1-methyl-4-hydroxy-6,7-dimethoxytetrahydroisoquinoline	CH_3	H	OH	H	OCH_3	OCH_3	H
II13	trans-1-methyl-4-hydroxy-6,7-dimethoxytetrahydroisoquinoline	CH_3	H	OH	H	OCH_3	OCH_3	H
II14	cis-1,2-dimethyl-4-hydroxy-6,7-dimethoxytetrahydroisoquinoline	CH_3	CH_3	OH	H	OCH_3	OCH_3	H
II15	trans-1,2-dimethoxy-4-hydroxy-6,7-dimethoxytetrahydroisoquinoline	CH_3	CH_3	OH	H	OCH_3	OCH_3	H
II16	longimammidine	H	CH_3	H	H	H	H	OH
II17	longimammamine	H	CH_3	OH	H	H	H	OH
II18	arizonine	CH_3	H	H	H	H	OCH_3	OH
II19	anhalamine	H	H	H	H	OCH_3	OCH_3	OH
II20	anhalonidine	CH_3	H	H	H	OCH_3	OCH_3	OH
II21	pellotine	CH_3	CH_3	H	H	OCH_3	OCH_3	OH
II22	anhalidine	H	CH_3	H	H	OCH_3	OCH_3	OH
II23	anhalinine	H	H	H	H	OCH_3	OCH_3	OCH
II24	O-methylanhalonidine	CH_3	H	H	H	OCH_3	OCH_3	OCH
II25	O-methylanhalidine	H	CH_3	H	H	OCH_3	OCH_3	OCH
II26	O,N-dimethylanhalonidine	CH_3	CH_3	H	H	OCH_3	OCH_3	OCH
II27	hydrocotarnine	H	CH_3	H	H	$O-CH_2-O$		OCH
II28	gigantine	CH_3	CH_3	H	OCH_3	OCH_3		H
II29	anhalonine	CH_3	H	H	H	OCH_3	$O-CH_2-O$	
II30	lophophorine	CH_3	CH_3	H	H	OCH_3	$O-CH_2-O$	
II31	peyophorine	CH_3	C_2H_5	H	H	OCH_3	$O-CH_2-O$	
II32	uberine	H	CH_3	H	OCH_3	H	OH	H
II33	deglucopterocereine	CH_2OH	CH_3	H	OH	OCH_3	OCH_3	H
II34	pterocereine	CH_2OH	CH_3	H	O-glu	OCH_3	OCH_3	H

FIG. 13. Isoquinoline alkaloids in cactus.

exchange resin (OH form) is usually used for separating the alkaloid extracts into phenolic and nonphenolic fractions (*214–217*). Lundström and Agurell separated these phenolic and nonphenolic fractions on TLC (*214*). Seven of the former alkaloids were chromatographed with silica gel on TLC with $CHCl_3$/EtOH/Et_2NH (85 : 5 : 10) or (85 : 10 : 5) and seven nonphenolic alkaloids with $CHCl_3$/EtOH/NH_4OH (85 : 15 : 0.4) or $CHCl_3$/*n*-BuOH/NH_4OH (50 : 50 : 2.5). The color reagent, dianisidine, produces a red color with phenolic tetrahydroisoquinolines and a yellow or brown fading color with nonphenolic alkaloids. Four phenolic alkaloids, a non phenolic alkaloid, and dolichotheline were detected in *Dolichothele sphaerica* by spraying DANS-Cl (Table II), tetrazotized benzidine, and a modified Pauly's reagent using eight different solvent systems (*215*). Fluorescamine used as a spray reagent on TLC produced stable fluorescent conjugates with primary amines and dark purple spots with secondary amines under UV. Tertiary amines, phenols, and imidazoles were not visualized with fluorescamine (*218*). Overspraying with 5-dimethylaminonaphthalene-1-sulfonyl chloride after spraying with fluorescamine gave yellow colors for secondary amines. Tertiary amines were then visualized as purple/blue colors when subsequently sprayed with iodoplatinate. This method was applied for the identification of seven alkaloids from *D. longimamma* (*219*) and ten from *D. uberiformis* (*216*). Five of the alkaloids from *D. longimamma* were new: longimammidine, longimammosine, longimammamine, longimammatine, and longimammine. Two of the alkaloids from *D. uberiformis* were new: uberine and ubine. The phenolic tetrahydroisoquinoline, depterocerine and its glucoside pterocerine were isolated from *Pterocereus gaumeri* (*217*). They reacted positively with iodine vapor, iodoplatinate, and Dragendorff's reagent, and negatively with fluorescamine on TLC, which indicated that they were tertiary amines. The glucoside gave no color but the free alkaloid did give an orange-red color with tetrazotized benzidine, specific for 5- or 8-hydroxy substituted tetrahydroisoquinoline (*220*). This suggests that glucose is bound to a phenolic hydroxyl group. A GC method was used to separate alkaloids from *Anhalonium lewinii* using a polar methylsiloxane column containing 5% phenyl substitution as the liquid phase (*221*). On GC separation of 18 closely related alkaloids, *N*-monomethyl secondary and tertiary amines, *O*-methyl bases, or *C*-monomethyl bases decreased the retention times. In contrast, alkaloids with hydroxyl or methoxyl groups, or a double bond in isoquinolines, and methoxy-substituted phenethylamines increased the retention times. Tetrahydroisoquinoline with hydroxyl groups had greater retention times than methoxylated analogs. Tetrahydroisoquinolines with methylenedioxyl groups showed a remarkable increase in their retention times compared with those having two methoxyl groups. It is noticeable that phenolic anhalonium alkaloids could be directly separated on GC since hydroxyl groups located *ortho* to a methoxyl group cause a strong intramolecular hydrogen bonding. Massingill and Hodkins and Lundström and Agurell used an SE-30 column to resolve phenethylamines of molecular weight (>200) but could not achieve a separation

of tetrahydroisoquinolines (*222,223*). An XE-60 column, however, achieved a good separation of tetrahydroisoquinolines giving shorter retention times for tertiary amines than for the corresponding secondary analogs. Under these conditions, a new trace alkaloid, 3,4-dimethoxyphenethylamine was found in *L. williamsii*. Kapadia identified 14 new peyote alkaloid amides by GC-MS, which are probably biosynthesized from primary amines such as mescaline and acids of the Krebs cycle (*224*). Mescaline was found in *Trichocereus bridgesii* and *T. macrogonus* using 5% SE-30 on Gas Chrom P150, and 5% XE-60 on Chromosorb W150, by GC. Detection of alkaloids from 14 species of *Trichocerus* and one of each species from *Echinopsis, Pachycereus, Pelecyphora,* and *Stetsonia* were examined on TLC and on two GC columns (SE-30, XE-60, or JXR) (*225*). Mescaline was newly discovered in *T. cuzucoensis, T. fulvilanus, T. taquimobalensis,* and *T. validus*. It is noteworthy that the macroscopic appearance of the new mescaline-containing species resembled markedly the previously known species. Mescaline was found in *S. coryne* but not in *Pachycereus pecten-aboriginum* and *Pelecyphora aselliformis*. HPLC was carried out for a separation of isomeric pairs of cactus alkaloids. Two isomeric hydroxymethoxyphenethylamines; 3-hydroxy-4-methoxy-phenethylamine and 4-hydroxy-3-methoxyphenethylamine, and the isomeric hydroxymethoxy-substituted tetrahydroisoquinolines salsoline, isosalsoline, and arizonine were detected with different retention times on normal phase HPLC (*226*). Addition of base to the neutral solvent systems gave a better separation.

When 500 mg of mescaline was given to humans, 87% of the dose was excreted in the urine within 24 hr; 60% was unchanged, and approximately 30% was converted to 3,4,5-trimethoxyphenethyl acetic acid (*227*). After intravenous injection of mescaline into rabbits, plasma mescaline was determined by GC-MS on a silanized glass column packed with 25% QF-1 (*228*). The alkaloid was extracted from plasma, derivatized with TFAA (Table II) and analyzed by GC-MS with ^{2}H mescaline as an internal standard.

F. Harmala Alkaloids

Harmala alkaloids are distributed in plants and have been consumed as hallucinogenic drinks, snuffs, and other forms in the Amazon basin, where they are found in *Banisteriopsis* spp. and *Tetrapteris* spp. (Malpighiaceae), *Virola* spp. (Myristicaceae), and *Anadenanthera (Piptadenia)* spp. (Leguminosae). *Peganuma harmala* (Zygophyllaceae), from which harmine and harmaline were first isolated, is native to dry areas from the eastern Mediterranean to northern India, Mongolia, and Manchuria and was, in the past, a hallucinogen in native religion or an agent in magic. The plant is still used as a spice and an intoxicant in India. *Passiflora incarnata* (Passifloraceae) which contains harman was used for many years as a central-nervous-system agent in Germany. Some harmala alkaloids are endogenously produced in mammals and in alcoholic patients.

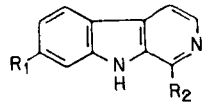
Banisteriopsis caapi grows in the Western half of the Amazon Valley and on the Pacific slopes of the Colombian and Ecuadorian Andes, known as Ayahusca, Capi, and Yaje, *B. caapi* contains three alkaloids, harmine, harmaline, and *d*-tetrahydroharmine (*229*). We isolated and determined six new β-carbolines from this plant (Figure 14) (*230,231*). They were isolated by continuous column chromatography and detected by fluorescence under UV light and by coloration with Dragendorff's reagent on paper electrophoresis and TLC (Table IV).

Harmine, harmaline, tetrahydroharmine, and harmol have been obtained as a mixture from stems of *B. inebrians* and purified by column chromatography over Florisil with gradient solvents of CH_2Cl_2-MeOH (*232*). TLC was carried out on silica gel GF_{254} using four different solvent systems: $CHCl_3$/MeOH (85 : 15), acetone/MeOH (85 : 15), EtOH/NH_4OH (99 : 1) and $CHCl_3$/MeOH/H_2O (8 : 19 : 1). Alkaloid spots were detected by UV visualization at 336 nm, staining with vapored iodine. 2-Methyl-tetrahydro-β-carboline (MTHC) of *B. rusbyana* was identified by GC and GC-MS, but harmine, harmaline, and tetrahydroharmine were not (*233*). *B. argentea,* which grows in the India Botanic Garden in Howrah, India, was examined for alkaloid content (*234*) on TLC, using MeOH/$CHCl_3$/AcOH (75 : 25 : 15) as developing solvent and Dragendorff's, Ehrlich's, and β-nitro-β-naphthol nitrous acid reagents for color visualization were used.

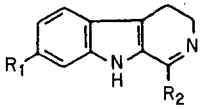
Many species of *Virola* are used as hallucinogenic snuff, and contain both β-carboline and indole alkaloids (see tryptamine derivatives) (*235*). MTHC and 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline(6-MeO-MTHC) were detected in the barks and roots of *V. theiodora* and *V. rufula* by GC, GC-MS, and PC. We obtained MTHC from *V. sebifera* by silica gel column chromatography eluting with 30% MeOH in $CHCl_3$ and using for detection its UV absorption at 254 nm, followed by sparying with Dragendorff's reagent on TLC (silica gel, Merck F_{254}) (*236*). The developing system, CH_2Cl_2/MeOH/H_2O (8 : 6 : 2) gave better separation of MTHC from other indole alkaloids on TLC (silica gel).

Anadenanthera (Leguminosae) (also named *Piptadenia*) contains β-carbolines besides indole alkaloids. 6-Methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THC) and 1,2-dimethyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-DMTHC) were found in *A. peregrina* as β-carboline alkaloid (*235*). Harman was detected in the snuffs inhaled by some South American Indians which was known to be from *A. peregrina* (*237*). This has been the only report in which harman was obtained from botanical sources of hallucinogens in South America.

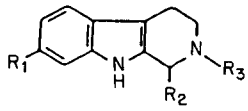
Harmine as a major alkaloid from *Peganum harmala* was obtained by column chromatography in addition to harmaline and non-β-carbolines as minor alkaloids (*238*). McKenzie *et al.* isolated harmol first from *P. harmala* (*239*). Carreras and Gonzales Alonso reported a separation of the major alkaloids, harmine and harmaline, from *P. harmala* by high voltage paper ionophoresis (*240*). Methylumbelliferone (0.1%) was used as a reference substance for measuring the electro-osmotic field. Difference in the migration velocity of the two alkaloids was achieved by moving of the tailed material and distorting the front.



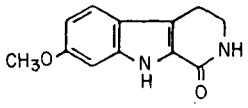
		R_1	R_2	
Ia	norharman	H	H	
Ib	harman	H	CH_3	
Ic	harmol	OH	CH_3	
Id	harmine	OCH_3	CH_3	
Ie	harmine Nb-oxide	OCH_3	CH_3	Nb-O
If	harmic acid methylester	OCH_3	$COOCH_3$	
Ig	harmic acid amide	OCH_3	$CONH_2$	
Ih	acetylnorharmine	OCH_3	$COCH_3$	



		R_1	R_2
IIa	harmalol	OH	CH_3
IIb	harmaline	OCH_3	CH_3
IIc	harmalinic acid	OCH_3	COOH



		R_1	R_2	R_3
IIIa	1,2,3,4-tetrahydronorharman	H	H	H
IIIb	1,2,3,4-tetrahydroharman	H	CH_3	H
IIIc	1,2,3,4-tetrahydroharmine	OCH_3	CH_3	H
IIId	*N*-methyl-1,2,3,4-tetrahydro-β-carboline	H	H	CH_3
IIIe	1,2-dimethyl-6-methoxy-tetrahydro-β-carboline	OCH_3	CH_3	CH_3



IV ketotetrahydronorharmine

FIG. 14. Harmala alkaloids.

TABLE IV

ELECTROPHORESIS AND TLC OF ALKALOIDS FROM *BANISTERIOPSIS CAAPI*

Alkaloid	Harmine	Harmaline	Harmic acid methylester	Harmic acid amide	Unknown	Acetylnorharmine	Harmine-*N*-oxide	Harmalinic acid	Ketotetrahydronorharmine
Migration distance (mm)[a]	151	150	146	144	141	123	115	53	50
R_f (×100) on TLC[b]	17	04	79	09	85	60	22	25	42

[a] On Toyo Roshi No. 51 paper in 5 N HOAc for 2 hr at 800 V and 0·38 mA/cm current.

[b] On Si gel G in $CHCl_3$–McOH (15:1).

This was improved by addition of 25% formamide to the Lorenz–Müller acetic acid–acetate buffer instead of ethyl and isopropyl alcohols or propylene glycol.

HPLC equipped with a fluorometric detector was used for determination of harmala alkaloids in *P. harmala* cell cultures (*241*). The alkaloids were divided into two groups according to their oxidation stage including, in one group, harmine and harmol, and in the other, harmaline and harmalol. The former had the same UV maxima at 355 nm with optical excitation at 304 nm, while the latter showed no emission under these conditions. The latter fluoresced maximally at 475 nm with excitation at 396 nm, whereas the former did not respond at this wavelength. Dilution of the methanol solution with water led to a bathochromic shift of the fluorescence spectra of harmine and harmol in a solution of MeOH/H_2O (1 : 9) from 355 to 425 nm with optimal excitation at 304 nm. An unknown compound was detected in the methanol extract of *P. harmala* in a suspension culture by measuring the fluorescence maximum at 335 nm by HPLC (*241*).

Harmala alkaloids in *Passiflora* species were examined by qualitative TLC. Poethke *et al.* found only harman in *P. incarnata* and *P. bryonioidis* when comparing it with authentic material on silica-gel TLC with $CHCl_3$/MeOH (75 : 25) (*242*). By the similar method of Poethke *et al.*, Löhdefink identified harman in *P. coerrulea, P. decaisneana, P. edulis, P. foetida, P. incarnata, P. subpeltata,* and *P. warmingii,* but not harmine, harmaline, harmol, or harmaolol with $CHCl_3$/MeOH (8 : 2) (*243*). Quantitative analysis of harman in an extract from a plant mixture which contained *P. incarnata* was carried out by spectrophotofluorometry on TLC (*244*). Paper chromatography of alkaloids in *P. edulis* was performed on Schleicher and Schüll paper (Nr.20436 *M*) developed with formamide/acetone/benzoic acid (18 ml : 6 ml : 0.4 g) (*245*). Comparison was carried out with standards of harman, harmine, harmol and harmalin. Harman alone was detected in extracts of leaves and stems in addition to other unknown alkaloids. Hultin, however, found harmol, harmaline, and harmalol as well as harman in *P. incarnata* by a simple countercurrent method using their partition coefficients (*246*). Phenolic alkaloids were separated by counter current extraction with ether at pH 12.2, harman was separated at pH 4, and unidentified alkaloids separated from other material at pH 1.2.

Harman and norharman were isolated from cured tobacco and its smoke (*247*). Harman alkaloids are also formed from tryptophan in cigarettes during burning. Further studies showed that enough tryptophan is present in tobacco to account for the quantities of harman alkaloids found in smoke. The Rf values of zones isolated from smoke on PC agreed with those of reference alkaloids. In contrast, only traces of harman alkaloids were present in the uncured leaf (1% of that in smoke).

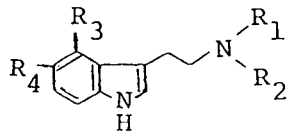
As a result of research in neurochemistry a discovery of endogenous harmala alkaloids in man and rat was made. Rommelspacher *et al.* found 1,2,3,4-tetrahydro-β-carboline (THC) in urine in man and in the forebrain of the rat

(*248*). THC in the sample was extracted into Et_2O and separated by TLC (solvent system I: $CHCl_3$/acetone/Et_2NH, 50 : 40 : 10), acetylated with ^3H-Ac_2O and further developed in a two-dimensional TLC system using solvent system I followed by solvent system II ($CHCl_3$/MeOH/AcOH, 93 : 7 : 1). The quantity isolated was determined by TLC scanning using seven different solvent systems. 6-Hydroxy-1,2,3,4-tetrahydro-β-carboline (6-OH-THC) was measured in the brain and platelets of rats (*249*). The separation of 6-OH-THC from other extracted substances was performed on HPLC with a C8 column using 8 m*M* AcOH, 11.4 m*M* Et_3N buffer, pH 8, as eluent A, and MeCN as eluent B. The fraction containing 6-OH-THC was acetylated with ^3H-Ac_2O and spotted on silica gel TLC plates developed by $CHCl_3$/acetone/Et_2NH (50 : 40 : 10). The area running isographically with reference mono- and di-acetylated 6-OH-THC was scraped off. The concentration of 6-OH-THC in the forebrain of rats was not sufficient for quantition but was found to be 51.67 ng $\pm 22.64/1 \times 10^8$ in platelets by counting under a microscope. Shoemaker *et al.* identified harman in human platelets and rat arcuate nucleus by GC and GC-MS/TLC (*250,251*). β-Carbolines were extracted from the platelet pellet by homogenization with a borate buffer at pH 9.5, followed by extraction with Et_2O. The ether extract was subjected to column chromatography on silica gel. β-Carboline was eluted with 4.35 *N* AcOH and its content measured by fluorometry. The β-carbolines were separated by preparative TLC on silica gel with *n*-BuOH/AcOH/H_2O (4 : 1 : 1) and/or $CHCl_3$/MeOH (9 : 1) and detected by UV fluorescence and identified by their characteristic colors and Rf values. The β-carbolines were scraped off the TLC plate, extracted with MeOH, and analyzed by GC (3% OV-12 on a Gas Chrom Q, 100/120 mesh, 6 foot × 2.0 mm i.d. column, operated isothermally at 246°C, FID) (*251*). Harman was identified in extracts of rat arcuate nuclei by its color and Rf value on TLC, and its retention time on GC. THC, MTHC and 6-MeO-THC were identified in rat brain and adrenal gland by GC and GC-MS by conversion to their HFB (Table II) derivatives; THC and 6-MeO-THC were quantitated by comparing their ion mass ratios with those of added deuterated standards (*252*). After intake of excessive ethanol, concentrations of tetrahydroharman and harman in man and rats are usually greatest at the time of "hangover." This would suggest that they are formed in the mammalian body by condensation of acetaldehyde (originating from ethanol) with tryptamine (*253*). Tetraydroharman and harman isolated from the urine of a subject given 100 g of ethanol were separated by gel-filtration and HPLC and identified by UV and MS in comparison with standards (*254*).

G. Tryptamines

Hallucinogenic simple indole alkaloids such as tryptamine and its anlogs were found in many species of sacred mushrooms in Mexico and elsewhere. Some of the plants in South America that contain harmala alkaloids also contain tryp-

tamines (see harmala alkaloids). Two active principal ingredients isolated from the sacred mushroom "teonanacatl" (Psilocybe mexicana) from Mexico were found to be 4-hydroxydimethyltryptamine (psilocin) and its corresponding 4-phosphate psilocybin (Figure 15). Other *Psilocybe* species belonging to the teonanacatl group containing psilocybin, usually together with a small amount of psilocin, are *P. caerulescens var. mazatecorum, P. zapotecorum, P. aztecormum, P. semperviva, P. bonettii* and *P. candipedes*. North American and European mushrooms in which psilocin and psilocybin are found are: *P. pelliculosa, P. cyanescens, P. baeocystis, P. quebecensis, P. stuntzii,* and *Conocybe cyanopus* in North America; and *P. semilanceata, P. coprinifacies, Panaeolus sphinctrinus, P. foenisecii, and P. subbalteatus* in Europe (*255*). Baeocystin and norbaeocystin (the *N*-demethylated derivatives of psilocybin) were first isolated from *Psilocybe baeocystis* (*256, 257*). Subsequently, baeocystin was also isolated from *Psilocybe semilanceata* (with psilocybin), *Paneolus renenosus, P. subbaltealus,* and *Copelandia chlorocystis* (*258,259*). Leung *et al.* (*256*) applied TLC to a separation and identification of these tryptamine analogs, using Ehrlich's reagent (5% *p*-dimethylaminobenzaldehyde in concentrated HCl) as a spray for color visualization of the alkaloids. This reagent reacts with active hydrogens present in compounds to give a blue-violet color. Dragendorff's reagent, as well as Ehrlich's reagent, is often used for the detection of tryptamine alkaloids not substituted on the C-2 position of the indol ring present in plant



		R_1	R_2	R_3	R_4
Ia	tryptamine	H	H	H	H
Ib	N-monomethyltryptamine	CH_3	H	H	H
Ic	N,N-dimethyltryptamine	CH_3	CH_3	H	H
Id	norbaeocystin	H	H	OH_2PO_3	H
Ie	baeocystin	CH_3	H	OH_2PO_3	H
If	psilocin	CH_3	CH_3	OH	H
Ig	psilocybin	CH_3	CH_3	OH_2PO_3	H
Ih	5-hydroxytryptamine	H	H	H	OH
Ii	5-hydroxy-N,N-dimethyltryptamine	CH_3	CH_3	H	OH
Ij	N-methyl-N-formyltryptamine	CH_3	CHO	H	H
Ik	N-methyl-N-acetyltryptamine	CH_3	CH_3CO	H	H

FIG. 15. Tryptamine derivatives.

extracts (*236*). A quantitative analysis of alkaloids in *Psilocybe* was examined by PC using *n*-BuOH saturated with water on Whatman paper and detection with *p*-dimethylaminobenzaldehyde in benzene saturated with HCl gas (Rf: psilocin 0.5, psilocybin 0.1) (*260*). Psilocybin and psilocin present in submerged cultures of *P. cubensis* were examined on PC by a two-dimensional procedure: *n*-BuOH/AcOH/H_2O (4 : 1 : 5) in the first direction and *n*-BuOH/1*N* NH_4OH (5 : 1) in the second direction (*261*). Psilocybin and psilocin in an extract of *P. cubensis* were detected by the combined technique of GC-MS (OV-101 on a Gas Chrome Q column), after TMS derivatization (*262*). The retention times for *bis* (TMS) psilocin and *tris* (TMS) psilocybin were 8.45 and 13.10 min, respectively. They were clearly separated from other components in the natural extract. HPLC (column, Partisil, with MeOH/H_2O/1N NH_4NO_3 = 240 : 50 : 10 and buffered to pH 9.7 with NH_4OH, detection 254 nm) was used for a convenient and speedy analysis of psilocin, psilocybin, and baeocystin in *P. semilanceata* (*263*). Psilocybin and baeocystin gave very similar retention times under these conditions. Greater resolution could be obtained by increasing the ammonia content of the solvent (*264*). Normal-phase HPLC has disadvantages since the column is susceptible to contamination by polar materials and solvents containing water, which shorten column life. Thomson used reversed-phase HPLC for quantification of these alkaloids of mushrooms in New Zealand (*264*), which proved more versatile and less restrictive in terms of requirements for sample size and solvent purity. Two different extracts were obtained from mushroom materials dried, ground, and preserved, without drying, in sugar by a roller-mixed extraction with MeOH for 24 hr. The mushrooms used were botanically not identified beyond the genus *Psilocybe*. Psilocybin and psilocin exist as zwitterions. There was no significant change in the retention volume for the former with either cationic or anionic reagents, but a marked increase was seen in that for the latter. It is suggested that either a complex was formed or that the complex was not partitioning between the mobile and the C18 column phases. The retention volumes of psilocybin and psilocin varied with the pH of the mobile phase, showing that solvent pH and phosphate concentration were less important and that the presence of phosphate was essential to delay the elution of psilocin. For analysis of psilocybin in MeOH extracts of dried ground mushrooms, fluorescence spectroscopy was investigated as a method for the selective detection and quantification on an ion-exchange column by HPLC (*265*). Psilocybin fluoresces strongly at 335 nm, with excitation at 267 nm, whereas psilocin displays weak fluorescence at 312 nm, with excitation at 260 nm. *N,N*-Dimethyltryptamin (DMT) was used as the internal standard. The minimum detectable quantities of psilocybin and psilocin are 250 pg and 30 ng, respectively, with fluorescence detection at the optimum wavelengths for each component, and 7 ng and 150 ng, respectively, by using UV absorption at 267 nm. Norwegian *P. semilanceata* was extracted with MeOH containing 10% 1*N*

NH_4NO_3 to examine HPLC for the quantification of psilocybin (*266*). Psilocybin was detected, in addition to, three unknown compounds by UV absorption at 254 nm and fluorescence at 335 nm (excitation at 267 nm). The results obtained by both UV absorption and fluorescence detection of psilocybin in dried mushrooms correlated well. C18 columns and the more polar Spherisorb Phenyl columns were used for separation of the components, but the very polar and soluble psilocybin did not seem to be sufficiently retarded.

Abuse of mushrooms has increased in Western countries. There have been two reports of psilocybin and *Psylocibe cubensis* found in chocolate cookies (*267, 268*). One report identified psilocybin in chocolate by TLC, HPLC, and GC (TMS derivatized). The other report isolated fruit bodies of *P. cubensis* appearing on chocolate grain cookies after 30 days cultivation.

Tryptamine derivatives are also found in species of South American hallucinogenic plants in which harmala alkaloids were found, for example, *Banisteriopsis rusbyana, Virola* spp. and *Anadenanthera* spp. (see harmala alkaloids). *N,N*-dimethyltryptamine (DMT), *N*-monomethyltryptamine (MMT), 5-methoxy-DMT (5-MeO-DMT), and 5-hydroxy DMT (bufotenine) were found in *B. rusbyana* leaves by GC and GC-MS. Contents of DMT were 98% of the total amounts of the alkaloids (*233*). The only plant species of *Banisteriopsis* where tryptamines were detected is *B. rusbyana*. Agurell *et al.* (*235*) detected DMT, MMT, 5-MeO-DMT, and 5-methoxy MMT (5-MeO-MMT) in various *Virola* species by GC and GC-MS. Corothie and Nakano detected DMT in *Virola sebifera* on TLC using benzene/$CHCl_3$/Et_2NH (25 : 23 : 2) (*269*). From the same species, the authors have isolated the amides of tryptamine base, *N*-formyl-*N*-methyl-tryptamine and *N*-acetyl-*N*-methyl tryptamine (*236*). Each of them was detected as two-spot and two-peak rotamers on TLC and HPLC, respectively. No attempt was made to isolate them preparatively by HPLC because their rate of isomerization was expected to be too fast to allow isolation at room temperature. Twenty tryptamine derivatives were separated by GC (two columns, F60-2 and NGS) (*270*). Bufotenine, DMT, and 5-MeO-DMT were determined from the South American snuff, epená (*V. Aublet*), by a comparison of their retention times. Agurell *et al.* (*235*) also examined *Anadenanthera peregrina* (Leguminosae) (barks) to find DMT, MMT, 5-MeO-DMT, and 5-MeO-MMT. Legler and Tschesche isolated MMT, 5-MeO-MMT, and 5-MeO-DMT by using TLC (silica gel G, 5% MeOH saturated with NH_3 in $CHCl_3$, or AcOEt/ MeOH/ 25% NH_4OH, 88 : 10 : 2 using *p*-dimethylaminobenzaldehyde for visualization (*271*). Four indole bases were found to occur in the seeds and pods of *A. peregrina* by PC by using four different solvent systems (*272*). Our study on alkaloids in *Anadenanthera* (*Piptadenia*) spp showed four unknown compounds which reacted positively with Dragendorff's reagent in addition to bufotenine and DMT, on paper electrophoresis (Table V) (*273*).

TABLE V
PAPER ELECTROPHORESIS OF BASIC COMPONENTS IN PIPTADENIA SPP.

	Migration distance (cm)[a]					
Piptadenia Benth.	I	II	Bufotenine	DMT	V	VI
P. colubrina Benth.		5·5	14·0	15·4	17·3	
P. contorta Benth.		5·5	14·5	15·0		
P. leprostachya Benth.	0	5·0				29
P. moniliformis Benth.		5·5	14·1			
P. peregrina Benth.		5·5	14·0	15·4	17·3	

[a] Condition; on Toyo Roshi No.52 paper in 5 *N* HOAc for 2.5 hr at 800 V. I,II,V, and VI are unknown.

In searching for a biosynthetic scheme for bufotenine in possible precursors labeled with ^{14}C or ^{3}H were incubated with tissues of *A. peregrina*. Products were detected by Rf values on PC, ionic mobilities on high-voltage electrophoresis, and color reaction with Ehrlich's reagent (Table I), K_2SO_4, and Pauly's reagent (*274*). Benington *et al.* (*275*) showed the presence of trace levels of *N*- and/or *O*-methylated derivatives of tryptamine and 5-hydroxytryptamine (serotonin) in the cerebrospinal fluid of schizophrenic patients. Indole alkylamines were converted to HFB derivatives by acyl transfer using *N*-heptaflurobutyrylimidazole (HFBI) as a derivatizing reagent for detection on GC.

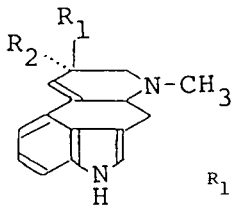
Metabolism of DMT in man was evaluated to explain its psychotic effect (*276*). The urine samples collected before and after injections were analyzed for indolic compounds by PC and colorimetry. The extracts were analyzed by two-dimensional PC (ascending) with *n*-BuOH saturated with 10% NH_4OH in the first, and *n*-BuOH/AcOH/H_2O (4 : 1 : 5) in the second, dimension. Chromatograms were sprayed with 2% *p*-dimethylaminobenzaldehyde in 1.2*N* HCl. 5-Hydroxyindole acetic acid was found 2–4 times more often in the extracted urine after the administration of DMT than before. α,α, β,β-Tetradeutero-DMT was incubated with rat brain homogenates and the metabolites identified as indole acetic acid, tryptamine, DMT-*N*-oxide, MMT, MTHC, and THC by TLC and GC-MS (Chromasorb W-HP) (*277*). DMT and its metabolites were converted to their corresponding HFB derivatives for GC analysis.

H. ERGOT ALKALOIDS AND LSD

More than forty ergot alkaloids were isolated from ergot, the dried sclerotium of a fungus, *Claviceps purpurea*, found to be parasitic on Graminacious and Cyperacious plants. Many ergot species appear to be extremely toxic and to cause plagues of ergotism, as by fungus-infected rye kernels milled into flour.

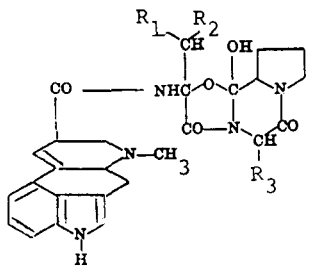
On the other hand, the alkaloids in ergots are medicinally useful. They are commonly divided into two groups: a series of simple clavine derivatives and a series of amides of lysergic acid, including dipeptides (Figures 16, 17, and 18). Derivatives of L-lysergic acids are used clinically, while those of isolysergic acids are not. D-lysergic acid diethylamide (LSD-25), prepared by partial synthesis from lysergic acid, is a potent psycotomimetic drug with powerful hallucinogenic properties; it is one of the most widely abused drugs. An alkaloid closely related to LSD, and occurring in nature, is ergine. Lysergic acid amide is found in 16 different varieties of morning glory seeds (*278*).

For isolation, identification, and determination of ergot alkaloids, LSD, and related compounds, various kinds of chromatographic methods were carried out.



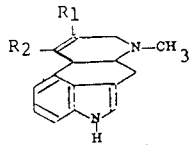
		R_1	R_2
Ia	ergine	$CONH_2$	H
IIa	isolyergine	H	$CONH_2$
Ib	lysergic acid dimethyl amide	$CON(CH_3)_2$	H
IIb	isolysergic acid dimethyl amide	H	$CON(CH_3)_2$
Ic	lysergic acid diethyl amide	$CON(C_2H_5)_2$	H
IIc	isolysergic acid diethyl amide	H	$CON(C_2H_5)_2$
Id	lysergic acid methyl propyl amide	$CON(CH_3)C_3H_7$	H
IId	isolysergic acid methyl propyl amide	H	$CON(CH_3)C_3H_7$
Ie	lysergic acid ethyl propyl amide	$CON(C_2H_5)C_3H_7$	H
IIe	isolysergic acid ethyl propyl amide	H	$CON(C_2H_5)C_3H_7$
If	lysergic acid dipropyl amide	$CON(C_3H_7)_2$	H
IIf	isolysergic acid dipropyl amide	H	$CON(C_3H_7)_2$
Ig	ergometrine (ergobasine) (ergonovine)	$CONHCH(CH_3)CH_2OH$	H
IIg	ergometrinine	H	$CONHCH(CH_3)CH_2OH$

FIG. 16. Simple lysergic acid and isolysergic acid amides.



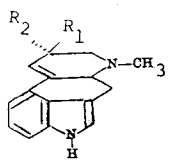
8β-ergotamine group		8α-ergotamine group		dihydroergotoxine group		R_1	R_2	R_3
IIIa	ergotamine	IVa	ergotaminine	Va	dihydroergotamine	H	H	$CH_2C_6H_5$
IIIb	ergosine	IVb	ergosinine			H	H	$CH_2CH(CH_3)_2$
IIIc	ergostine	IVc	ergostinine			H	CH_3	$CH_2C_6H_5$
IIId	ergocristine	IVd	ergocristinine	Vd	dihydroergocristine	CH_3	CH_3	$CH_2C_6H_5$
IIIe	ergocornine	IVe	ergocorninine	Ve	dihydroergocornine	CH_3	CH_3	$CH(CH_3)_2$
IIIf	α-ergocryptine			Vf	dihydro-α-ergokryptine	CH_3	CH_3	$CH_2CH(CH_3)_2$
IIIg	β-ergocryptine			Vg	dihydro-β-ergokryptine	CH_3	CH_3	$CH(CH_3)C_2H_5$

FIG. 17. Derivatives of lysergic, isolysergic, and dihydrolysergic acids of the peptide type.



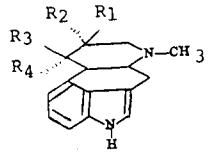
ergolene(8) group

		R_1	R_2
VIa	agroclavine	CH_3	H
VIb	elymoclavine	CH_2OH	H
VIc	molliclavine	CH_2OH	OH



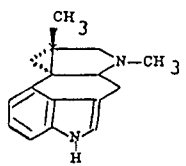
ergolene(9) group

		R_1	R_2
VIIa	lysergine	CH_3	H
VIIb	lysergol	CH_2OH	H
VIIc	isolysergol	H	CH_2OH
VIId	lysergene	CH_2	-
VIIe	setoclavine	CH_3	OH
VIIf	isosetoclavine	OH	CH_3
VIIg	penniclavine	CH_2OH	OH
VIIh	isopenniclavine	OH	CH_2OH

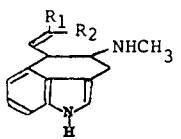


ergoline group

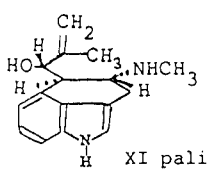
		R_1	R_2	R_3	R_4	10H
VIIIa	festuclavine	CH_3	H	H	H	α
VIIIb	α-dihydrolysergol	CH_2OH	H	H	H	α
VIIIc	pyroclavine	H	CH_3	H	H	α
VIIId	costaclavine	CH_3	H	H	H	β
VIIIe	fumigaclavine A	CH_3	H	$OCOCH_3$	H	α
VIIIf	isofumigaclavine A	CH_3	H	H	$OCOCH_3$	α
VIIIg	fumigaclavine B	CH_3	H	OH	H	α
VIIIh	isofumigaclavine B	CH_3	H	H	OH	α



IX cycloclavine



		R_1	R_2	10H
Xa	chanoclavine I	CH_2OH	CH_3	α
Xb	chanoclavine II	CH_2OH	CH_3	β
Xc	isochanoclavine I	CH_3	CH_2OH	α



XI paliclavine

FIG. 18. Clavine alkaloids.

A number of PC procedures for their separation have been reported, but TLC procedures are advanatageous since time of equilibration and development is shorter and gives better resolution, requiring smaller amounts of samples and affording a wide choice of reagents. Agurell (*279*) analyzed both ergot alkaloids (amide series) of lysergic acid and clavine series on silica gel or alumina TLC with different solvent systems and silica gel thin-layer electrophoresis at 1500 volts with pyridine–acetate buffer of pH 5.6. Thin-layer electrophoresis and TLC were also combined for a two-dimensional separation. Agurell found a close relation between structure and relative migration value and also a correlation of structures with stereochemical relations in the clavine series of alkaloids by TLC with the solvent system MeOH/$CHCl_3$ (2 : 8). Twenty clavine alkaloids were gas chromatographed with satisfactory results and identified as TMS, HFB, or TFA derivatives on JxR and SE 30 columns, but most satisfactorily on an XE-60 column (*280*). None of these columns, however, was able to separate stereoisomers. In the group of compounds which have a methyl group on C-17, cycloclavine, costaclavine, pyroclavine, and festuclavine, which have no double bond on the *D*-ring, had the shortest retention times, followed by agroclavine and lysergine, which have double bonds. Chanoclavines-I and II and isochanoclavine-I (which are secondary amines with an open *D*-ring) were detected in addition to fumigaclavine, or setoclavine, and isosetoclavine, which have secondary or tertiary hydroxyl groups. α-isolysergol, lysergol, and elymoclavine, which have a hydroxymethyl group of C-17, showed longer retention times; penniclavine and isopenniclavine, which both have a primary and tertiary hydroxyl group, were found to have the longest retention times in the series of clavine alkaloids. LSD was chromatographed on SE-30 and JxR columns at comparatively high temperature. Analytical GC determination of agroclavine, festuclavine, and pyroclavine was satisfactory as TMS-derivatives, which was not advantageous for hydroxylated clavine alkaloids (*281*). HPLC analysis of clavine alkaloids mixed with simple derivatives of lysergic acid was undertaken by Wurst *et al.* (*282*). They chromatographed on a Micropak NH_2 column using three different solvent systems: Et_2O/EtOH, Et_2O/isoPrOH, and $CHCl_3$/isoPrOH. The compounds containing a tertiary hydroxyl group such as setoclavine and isosetoclavine were eluted quickly, followed by the compounds containing a secondary hydroxyl group such as paliclavine, and then the compounds containing a primary hydroxyl group such as lysergol, elymoclavine and chanoclavine. Penniclavine, containing two hydroxyl groups (primary and tertiary), required smaller elution volumes than compounds containing a single tertiary hydroxyl group and, thus, a decreasing polarity in the systems used. Steric isomers such as setoclavine and isosetoclavine, or pyroclavine and festuclavine, were separated similarly in all three systems. It was found that a gradient elution (Et_2O and EtOH) was more useful than an isocratic procedure. Clavine alkaloids produced by the submersed culture of *Claviceps purpurea* were determined by using a gradient system.

It is important in the forensic field to identify LSD in drug seizures and biological samples because LSD is one of the most potent hallucinogens; it has thus become an important drug in narcotics traffic, requiring sensitive methods for its detection. Good recoveries of LSD are achieved from solids, such as sugar cubes impregnated with LSD, tablets, or contents of capsules which are crushed, extracted with $CHCl_3$/MeOH (9 : 1) and filtered through filter paper. All operations are conducted in subdued light because LSD is unstable in the presence of moisture and UV light. TLC on silica gel G with CH_2Cl_2/MeOH (93 : 7) gave Rf values of about 0.6, a blue fluorescence under long-wave UV light, and blue–violet spot with *p*-dimethylaminobenzaldehyde as a color-producing reagent (*283*). LSD was also examined by GC after derivatization. The TMS derivative (Table II) is superior to TFA derivative for analytical use (*283*). Many conditions for PC and TLC have been adapted for identification of LSD, and TLC is the most widely applied, using many different solvent systems. Bailey *et al.* (*284*) distinguished LSD from iso-LSD dimethyl, and ethylpropyl analogs by TLC developed with $CHCl_3$/MeOH (9 : 1, system I) and $CHCl_3$ saturated with NH_4OH/MeOH (18 : 1, system II) on silica gel G. Sperling reported the differentiation of LSD from other ergot alkaloids and its analogs of the lysergic acid type by TLC with the same conditions (system I and II) (*285*). For separation of LSD from its methylpropyl analog, GC on 0.25% OV-17 after silyl derivatization was used. Detection of trace amounts of LSD in sugar cubes was achieved by hydrogenation of the drug prior to TLC and GC analysis, giving more reproducible retention times on GC (*286*). It was also found that TLC was superior to GC, since only a shoulder of hydrogenated LSD appeared on the descending portion of the solvent peak on GC. Alternatively, a successful analysis of LSD in sugar cubes by GC on 0.3% SE-30 requires neither hydrogenation nor derivatization, since the chromatogram showed a nice, symmetrical peak (*287*). The quantitative analysis proved to be linear at low concentrations (0.5 μg). GC was effective for detecting LSD in tablets after silylation, but the chromatograms often showed many peaks which obscured the LSD peak either partially or completely (*288*). The use of a fluorometric detector on HPLC analysis obliterated coextractives contained in tablets and detected by the use of a UV detector, and gave chromatograms displaying a linear relationship. The HPLC conditions for the separation of LSD from other ergot alkaloids was MeOH/0.1% $(NH_4)_2CO_3$ (6 : 4) using C18 as the column which improved the resolution and shortened retention times in comparison with GC. HPLC, when eluted with MeCN/isoPr$_2$O (40 : 60) on sil-X and MeCN/isoPr$_2$O (25 : 75) on Corasil II, gave sensitive, accurate, and reproducible separation of LSD from 14 other ergot alkaloids, phencyclidine, STP, and strychnine (*289*). The last three drugs were investigated because of their possible use in combination with LSD. HPLC was also used to distinguish between LSD and lysergic acid methylpropylamide present in street samples and difficult to analyze because only small amounts of material were available and because

purification gave inconsistent results. Siefert and Collins succeeded in separating the two compounds by a retention-time difference of 0.3 min using GC-MS on an SE 54 capillary column and an oven temperature of 235°C (*290*). Other methods of separation of LSD from lysergic acid methylpropylamide in illicit samples were undertaken by Nichols *et al.* by GC on a fused column coated with a film of SE-30 at isothermal (260°C) or programmed temperatures (100–295°C) (*291*).

Numerous chromatographic methods for the detection of natural ergot alkaloids, isomers, and their products of degradation have been conducted, although there is as yet no satisfactory separation between ergocristinine and ergocryptine, ergosinine and ergocristine, or D- and L-alkaloids. Gibson separated all D- and L-alkaloids of the ergotoxine group on silica gel G on TLC using hexafluorobenzene as the solvent but this method has several disadvantages, including long lasting development and relatively small differences between Rf values (*292*). Reichelt and Kudrnác developed a system for the separation of 16 ergot alkaloids by TLC on silica gel impregnated with formamide using iso-Pr_2O/THF/toluene/Et_2NH (70 : 15 : 15 : 0.1) or isoPr_2O/toluene/EtOH Et_2NH (75 : 20 : 5 : 0.1) as mobile phases (*293*). In their solvent systems, both D-lysergic and D-isolysergic acids remained at the origin. Ergometrine and ergometrinine gave the same Rfs as ergoine and isoergine, respectively. Lysergic and isolysergic alkaloids in ergot, in single sclerotia of ergot, in the medium of saprophytically grown *Claviceps purpurea,* and in medicaments were separated by TLC using silica gel G as an adsorbent and EtOAc/ dimethylformamide/ EtOH/25% NH_4OH (131 : 19 : 1 : 0.2) as a solvent system for a fluorometric determination. This procedure required 15–200 ng for determination with high precision (*294*). Ergot alkaloids in individual sclerotium of *Claviceps* were determined with a limit of detection of 10 ng on TLC by a fluorodensitometric method using silica gel G and NH_4OH/MeOH/$CHCl_3$ (1 : 9 : 90) (*295*). Ergonovine may be degraded by oxygen, light, and temperature at pH 7, and it is thus a requirement to handle with excessive attention to these factors. The sample is prepared in the dark, derivatized with TMSDEA and TMSI (Table II) and gas chromatographed on a column containing a nonpolar methyl silicone liquid phase which minimizes formation of degradation products (*296*). GC analysis of ergotoxine alkaloids (dihydroergocristine, dihydroergocryptine, and dihydroergocornine) as bases or salts could not be achieved because of their low volatility and because thermal breakdown occurred in the GC. Szepesi and Gazdag determined dihydroergotoxine alkaloids on GC quantitatively through the catalyzed decomposition on metal surfaces and measuring the different migration rates of the peptide moieties formed (*297*). The optimal temperature to decompose these alkaloids on a metal surface is between 230 and 250°C carried out on spiral stainless steel columns packed with 20% Dexsil 300 (80–100 mesh) on a Gas Chrom Q. Franc applied this method for separation of the ergot–peptide alkaloids: ergotamine, ergosine, ergostine, ergocristine, α-ergocryptine and

ergocornine on GC (Figure 17) (*298*). The optimal injection-port temperature was 300°C giving decomposition products which were further characterized by GC-MS and high-resolution MS and comparison with reference samples. Gas chromatography of a mixture of six ergot–peptide alkaloids showed that each individual alkaloid was present in the form of two or three characteristic degradation products. Various street-drug samples were investigated by this GC technique and by TLC. Plomp *et al.* described a rapid, sensitive, and specific method for the analysis of the dihydroergotoxine alkaloids by GC on 3% SE-30 (*299*). They employed a temperature of 225°C for decomposing the alkaloids into two components, dihydrolysergic acid amide and the peptide moiety. The structure of each peptide moiety was determined by GC-MS.

Analysis of ergot alkaloids by HPLC seems to be a more appropriate method than GC, even though the thermal peptide degradation allows identification of the parent alkaloid which is separated reasonably well from the peptide moiety by GC. Preliminary investigation by HPLC using a fluorescence detector for separating ergotamine from ergocristine was carried out on Corasil column using $CHCl_3$/MeOH/AcOEt/AcOH (60 : 20 : 50 : 3) as a solvent system (*300*). A variable-wavelength UV detector was used for determination of ergot alkaloids on HPLC with maximum of 310 or 320 nm for ergot alkaloids and 280 nm for hydrogenated alkaloids (*301*). In this report, reversed-phase packing silica gel with different bonded hydrocarbon groups C2, C8, and C18 were investigated as well as normal-phase packing silica gel in the analysis of 13 alkaloids. Good separation of α- and β-ergocryptine was achieved on the silica gel C18 column eluted with 40% MeCN in a 0.01 *M* $(NH_4)_2CO_3$ solution using a UV 320 nm detector. Separation of dihydroergotoxines, dihydroergocristine, dihydroergocornine, and α- and β-dihydroergocryptines was achieved under varying HPLC conditions. A pH variation (2–13) of the aqueous part of the mobile phase influenced chromatograms. H_2O/MeCN/Et_3N (32 : 8 : 1) as a mobile phase and Lichrosorb C18 as a column were used for a successful separation of four alkaloids with ^{13}C NMR investigations for a study of the influence of basicity on the carbon skeleton of the molecules (*302*). Ali and Strittmatter also separated the dihydroergotoxine alkaloids, dihydroergotamine, dihydroergocornine, dihydroergocryptine, and dihydroergocristine by HPLC with an ion-pair mechanism by using reversed-phase columns (C8 and C18) with five different mobile phases (*303*). Citric acid, AcONa and AcOH, tetradecyltrimethylammonium bromide, or KBr can be added advantageously to the mobile phase using MeCN and water as eluants on C8 and C18 columns. Separation of α- and β-isomers of dihydroergocryptines was obtained in a strongly alkaline medium with a mobile phase of MeCN/H_2O/Et_2NH (37.5 : 62. 5 : 2.1, pH 12.5) on a C18 column.

Wurst *et al.* (*304*) suggested a relationship between the structures of ergopeptines and measured retention volumes using LiChrosorb NH_2 as a column and Et_2O/EtOH as a solvent on HPLC. The elution volumes of series "-ines" of

ergopeptines, which are in the equatorial position with respect to the D-ring of the ergolenes, are substantially higher than those of "-inines," which are in the axial position. Intramolecular hydrogen bonding between the basic nitrogen in position N-6 and the hydrogen of the amide group in position C-18 of "-inines" seems to be the reason. Another interaction originates from alkyl groups positioned at C-5′ and C-2′ on the cyclic peptide of ergopeptines to influence elution volumes. Alkaloids with a benzyl group at C-5′, together with an alkyl group at position C-2′, are greater than those with alkyl groups at both positions, suggesting an interaction between the alkaloid and the stationary phase. The smaller the alkyl group is at position C-2′, the higher the elution volumes. Elution volumes of alkaloids with aliphatic groups at C-2′ and C-5′ are lower than those of alkaloids with a single aromatic group at C-5′. Substitution of the aromatic group by branched alkyl groups at C-5′ and by ethyl and isopropyl groups at C-2′, decreased the elution volumes because of greater steric hindrance on the peptide moiety of the alkaloids interacting with stationary phases. Alkaloids with only hydrogen at position C-8 produced higher elution volumes than those having a tertiary hydroxyl group and thus having hydrogen bonding between the carbonyl group at C-18 and the hydroxyl group at C-8. A primary amino group, a primary hydroxyl group, or an isopropyl group at C-18 influenced elution volumes.

Normal-phase dynamic molecular complexation chromatography also was investigated to separate alkaloids (*305*). Cyanopropyl-silica as a polar bonded stationary phase and, less polar eluent mixtures such as hexane/$CHCl_3$/MeCN or hexane/isoPrOH containing a small amount of anionic ion exchanger such as di-(2-ethylhexyl) phosphoric acid/or α-camphor-10-sulphonic acid, were developed for separation of 11 ergot alkaloids. It was proposed that molecular complexation products between the uncharged alkaloid base and the ion exchanger increase retention times then the free unchanged alkaloid base. The ergot alkaloids are high molecular weight, thermally unstable compounds which were administered in doses varying from 0.5 to 10 mg/day. After such doses, the concentration in plasma was found to be lower than 1 ng/ml by radioactive tracer studies (*306*). The absolute detection limits for pharmaceutical preparations, biological samples, and street sampled drugs were in the microgram range when a UV absorption detector with a standard xenon lamp was used as an excitation source for fluorometric detection on HPLC. Perchalski *et al.* evaluated a 150 W Eimac lamp (high pressure xenon arc lamp with an interal collimating mirror) as an excitation source, which would increase sensitivity by gathering more efficient light at the source (*306*). They showed that an Eimac lamp gave detection with limits below 1 ng/ml for ergot alkaloids extracted from plasma. Toxicological reinvestigation of whole blood hydrolyzed with acid was attempted (instead of the usual separation of plasma). Whole blood was extracted at pHs 1.5, 9.5, and 12.0 and assayed by GC and GC-MS on glass columns (1.8 m × 2 mm) filled with 3% SE-30 or 3% SP-2250 (*307*). The GC-MS showed the

presence of pentazocine and two characteristic compounds, the L-phenylalanine and D-proline originating from ergotamine in the alkaline extract at pH 9.5 after the acid-hydrolysis of blood. LSD (2–8 ng/ml) in serum was found (about 1 hr after administration of 160 μg LSD into seven persons) by HPLC equipped with a fluorescence spectrophotometer (column, Merck Hibar Fertigstahlsaule EC 250-4 with C8 reversed-phase material LiChrosorb, using both alkalic and acidic solvent systems) (*308*).

I. Arecolines

The seed of *Areca catechu* (Palmae) is commonly known as areca nut or betel nut. This plant is cultivated widely in the southern parts of Asia such as Malay, India, the Philippines, Indonesia, Papua New Guinea, and the southern part of China. Areca nut contains several alkaloids, arecoline, arecaidine, guvacoline, and guvacine, the first one being the primary pharmacologically active component. Areca nut is a potent anthelmintic and an important traditional medicine in China and India. In the southern part of Asia, Papua New Guinea, the Philippines, and Burma, the Areca nut has been used as masticatory. Its method of preparation differs in the different regions. In Papua New Guinea, where the method is most primitive, the shelled kernel of the nut is dusted with lime and masticated. Since areca nut is highly astringent due to tannins, the fruit of the Piper betel (kimma) is quite often chewed together with it to make the preparation tasty. In Malay, the preparation is often wrapped with the leaf of Piper betel and used. Several other allied plants, *A. concinna, A. nagensis,* and *A. triandra,* are also sometimes utilized as substituents. Arecolin is a potent parasymphathomimetic (cholinergic) agent whose action resembles pilocarpine (a major alkaloid in *Pilocarpus jaborandi*). The preparation promotes the secretion of saliva and is an effective aid for digestion. Although the chewing of betel nuts was used to achieve anthelmintic and stomachic properties in tropical Asia, the undesirable side effects are not negligible. Arecoline and arecaidine have been reported to be potent carcinogens (*309–311*), but the carcinogenic action of arecaidine is somewhat questionable (*312*). It is thought that during the chewing of betel nut quid, arecoline gives rise to carcinogenic *N*-nitrosoamines (*313*). Among three possible nitrosoamines, *N*-nitrosoguvacoline (NG), 3-(methylnitrosoamine)-propionitrile (MNPN), and 3-(methylnitrosoamino) propionaldehyde, MNPN is suspected to be potent carcinogens (*314*). Other constituents such as polyphenols (tannins) are also possible carcinogens (*315*). The strongly alkaline lime will also promote cancer. Thus, cancer, especially oral cancer, is apt to be induced by chewing betel nut quid. In addition, it is believed that excess uptake of the nut may induce other undesirable side effects such as temporary giddiness and intestinal irritations. The chronic use of betel nut may also induce an excessive secretion of saliva leading to a concomitant decrease in enzyme and electrolyte content in saliva (*316*).

Although pharmacological studies of arecolines have been rather extensively carried out, few reports have appeared on the chromatographic analysis of arecolines. In systematic TLC studies of alkaloids, Waldi *et al.* examined the chromatographic behavior of arecoline along with numerous other alkaloids on silica gel, using several basic solvent systems (*30*). Arecoline can be detected by the color reagents normally used for alkaloids, such as Dragendorff's reagent (Table I) (*317*). Color reactions of arecolines by various reagents are reviewed along with synthetic and pharmacological studies (*318*). Direct microdetection by HPLC is rather difficult, since arecolines have only weak absorption in the UV region. Derivatization is also not easy because arecoline is an *N*-methylated compound and possesses neither –OH nor –NH groups. Analysis by GC also seems to be difficult, since arecolines are rather volatile compounds with strong basicity; direct GC analysis, therefore, gives tailing peaks. The development of chromatographic methods for the analysis of arecolines and their metabolites, in plant materials and biological samples, is needed.

J. Tobacco Alkaloids

The smoking of tobacco (the leaf of *Nicotiana tabacum*) is the most widespread drug addiction worldwide along with the drinking of alcohol. The habit was introduced into Europe in the beginning of the Sixteenth Century by the crewmen who accompanied Columbus to the New World. In the following century, the smoking of tobacco spread throughout the world, in spite of vigorous official opposition and, in some cases, penalties. Tobacco leaf contains several alkaloids, including nicotine, nornicotine, anabasine, and anatabine: among these nicotine, whose content ranges 1–5%, is the main alkaloid.

The chronic toxicity of tobacco has been extensively investigated, including lung cancer and coronary artery disease which are the most dangerous. Among the 4000 compounds which are generated by the burning of tobacco, nicotine, along with several other substances such as carbon monoxide and "tar," is chiefly responsible for its toxity. Tar contains strong carcinogens such as nitrosoamines, aromatic amines, and benzo(a)pyrenes. Since the recognition of the hazard to health induced by the smoking of tobacco, the consumption of tobacco has decreased in recent years in the United States, Europe, and Japan.

The chromatographic analysis of nicotine and related compounds has been extensively carried out. Since nicotine is commonly found in urine, it is often included in the analysis of biological materials of drug addicts. The analysis of nicotine is also necessary in forensic chemistry because nicotine is a deadly poison (the fatal dose of nicotine for adult is as small as 60 mg). Nicotine poisoning has been observed in several countries.

For the analysis of tobacco alkaloids, GC and GC-MS are suitable methods, especially for the analysis of tobacco alkaloids, Quin gas chromatographed several tobacco alkaloids on polyethylene glycol columns on alkali-wahsed firebrick

(*319*). Because nicotine belongs to one of the most volatile groups of alkaloids, GC analysis without derivatization is not difficult when columns are treated with strong bases. Neutral column packings tend to cause tailing, and strongly basic columns treated with potassium hydroxide give better results (*320*). The routine determination of nicotine and nornicotine in tobacco was devised by Quin and Pappas (*321*) and includes determination of minor alkaloids (*322,323*). Quin also carried out the GC analysis of tobacco alkaloids in smoke (*324*). Since then, several reports have appeared regarding the analysis of tobacco smoke. In the analysis of tobacco smoke, the method for collecting volatile components should be chosen. In one approach, cigarettes are smoked through a filter, and the adsorbents to the filter are then extracted and analyzed (*325*). The other method is a head-space technique, in which gaseous volatile components are collected in the vessel. The head-space volatiles are either directly introduced to the GC or gas chromatographed after condensation by a suitable trapping method. Apparatus for head space GC is now commercially available, and rather widely applied in the analysis of various volatile components. To determine nanogram to picogram amounts of nicotine, PFPA (Table II) derivatization after hydrogenation (*326*), and trichloroethylcarbamate derivatization (*327*) were reported. In these derivatization methods, cleavage of the pyrrolidine ring is involved. The use of capillary columns and capillary GC-MS (*328–330*) is advantageous because numerous compounds are present in tobacco smoke. Capillary GC analysis facilitates the fingerprint analysis of different tobacco samples (*331,332*).

Analysis of nicotine in biological samples by GC (*333–340*), glass capillary GC-MS (*341,342*), and fused-silca capillary GC-MS (*343,344*) has also been extensively carried out. In the analysis of biological samples, detection of the major metabolite, cotinine, in addition to nicotine, is often of concern.

The number of reports of HPLC analysis of nicotine are relatively small compared to GC and GC-MS. HPLC analysis of tobacco alkaloids in cigarettes and the tobacco leaf has been carried out by reversed-phase chromatography on C18 columns (*345,346*) and on C8 (*347*) columns. Nicotine and various minor alkaloids such as nornicotine, anabasine, anatabine, and myosmine were separated (*345*) by gradient elution. HPLC determination of nicotine and its metabolites in biological samples was also carried out on a cation-exchanger (*348*) and on C18 (*349*). Although the detection of nicotine is usually carried out by UV detector (near 254 nm), the use of an electrochemical detector (*349*) facilitates the microdetection in the microgram to subnanogram range of levels. The use of silica gel has also been reported (*350*) for the separation of nicotine and conine in urine, and a basic eluent gave better results.

K. Purine Alkaloids

The purine alkaloids caffeine, theobromine, and theophylline are naturally occurring and are found, for example, in cocoa seeds (cocoa beans) such as

Theobroma cacao (Sterculiaceae) in South and Central Americas, the West Indies, West Africa, Sri Lanka, and Java; kola seeds (cola seeds) such as *Cola acuminata, C. astrophora, C. alba,* and *C. vera* (Sterculiaceae) in West Africa, the West Indies, Brazil, and Java; guarana (Pasta Guarana or Brazilian cocoa), *Paullinia cupana* (Sapinodaceae) in the upper Amazon basin; coffee, *Coffea arabica, C. liberica, C. canephora* (Rubiaceae); tea, *Thea sinensis* (*Camellia sinensis*) (*Theaceae*) cultivated in India, Sri Lanka, China, and Japan; and Mate leaf (Yerba Mate or Paraguay Tea), *Ilex paraguariensis,* and other species of Ilex (Aquifoliaceae) in Argentine, Paraguay, and Brazil.

Aqueous extracts of these plants have been used for beverages from the earliest times and are now popular in soft drinks throughout the world. Kola nuts were used as masticatories in the Sudan.

Caffeine is the most powerful central nervous system stimulant of the three methylxanthine derivatives, varying in potency depending on the dose and the individual. Most people are unaware of any stimulation from daily drinks, but children are more susceptible than adults. These methylxanthines may induce a psychiatric dependence (habituation). Caffeine has been more often used as an adulterant than any other agent.

There have been numerous reports of TLC analysis for purine alkaloids under neutral, basic, and acidic conditions with mixtures of organic solvents on normal-phase silica gel plates (*351*). Detection is made from their quenching properties with UV light on fluorescent plates. The reagents used to detect purine alkaloids on plates are combinations of Dragenforff's reagent with 10% H_2SO_4/20% HNO_3, and iodine (because there is no detectable reaction with Dragendorff's reagent itself). On reversed-phase ion-pair chromatography, the relationship between the Rf values of the alkaloids and the chain length of the alkyl group was examined with the ion-pair reagent alkyl-SO_3Na (*352*). The Rf values for most of the alkaloids decreased with increasing chain length of the alkyl-SO_3Na, but these fluctuated within the range of 0.5–0.56 because it is a neutral molecule. Caffeine as a commercially available stimulant was analyzed by high-pressure TLC (*353*). The chamber for increased-pressure TLC has an operating membrane pressure of 1 million Pa (Pascals). A fast and accurate procedure to determine the content of caffeine in coffee, cocoa, and other beverages has been devised. To determine caffeine and theobromine in cocoa powders, fats were removed through paper chromatography by development in light petroleum for about 1.5 hr. Caffeine and theobromine were then detected on the paper chromatogram after developing with *n*-BuOH saturated with NH_3 for 1.5–2 hr (*354*). There have been many conditions on GC for purine derivatives: SE-30, SE-52, QF-1, OV-1, OV-17, and XE-60 and the more polar neopentyl glycol sebacate and polyvinyl pyrrolidone have been developed as the stationary phase for columns; FID, nitrogen sensitive FID, and alkaline flame detector have been used as detectors, and TMS- and TFA-derivatives prepared for better chromatographic properties (*355*). *n*-Propyl derivatives gave advantageous separa-

tions over other alkyl derivatives such as butyl or pentyl (*356*). *n*-propyl derivatives of purines in biological samples were most conveniently analyzed by GC-MS on a 3% mixed phase of phenyl silicone and methyl silicone column (*357*).

For a spearation of purine derivatives by HPLC the following have been employed: a standard UV detector (254/275/280 nm); normal-phase columns (silica gel) in combination with a mixture of CH_2Cl_2/isoPrOH/ MeOH/ AcOH/ HCOOH as mobile phases; ion-exchange columns (cation-exchange resins, a chemically bonded strong-cation exchanger on pellicular beads, and a cross-linked cation-exchange resin) in combination with acidic aqueous solutions and buffers for mobile phase; and, finally, reversed-phase C18 columns in combination with mixtures of MeCN-MeOH and acidic aqueous buffers with the tetrahydroammonium hydrogen sulfate ion-pair reagent added to those solvent systems (*358*). HPLC conditions, using a reversed-phase column and solvent system mixture of 1% AcOH (1.2 ml/min) and 100% MeCN (0.3 ml/min), and a UV detector (254 nm) were set up to determine caffeine in black leaf tea and tea extracts (*359*). Argoudelis determined the caffeine content of soft drinks by HPLC with condition of a strong cation exchanger, Partisil-10SCX, eluted with 0.1 *M* $NH_4H_2PO_4$ and detected at 214 nm (*360*). Reld and Good examined the recovery of three methylxanthines from 5-ml water samples extracted with different bonded-phase silicas (*361*). They used an HPLC equipped with a UV detector at 280 nm and eluted with MeCN/MeOH/7.0 m*M* H_3PO_4 (4 : 4 : 92) on Sepralyte C18 after extraction through a C18-bonded eluting column, since C18 bonded phase gave the best recovery. Terada and Sakabe used a Sep-Pak C18 cartridge for pretreatment to get cleaner extracts for HPLC samples (*362*). Three methylxanthine derivatives from the food products cocoa, cola, coffee, chocolate, green tea, and black tea were analyzed by HPLC equipped with a UV photometer at 275 nm on LiChrosorb C8, eluted with MeOH, H_2O, 0.2*M* phosphate buffer of pH 5.0 after pretreatment (*362*).

Three methylxanthines and their metabolites in biological samples were examined by HPLC, on phenyl, C8, and C18 columns and evaluated for achieving a separation and resolution of theophylline and caffeine on HPLC (*363*). The HPLC conditions were optimized as follows: the phenyl column, a gradient system of phosphate pH 4.0-MeCN for the mobile phase, and monitored at 280 nm. Linear correlations for theophylline and caffeine were 2.5 to 20 or 50 mg/liter. Three methods of extraction from biological samples were used: first, a simple solvent extraction with CH_2Cl_2/isoPrOH and $CHCl_3$/isoPrOH; second, a more polar solvent mixture with salts, and third, *N*-alkylation followed by extraction (*357*). The first method is used more often than the others. Ou and Frawley extracted theophylline and caffeine from serum samples with $CHCl_3$/ isoPrOH (95 : 5) and analyzed them by HPLC (*364,365*). The HPLC system consisted of a compressed C18 reversed-phase column, MeCN/0.1 *M* K_3PO_4 buffer, pH 4.0 (9.5 : 90.5) as the mobile phase, and 254 nm for detection. A

detection limit of as low as 0.5 mg/liter in 50 μl of serum was observed (*364*). Theophylline and its metabolites ,1,3-dimethyluric acid, 1-methyluric acid and 3-methylxanthine, from serum and urine were analyzed by reversed-phase HPLC (*366*). HPLC samples were prepared from serum with acetone extraction and from urine with anion exchange cleanup. The lower limits of sensitivity were 0.04 μg/ml and 1 μg/ml for serum and urine metabolites, respectively. Chang *et al.* investigated the analysis of theophylline in plasma with the advantage of no interference from endogenous substances by using a multidimensional column chromatography technique (*367*). Their HPLC conditions were MeOH/ THF/ 0.01 *M* NaOAc buffered to pH 5.0 (7 : 1 : 92) for the mobile phase, an ultrasphere C18 column, and a 280 nm detector. As little as 30 μl of plasma samples were required and a range of 0.25–30 μg/ml gave linear results.

L. OTHERS

Alkaloids and related compounds are described in the previous sections which concern drugs of abuse. There are several other alkaloids such as strychnine, brucine, atropine, and scopolamine, which are the object of analyses, although they are not always the object of addiction. In addition, there are several alkaloids such as strychnine, aconitine, coniine, tropane alkaloids, and others which are of forensic interest as poisons. Accidental or willful uptake of these poisonous alkaloids sometimes occurs.

Strychnine and brucine were found in *Strychnous nux-vomica* (Loganiaceae), which is native to India. There are about 300 species of *Strychnos* in Asia, Africa, and America. Strychnine is employed less frequently in pharmaceutical preparation than previously. It has been used as an ingredient of grain baits for rat, and dog poisons, but has thus been the source of accidental poisoning of children. Strychnine is also one of several agents used as an adulterant in street drugs.

The structures of *Strychnos* alkaloids are classified into four series: (1) normal series, (2) the *N*b-oxide series, (3) the pseudo series with a 16-hydroxy function, and (4) the *N*b-methylates of the pseudo series (*N*-methyl-*sec*-pseudo compounds). Bisset and Phillipson screened 234 samples from 36 Asian species of *Strychnos* on TLC and GC (*368*). They used three solvent systems on TLC: (1) AcOEt/isoPrOH/5.5% NH_4OH (45 : 35 : 20) for separation of polar bases with *N*b-oxides, (2) AcOEt/isoPrOH/NH_4OH (80 : 15 : 5) for separation of alkaloids between normal series and those of the less polar pseudo *N*-methyl-*sec*-pseudo compounds, (3) EtOAc/isoPrOH/NH_4OH = 100 : 2 : 1, and (4) *n*-BuOH/0.1 *M* HCl/7.4% aq. $K_3Fe(CN)_6$ (100 : 15 : 34) for separation of all the compounds.

Dragendorff's reagent, 0.025 *M* $Ce(SO_4)_2$ in 1 *M* H_2SO_4, and 0.2 *M* $FeCl_3$ in 3.5 *M* $HClO_4$ was used as a spray. Strychnine, aromatic substituted strychnine, and their derivatives with a *N*a-carbonyl function, gave different colors accord-

ing to the substitution pattern and independent changes in the molecule with the last two reagents. The compounds with an acyl group moiety such as a *N*a-carbanoyl function rather than a lactam moiety yielded slightly different colors. Phillipson and Bisset examined correlations between Rf values on TLC (silica gel and alumina using seven solvent systems) and chemical structures of tertiary *Strychnos* alkaloids (*369*). The factors affecting adsorption on TLC were proposed as follows: type of aromatic substitutent, inductive effect on *N*a-carbonyl function, adjacent oxygen atoms at carbons (C-16 and C-18) bonded next to *N*b, *N*b-oxides, reduced compounds on the C-21–C-22 double bond, and 14-hydroxyl compounds. Bisset and Fauche also investigated tertiary *Strychnos* alkaloids in *Strychnos nux-vomica* and *S. icaja* on GC and discussed the relationships between the relative retention times and chemical structures (*370*). A more polar stationary phase gave better separation, however slightly polar SE-52 (methylphenylsilicone polymer) (FID) stationary phase was used in order to achieve increased Rfs. Separation of strychnine and brucine was studied on HPLC equipped with a UV detector (254 nm) on Merckosorb Si-60 (*371*). Et_2O/MeOH (1 : 1) as an eluent solvent gave a separation with high retention times and much tailing. The addition of 1% Et_2NH into the above solvent system improved the chromatogram. This solvent system was applied for the identification of other *Strychnos* alkaloids (*372*). The concentration of strychnine in grain baits was determined by HPLC (μPorasil, detector 254 nm) eluted with MeOH/$CHCl_3$ (10 : 90). Under these conditions, the concentration of brucine was much greater as a contaminant in the preparations (*373*). Reversed-phase HPLC was carried out for separation of strychnine and brucine using a gradient of phosphate buffer/MeOH from (80 : 20) to (60 : 40) (*374*). The concentration in grain bait and stomach contents was then determined. Alliot *et al.* (*375*) analyzed the concentration of strychnine in plasma and urine samples by HPLC, using as elution buffer NH_4OH/MeOH (0.75 : 99.25) on a silica gel column (254 nm detection).

Atropine (*dl*-hyoscyamine), scopolamine, and related alkaloids are widly distributed in the Solanaceae family (*Atropa belladonna, Datura stramonium, Hyoscyamus niger, Scopolia carniolica,* etc.). *l* Hyoscyamine/*l*-scopolamines are isomerized to the *dl* forms, then hydrolyzed into tropine/scopoline and tropic acid, or dehydrated to apoatropine/aposcopolamine, which are hydrolyzed into tropine/scopoline and atropic acid. Atropine and scopolamine are the most important drugs. Antimuscarinic actions are quantitatively different between atropine and scopolamine. The latter is usually considered more toxic than the former. Infants and young children are especially susceptible to them. The berries and seeds containing the alkaloids may cause intoxication in children. There are a number of reports for TLC of tropine alkaloids and their degradation products. One such report used a mixture of $CHCl_3$/acetone/alcohols and NH_4OH/Et_2NH as solvent systems, and silica gel as a thin layer technique (*376*). Acidic conditions such as HCl/AcOH for solvent systems have also been em-

ployed. Modified Dragendorff's, iodine (vapor), or idoplatinate are used for detection of the alkaloids on TLC because of their poor sensitivity by UV (254 nm) and Dragendorff's reagent when used alone. The pH indicators Bromophenol Blue and Bromocresol Green were sprayed to detect the nonnitrogenous degradation products of tropine alkaloids (*376*). Nonpolar and polar stationary phase columns are both used for separation of tropane alkaloids on GC (*377*). Polyethylene glycol as a stationary phase gave the best separation for nine alkaloids in five different polar columns (*378*). Windheuser applied GC (on OV-17) for analysis of scopolamine and its four degradation products as mentioned above, after derivatization with TMSA (*379*). Numerous methods for separation of tropine alkaloids by both normal- and reversed-phase HPLC have been reported (*380*). Various ion-pairing reagents have been added to the solvent systems on reversed-phase HPLC (sodium dodecylsulfate, octanesulfonic acid, heptanesulfonic acid, dictoylsulfosuccinate, tetrabutylammonium sulfate, picric acid, etc.). Picric acid improves by 100–300 times the sensitivities for tropane alkaloid detection (254 nm). 9,10-Dimethoxyanthracene-2-sulfonate is used as pairing-ion and improves the detection limit (*381*). Duez *et al.* added *d*-10-camphorsulphonic acid as a counter-ion in the mobile phase MeCN-$PrNH_2$ to analyze alkaloids in the leaves and fruits of *D. innoxia* (Zorbax TMS column, 259 nm detection) (*382*). Pasphassarang and Raynaud determined the content of hyposcyamine, atropine, and scopolamine in three species of in Solanaceae using C18 as the column, 3% AcOH/MeOH (75 : 25) as the mobile phase, and 254 nm as detector (*383*).

Solanine and its congeners are glycosides of steroidal alkaloids which are distributed in several solanaceous plants. In the sprout or green tuber of potato, solanine is found to be present in high concentrations. Solanine is poisonous and too much uptake results in poisoning and even death. In recent years, the nightshades, which contain solanum alkaloids, have become pests in various crops throughout the world. TLC of solanum alkaloids and steroidal sapogenines was carried out on silica gel in cyclohexane/AcOEt (1 : 1), by which the best resolution was attained (*384*). Rönsch and Schreiber reported TLC separation of solunum alkaloids on $AgNO_3$-impregnated silica gel following the observation that $AgNO_3$-treated silica gel is useful for the separation of steroids with and without double bonds in the steroidal core (*385*). A similar technique has also been found useful for HPLC. Hunter *et al.* (*386*) scrutinized TLC behavior of 26 steroidal alkaloids on silica gel under various solvent systems. GC analysis of solanum alkaloids in the potato was undertaken by Herb *et al.* on an OV-1 column in which nonvolatile alkaloids were permethylated with CH_2N_2 (*387*). Analyses by HPLC have been reported by several authors in which tedious derivatization procedures are unnecessary. Since solanum alkaloids are poor in UV absorption, however, detection is carried out at shorter wavelengths (200–215 nm). Separation has been carried out on C18 or C8 by an ion-suppression mode (*388–390*),

amino silica (*391, 392*), and silica gel (*393*). Bushway *et al.* found that amino silica gave better separation (THF/MeCN/H_2O/MeOH) than C18 for three major potato alkaloids, α-solanine, α-cachonine, and β-cachonine (*391*).

Aconitine and related alkaloids are contained in *Aconitum spp.*. *Aconitum* tuber has been an important crude drug in many countries such as China, Japan, and others. Aconitine, however, belongs to a group of notorious deadly poisons, and overdose causes death. In utilizing *Aconitum* tuber as drugs, the content of poisonous alkaloids has been reduced and strictly regulated by suitable treatment. Kurosaki *et al.* separated four major poisonous diterpene alkaloids on silica gel TLC by Et_2O saturated with 25% NH_4OH (Rf for hypaconitine, aconitine, jesaconitine, and mesaconitine was 0.92, 0.54, 0.32, 0.25, respectively) (*394*). They also carried out densitometric determinations. The HPLC separation of aconitine alkaloids in crude drugs was undertaken by Hikino *et al.* on C18 packings with phosphate buffer, pH 2.7/THF in the absence and presence of an ion-pair reagent (sodium hexanesulfonate) at 254 nm (*395*). Both systems gave satisfactory separation of ten aconitine alkaloids. The use of -CN packings was also reported with MeCN/phosphate buffer containing dibutylamine (*396*).

Colchicine and congeners are tropoid alkaloids which are distributed in the seeds of *Colchicum autumnale* and related species. Colchicine is a very important antipodagric, but it is highly toxic. Colchicine poisoning of cattle sometimes occurs by contaminating animal food sources with *Colchicum spp.* Potesilova *et al.* (*397,398*) scrutinized TLC of numerous colchicine alkaloids in plant materials on silica gel using several solvent systems. Aside from the usual color reagents such as Dragendorff's reagent and iodoplatinate reagent, they found that a characteristic Oberlin–Zeisel reaction was applicable for alkaloids with a tropolone ring: alkaloids with a methoxyl group attached to the tropolone ring develop a brown color by $FeCl_3$ spraying reagent after treatment with HCl vapor for 30 min. The HPLC determination of colchicine was carried out on a C18 column (MeOH/MeCN/Et_3NH in phosphate buffer, pH 2.2, or MeOH/ MeCN/ phosphate buffer, pH 6.0) for the analysis of metabolites of colchicine in microbial culture (*399–401*). Detection was carried out by UV detector (350–370 nm). Analysis of colchicine and demecolcine in contaminated food sources and in the tissue of posioned bovine such as milk, urine, muscle, and liver was carried out (*402*) under similar HPLC conditions, described in (*399*).

Pyrrolizidine alkaloids are distributed in *Senecio spp.*, in which senecionine and retrosine are most widely found. These alkaloids are important in veterinary science because poisoning of cattle by contaminated weeds sometimes take place. GC analysis of *Senecio* alkaloids was carried out on an SE-30 column without derivatization (*403*). Seven alkaloids in several *Senecio* plants were determined, but separation of senecionine and senecivernine was incomplete. In a series of research publications on HPLC analysis of senecio alkaloids, Segall *et*

al. established the best HPLC conditions (MeOH/0.01 *M* KH_2PO_4 buffer, C18), and analyzed several plant materials (*404–406*).

Tubocurarine chloride is a potent neuromuscular blocking agent obtained from the stems of *Chondrodendron tomentosum,* one of the famous plants from which South American Indians have obtained the arrow poison curare. A few reports on TLC of curare alkaloids have appeared (*407–409*). Clarke and Raja (*409*) separated five major curare alkaloids on silica gel TLC. The developing solvent was the lower phase of $CHCl_3$/MeOH/12.5% (w/v) aqueous trichloroacetic acid (1 : 1 : 1). Rf values of isocondrodendrine, curine, chondrocurine, tubocurarine, and curarine were 0.52, 0.48, 0.44, 0.26, and 0.08, respectively. The HPLC separation of curare alkaloids was carried out on a C18 column using a gradient elution technique by a mixture of H_2O/MeOH containing phosphate buffer (*410*). Five of the alkaloids mentioned above were so separated. Since curare alkaloids are quaternary bases and, consequently, water soluble, separation was impossible without gradient elution on a C18 column. Ion-pair chromatography has not been applied, but these quartenary alkaloids may best be separated by ion-pair chromatography because bulky organic quarternary amines are well known as the most potent ion-pair agents.

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— CHAPTER 2 —

STEROIDAL ALKALOIDS OF APOCYNACEAE AND BUXACEAE

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I. Introduction

Comprehensive reviews on the steroidal alkaloids of Apocynaceae and Buxaceae have been published previously (*1–3*). The present chapter deals with recent advances made in this field, and it may be considered as a supplement to Volumes 9 and 14 of this series.

Steroidal alkaloids of Apocynaceae are conveniently classified into three main structural groups: the conanine group, the pregnane group, and the paravallarine group.

The alkaloids of Buxaceae are regarded as derivatives of 9β,19-cyclo- and 9(10→19)-*abeo*-pregnane and are further subdivided according to the number of nitrogen atoms incorporated.

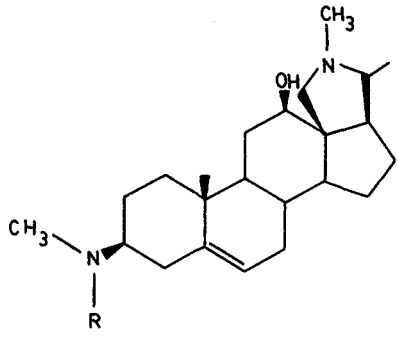
II. Steroidal Alkaloids of Apocynaceae

A. Isolation and Structure Elucidation

1. The Conanine Group

The alkaloids' holarrheline (**1**), holadienine (**2**), holaromine (**3**), holaline (**4**), and holarrhenine (**5**) possessing conanine-type structures, were isolated from the bark of *Holarrhena floribunda* (G. Don) Dur. *et.* Schinz (*4, 5*).

Holarrheline (**1**) exhibited absorption bands at 3300 and 3200 cm^{-1} in its IR spectrum, indicating the presence of OH and sec. NH groups. The ^{1}H-NMR spectrum of **1** showed a one-proton doublet at δ2.78 (J = 10.0 Hz) attributed to

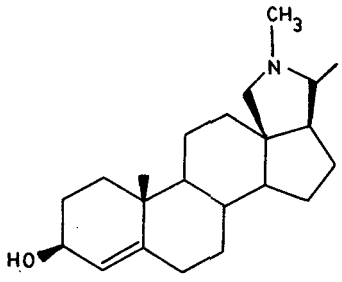


1 R = H

5 R = CH_3

the C-18 methylene protons. A triplet at δ5.33 revealed the presence of an olefinic proton. The 21-methyl protons appeared as a doublet at δ1.02 (J = 6.0 Hz). The structure of holarrheline (**1**) was confirmed by its conversion to holarrhenine (**5**) on methylation and by preparation of its *N*-acetyl and *O,N*-diacetyl derivatives (*5,6*).

Holadienine (**2**) exhibited vibrations at 1665, 1635, and 1605 cm^{-1} characteristic of a doubly conjugated ketonic group. The homoannular dienone system was also indicated by its UV absorption at 242 nm. The ^{1}H-NMR and mass spectral data, as well as the reduction of holadienine with KBH_4 to conan-3β-en-4-ol (**6**) and with H_2/Pt to conan-5αH,3β-ol (**7**), established its structure as **2** (*6*).



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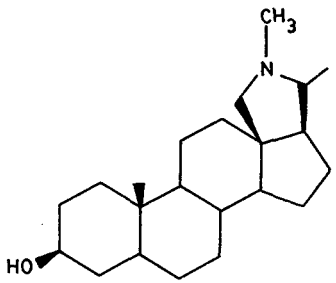

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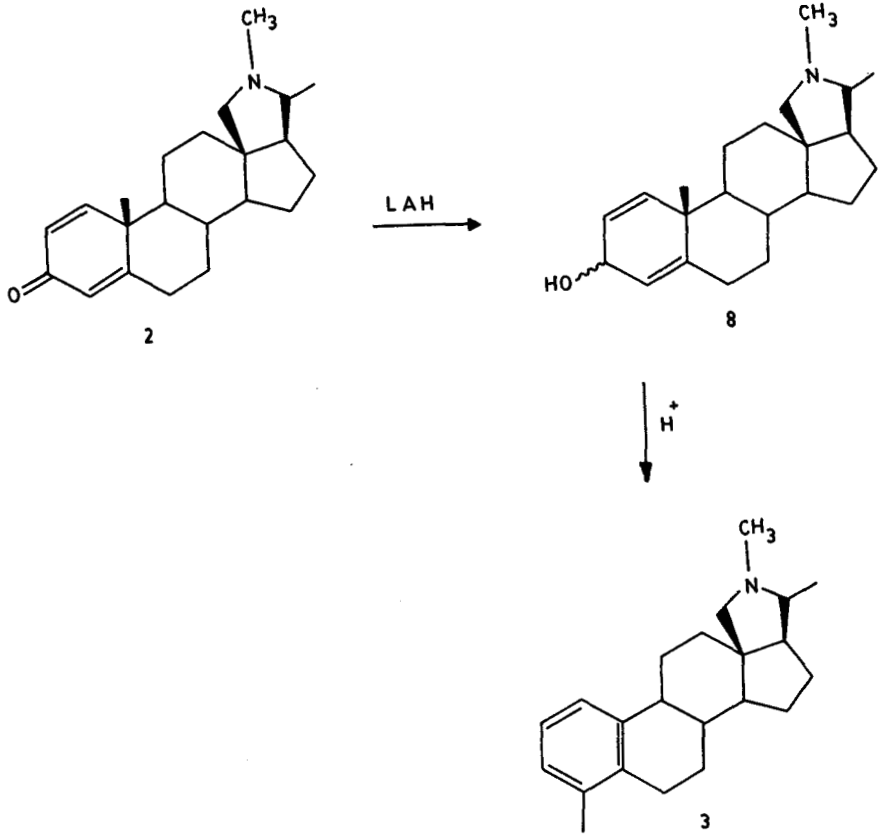
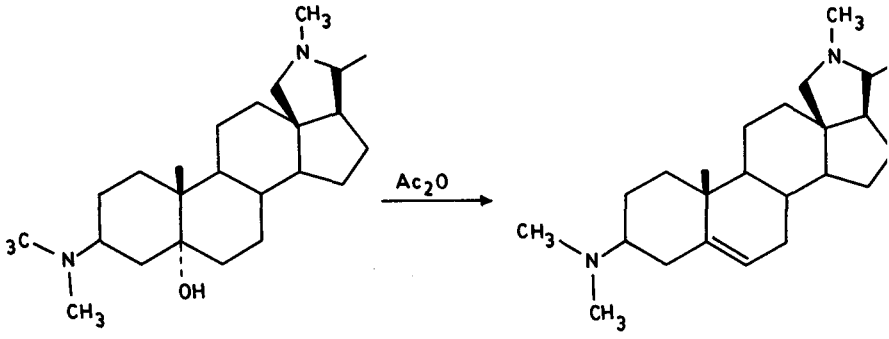
Holaromine (**3**), another alkaloid possessing a conanine skeleton, was suspected to have an aromatic ring from its ^{1}H-NMR spectrum, which indicated the presence of three protons resonating in the region δ6.90–7.20. The absence of a signal for the C-19 methyl group at δ1.00 and the presence of a three-proton

singlet at δ2.19 for a methyl group attached to an aromatic ring supported structure **3** for holaromine. This was confirmed by its preparation from holadienine (**2**) by reduction with LAH to the alcohol **8,** which on treatment with dilute acid afforded holaromine (**3**) (Scheme 1) (*5,6*).

Holaline (**4**) was dibasic and showed an absorption at 3125 cm^{-1} in its IR spectrum for the hydroxyl group. Its ^{1}H-NMR and mass spectra suggested that it was a derivative of conanine. It was readily dehydrated to conessine (**9**) on treatment with acetic anhydride (Scheme 2). The possibility of the –OH group being located at C-6 was eliminated by comparison of holaline with the corresponding 6α-hydroxy and 6β-hydroxy compounds (*5,6*).



SCHEME 1



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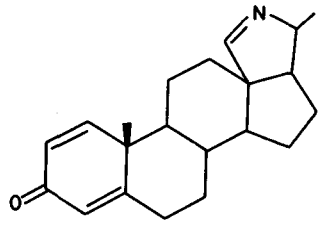
SCHEME 2

Structure **10** was assigned to maingayine, occurring in the bark of *Paravallaris maingayi* (Hook, F.) Kerr (*7*). Absorptions at 1660, 1621, and 1592 cm^{-1} in its IR spectrum indicated the presence of a 1,4-dien-3-one moiety, which was confirmed by the UV absorption at 244 nm and signals at δ7.00 (doublet, $J = 10.0$



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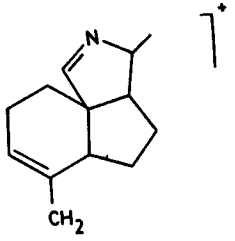
Hz), δ6.18 (double doublet, $J = 10.0$ Hz, approx. 2.0 Hz), and δ6.03 (broad singlet) in the ^{1}H-NMR spectrum. Signals for a tertiary methyl group (δ1.26, singlet), a secondary methyl group (δ1.34, doublet, $J = 7.0$ Hz), a proton α to the nitrogen, and a ketimine proton in the grouping R—CH=N—CH—R^1R^2 (δ7.58, doublet, $J = 3.0$ Hz) clearly established the presence of a pyrroline nucleus. The mass spectrum yielded the molecular ion at *m/e* 309.2082 ($C_{21}H_{27}NO$) and other significant peaks at *m/e* 188 ($C_{13}H_{18}N$, 100%, **11**), 122 ($C_8H_{10}O$ and $C_8H_{12}N$, **12** and **13**), and 121 (C_8H_9O, **14**). Maingayine underwent an acid-catalyzed rearrangement to a phenol.

The IR (absorption at 1660 cm^{-1}), ^{1}H-NMR (δ8.11), and mass (*m/e* 311, M^+-29) spectra of *N*-formylconkurchine (**15**) obtained from the leaves of *H. crassifolia* Pierre showed the presence of an *N*-formyl group. A doublet at δ7.58 ($J = 3.0$ Hz) in its ^{1}H-NMR spectrum indicated the presence of a ketimine



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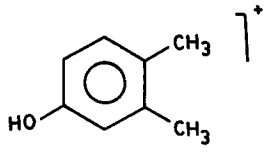

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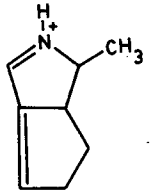

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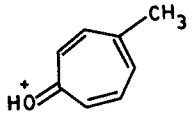
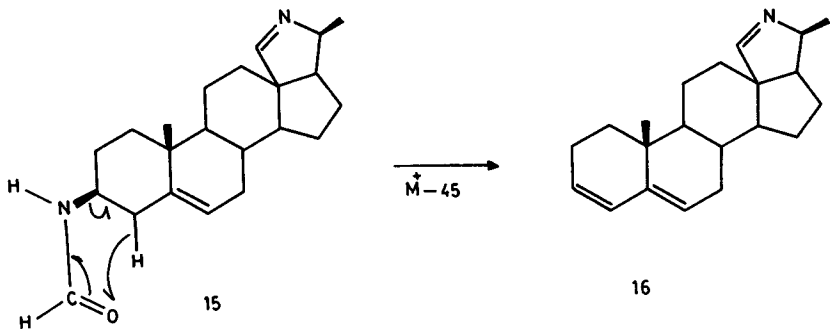


14

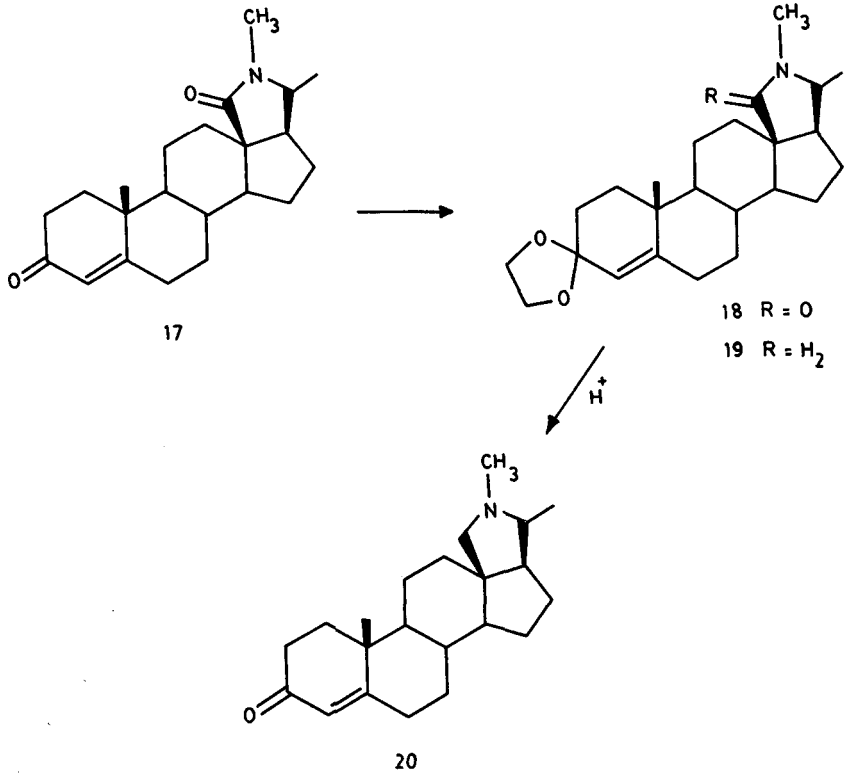
proton. The 19-methyl protons produced a singlet at δ1.05, while the 21-methyl group appeared as a doublet at δ1.35 (J = 7.0 Hz). The C-3 proton resonated as a multiplet at δ3.80. The C-20 proton yielded quartets of double doublets due to coupling with H-21, H-17, and H-18 ($J_{20,21}$ = 7.0 Hz, $J_{20,17}$ = 7.0 Hz, $J_{20,18}$ = 3.0 Hz). The C-6 proton gave a multiplet at δ5.40, while the NH proton gave a multiplet at δ5.71, which disappeared on deuteration (*8*). The mass spectrum showed the molecular ion at *m/e* 345, which readily lost a NH_2CHO fragment to give the ion **16** (see Scheme 3). This spectroscopic evidence led to the assignment of structure **15** for N-formylconkurchine.



SCHEME 3

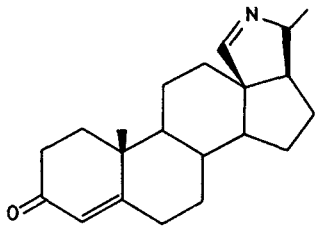
The conanine-type alkaloids malouetamide (**17**) and malouetafrine (**21**) have been obtained from *Malouetia hondelotii* A.D.C. (syn. *M. africana* K. Schum.) along with latifolinine, latifoline, and *N*-acetylconamine (*9*). Malouetamide (**17**) showed an absorption at 1675 cm^{-1} for the lactam carbonyl group. Absorption at 244 nm in the UV spectrum was characteristic of a 3-keto system conjugated with a 4(5)-double bond. The ^{1}H-NMR spectrum showed a singlet at δ2.75 for the N-CH_3 proton α to a carbonyl group, while the olefinic proton was observed at δ5.75. The structure was confirmed by reduction of **17** with LAH to **19** after protection of the keto group as a ketal **18,** which yielded latifolinine (**20**) on acid hydrolysis (Scheme 4) (*9*).

Malouetafrine (**21**) possessed bands at 1675 cm^{-1} for the conjugated C=O group in its IR spectrum and showed absorption at 241 nm, characteristic of the



SCHEME 4

conjugated ketone in the UV spectrum. The ^{1}H-NMR spectrum possessed signals at δ5.77 (assigned to H-4), δ7.60 (C*H* = N), and δ1.36 (doublet, J = 7.0 Hz, 21-CH_3), lacking the *N*-methyl signal at δ2.75 observed in malouetamide. These



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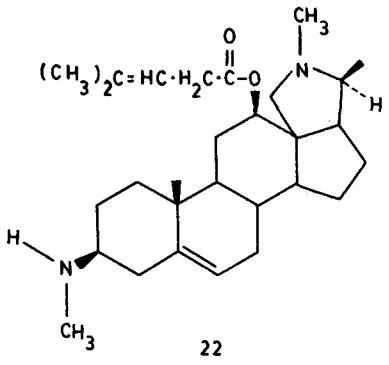
data, along with the mass spectral fragmentation pattern, led to structure **21** for malouetafrine.

Holarrhesine, isolated from the bark of *H. floribunda* together with the known alkaloids holadienine and conessine, was assigned structure **22** on the basis of spectroscopic studies and its conversion on base-catalyzed hydrolysis to holarrheline (**1**) (*10*). The fragment at *m/e* 340 (M^+-$(CH_3)_2C{=}CH{-}CH_2{-}CO_2H$) in the mass spectrum and the presence of signals at δ1.65, δ1.75 (singlets due to two tertiary methyl groups), and multiplets in the range of δ5.22–5.44 (attributed to C-6 proton) in the ^{1}H-NMR spectrum indicated the nature of the acylated side chain.

Holacine (**23**) was obtained from the bark and root of *H. antidysenterica* Linn. along with another alkaloid, holacimine ($C_{22}H_{36}N_2O_3$) (*11,12*). Structure **23** was proposed for holacine largely on spectroscopic evidence.



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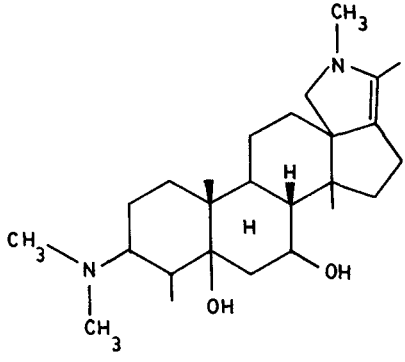

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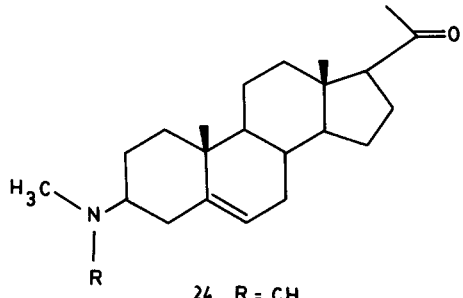
2. The Pregnane Group

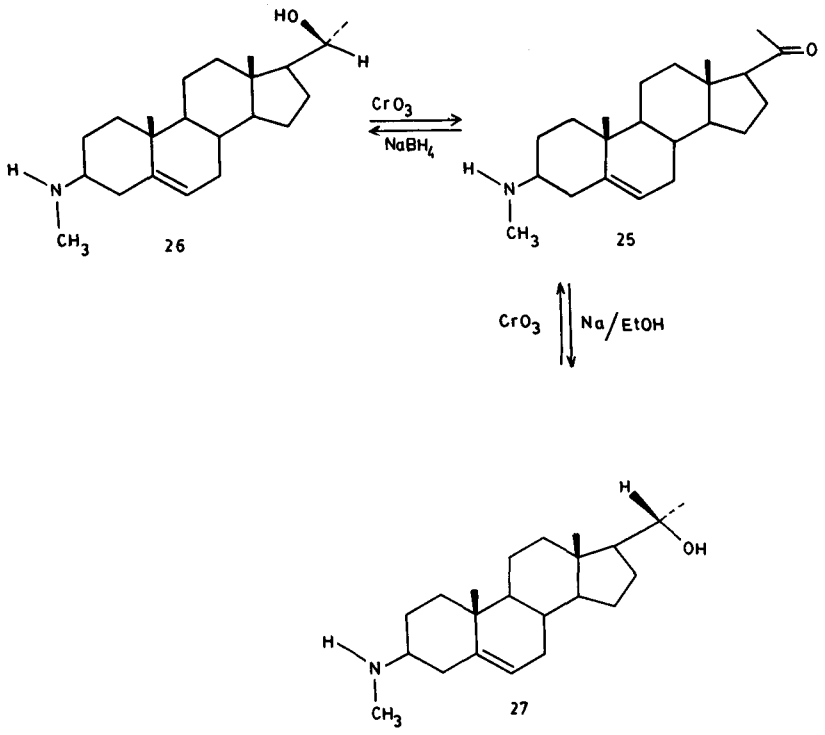
Methylholaphylline, extracted from the leaves of *H. floribunda,* was shown to possess structure **24** based on its spectral behavior (*13*). Its IR spectrum showed bands at 1695 (ketone), 1665, and 800 cm^{-1} (endocyclic double bond). The 18-, 19-, and 21-methyl groups resonated as singlets at δ0.64, δ0.99, and δ2.12. The protons of the *N*-dimethyl group resonated at δ2.29, while the C-6 olefinic proton resonated as a multiplet at δ5.30. The structure of methylholaphylline was confirmed by methylation of holaphylline (**25**) with $HCHO/HCO_2H$ (*14,15*), which gave an identical product.



24 R = CH_3
25 R = H

Holaphyllinol (**26**), obtained from the leaves of *H. floribunda* along with its isomer holaphyllidine (**27**), produced bands at 3410 (NH), 3210 (OH), 1670, and 800 cm^{-1} (C=C). The 18- and 19-methyl groups resonated as singlets at δ0.78 and δ1.01 while the 21-methyl protons resonated as a doublet at δ1.13 (J = 6.3 Hz). The *N*-methyl group yielded a singlet at δ2.46 while the C-6 olefinic proton gave a multiplet at δ5.31. Oxidation of holaphyllinol (**26**) and holaphyllidine (**27**) with CrO_3 produced holaphylline (**25**). Reduction of **25** with $NaBH_4$ gave holaphyllinol (**26**) while its reduction with Na/EtOH gave holaphyllidine (**27**) (Scheme 5) (*13*).

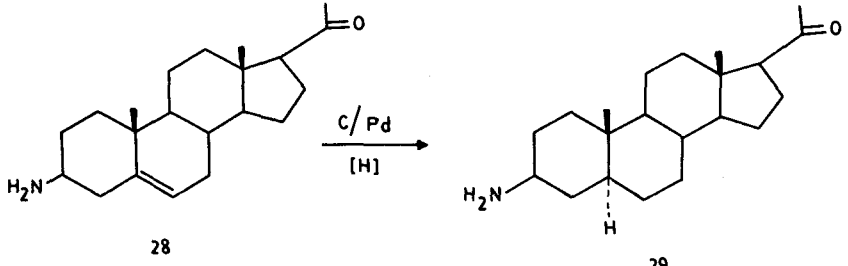
Dihydroholaphyllamine (3β-amino-20-oxo-5α-pregnane, **29**) has been isolated from the leaves of *H. floribunda.* It contains a keto group and a primary amine function. The IR spectrum of the corresponding hydrochloride showed bands at 3400, 2000 (NH) and 1705 cm^{-1} (C=O). Its ^{1}H-NMR spectrum showed singlets, each integrating for three protons, at δ0.61 attributed to the 18-methyl group, δ0.79, attributed to the 19-methyl group, and δ2.09, attributed to the 21-methyl group. A signal at δ1.55, which disappeared on shaking with D_2O, was assigned to the NH_2 group. Its structure was confirmed by catalytic hydrogenation of holaphyllamine (**28**), which gave a product identical to di-



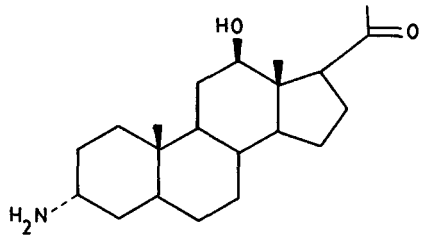
SCHEME 5

hydroholaphyllamine (**29**) (Scheme 6), and by acetylation to the corresponding *N*-acetyl derivative (*13*).

Bokitamine (12β-hydroxyfuntumine, **30**), isolated from the leaves of *H. wulfsbergii* Stapf., showed absorptions in the IR spectrum at 3420, 3240 (OH), 2040 (N-H of $NH_3^+Cl^-$), and 1690 cm^{-1} (C=O) (*16*). The mass spectrum



SCHEME 6



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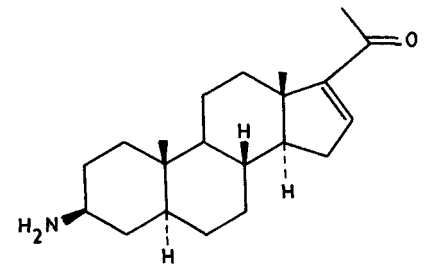
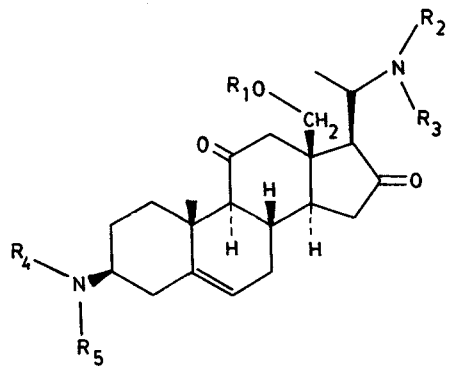
showed peaks at *m/e* 82 and 56 characteristic of 3-aminosteroids not possessing a 5(6)-double bond. The ^{1}H-NMR spectrum indicated that bokitamine was a 5α-pregnane derivative with three-proton singlets at δ0.78 for 19-CH_3 protons, δ0.70 for the 18-CH_3 protons, and δ2.20 for the $COCH_3$ protons. The α (axial) geometry of the amino group at C-3 was established by preparing *N*-acetylbokitamine which showed a signal at δ4.10 for the C-3 (equatorial) proton (*16*). Chromic acid oxidation of **30** resulted in the corresponding keto-compound which gave downfield displacements of the 18-methyl group to δ0.88 and 19-methyl group to δ0.93 in the ^{1}H-NMR spectrum. This showed that the hydroxyl group was present at C-12. The C-12α proton resonated as a double doublet at δ3.43 in bokitamine, and it was shifted downfield to δ4.73 in *O,N*-diacetylbokitamine (*17*). The structure of bokitamine was confirmed through its preparation by microbial hydroxylation of funtumine and by Ruschig deamination to the corresponding 12β-hydroxy-5α-pregnan-3,20-dione (*16,18,19*).

Kisantamine, isolated from the leaves of *H. congolensis*, was shown to possess structure **31.** The structure was assigned mainly on the basis of spectroscopic studies carried out on its *N*-acetyl derivative (*20*).



31

Holarricine (**32**) has been obtained from the bark and seeds of *H. antidysenterica* Linn. (*11,12*). Bromination of **32** to the dibromo derivative established that it possessed a double bond. Methylation of holarricine (**32**) with HCOOH/



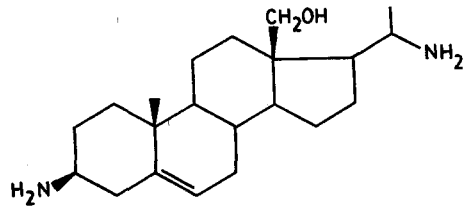
32 $R_1 = R_2 = R_3 = R_4 = R_5 = H$

33 $R_1 = H$, $R_2 = R_3 = R_4 = R_5 = CH_3$

34 $R_1 = COCH_3$, $R_2 = R_3 = R_4 = R_5 = CH_3$

HCHO afforded a tetramethyl derivative **33** which could be acetylated to the monoacetyl derivative **34.** On treatment with nitrous acid it was readily converted to the corresponding nonnitrogenous trihydroxy compound. Clemmensen reduction resulted in the reduction of both carbonyl groups with the formation of holarrhimine (**35**). On the basis of these chemical transformations, as well as spectroscopic data, structure **32** was assigned to holarricine.

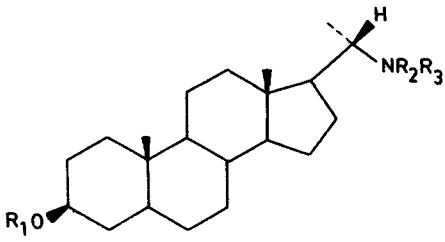
Holacetine (**36**), isolated from the root-bark of *H. antidysenterica* in the form of its *O*-acetyl derivative **37**, showed absorptions at 3280 (–OH) and 1635 cm^{-1} (amide C=O) (*21*). The NMR and mass spectra of *O*-acetylholacetine suggested a 20-acetamidopregnane skeleton for the alkaloid (*22*). The ^{1}H-NMR of *O*-acetylholacetine showed two singlets at δ0.73 and δ1.20 due to 18- and 19-



35

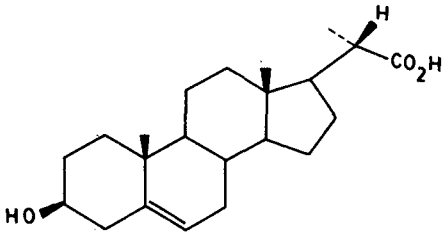
methyl groups, and a three-proton doublet at δ1.93 (J = 6.5 Hz) which was assigned to the 21-methyl group. The acetyl methyl group resonated as a singlet at δ1.93 while H-20 and NH were observed as multiplets at δ5.40. Holacetine possessed an NMR spectrum similar to its *O*-acetyl derivative except that it

showed a signal at δ1.52 for the –OH proton and lacked the singlet at δ2.02 present in the NMR spectrum of *O*-acetylholacetine (**37**). A peak at *m/e* 86 in the mass spectrum of **36** was diagnostic of a 20-acetamidopregnane skeleton. Acid hydrolysis of *O*-acetylholacetine (**37**) gave the corresponding amine **38,** which could also be obtained synthetically from **39** by Curtius degradation followed by catalytic hydrogenation (*23*). Methylation of **38** by Eschweiler–Clarke's method yielded **40** which was oxidized to funtumafrine-C (**41**) (*21*).



36 $R_1 = R_2 = H$, $R_3 = COCH_3$
37 $R_1 = R_3 = COCH_3$, $R_2 = H$
38 $R_1 = R_2 = R_3 = H$
40 $R_1 = H$, $R_2 = R_3 = CH_3$

20-Epiirehdiamine-I (**42**), a dibasic alkaloid possessing a 5(6)-double bond, was isolated from the seeds of *Funtumia elastica*. Its structure was confirmed by its synthesis from progesterone by formation of the dioxime, reduction with sodium in *n*-propanol to the corresponding diamino compound, and methylation with HCOOH/HCHO (*20*).



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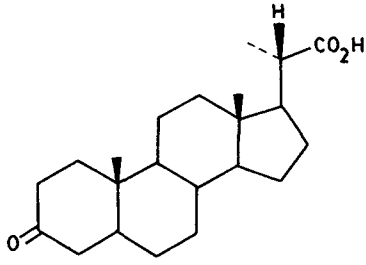

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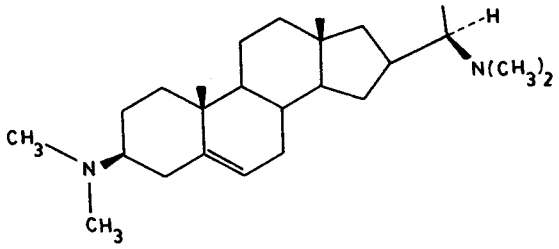
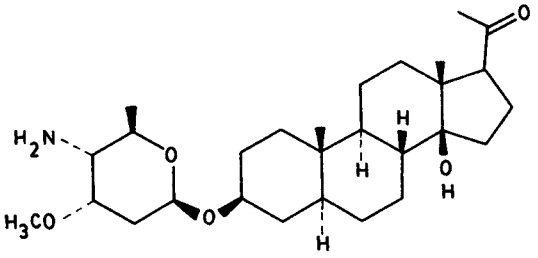


42

N-Desmethylholacurtine (**43**) was isolated from the leaves of *H. curtisii*. Its structure was assigned on the basis of spectroscopic comparison with holacurtine (*24*).

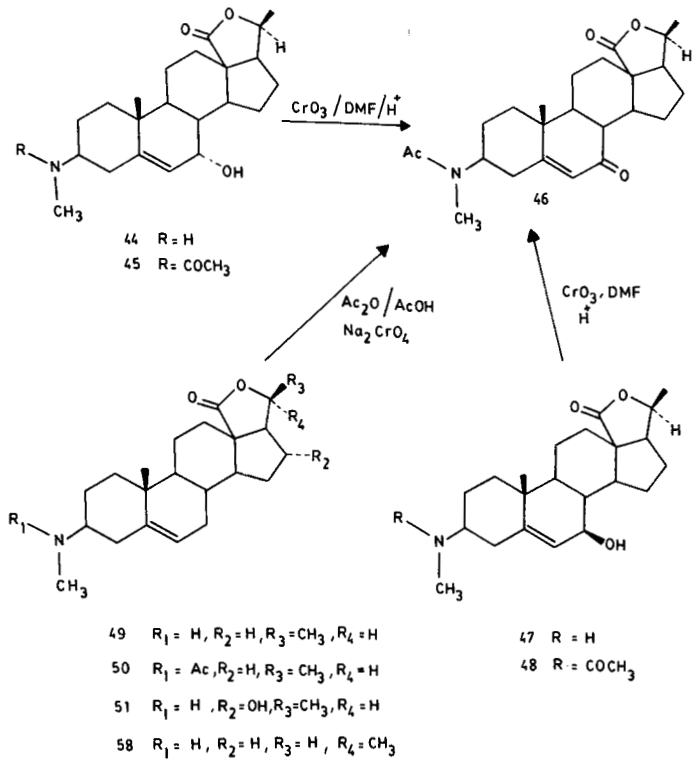


43

3. The Paravallarine Group

7α-Hydroxyparavallarine (**44**) and 7β-hydroxyparavallarine (**47**) have been isolated from *Paravallaris microphylla* (*25,26*). Both substances showed bands at 1750 cm^{-1} for the γ-lactone in their IR spectra. The C-6 proton in the NMR spectrum of 7α-hydroxyparavallarine (**44**) resonated at δ5.60 (J = 5.0 Hz), while the C-7 proton appeared at δ3.91 as a triplet. In 7β-hydroxyparavallarine (**47**) the C-6 proton appeared at δ5.31 (doublet, J = 2.0 Hz) and the C-7 proton at δ3.73. Chromic acid oxidation of the corresponding *N*-acetyl derivatives **45** and **48** yielded the same ketone **46** identical to the substance prepared by oxidation of the *N*-acetyl derivative **50** of paravallarine (**49**). Reduction of the ketone **46** with $NaBH_4$ resulted in the formation of **45** and **48** (Scheme 7). The configurations of the hydroxyl groups at C-7 were assigned on the basis of NMR spectroscopy and difference in optical rotations (*25–28*).

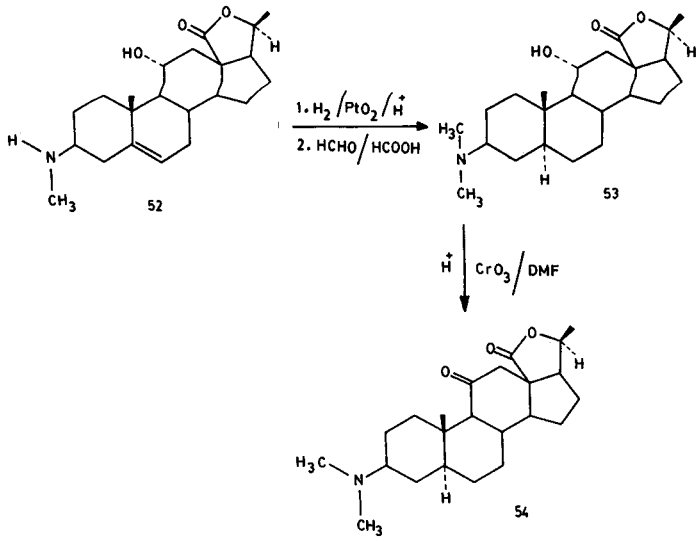
11α-Hydroxyparavallarine (**52**) was also isolated from *Paravallaris micro-*



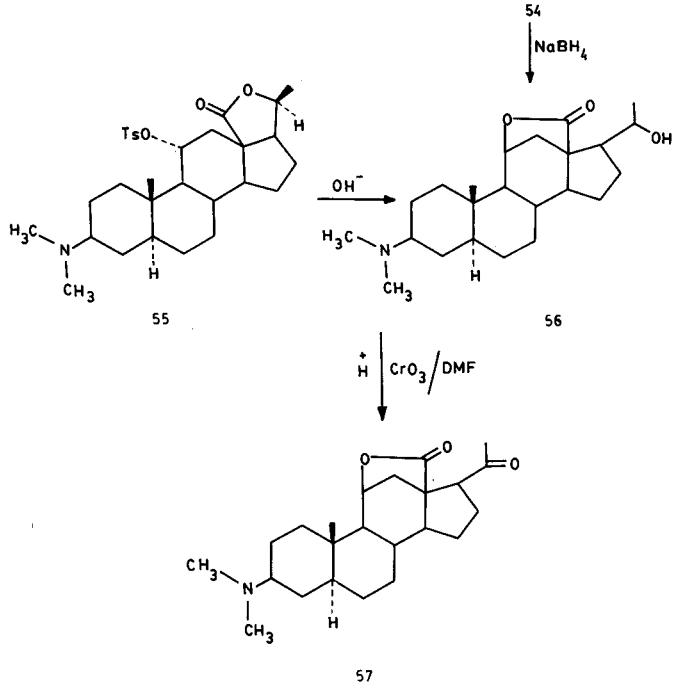
SCHEME 7

phylla together with paravallaridine (**51**) (*25,26*). The mass spectrum showed peaks at *m/e* 359 (M^+), 341 (M^+-18), and 70, and was generally characteristic of 3-*N*-methyl-amino steroids possessing a 5(6)-double bond (*29,30*). Its IR spectrum showed bands at 3240, 3320, and 1750 cm^{-1}, indicating the presence of a lactone ring.

Catalytic hydrogenation and methylation of **52** gave **53,** which could be oxidized to the corresponding ketone **54** as illustrated in Scheme 8. The hydroxyl group was assigned at C-11 in an α-configuration on the basis of its coupling constant in the NMR spectrum (*31*) and a comparison of its properties with other 11α-hydroxy-18→20-steroidal lactones (*32,33*). Reduction of the ketone **54** with sodium borohydride resulted in the alcohol **56** which was also obtainable by hydrolysis of the tosylate **55.** Chromic acid oxidation of **56** yielded the ketone **57** (Scheme 9). The stereochemistry at C-3 of the methylamino functionality in **52** was assigned as β on the basis of the NMR of the *N*-dimethylamino derivative **53,** which showed the equivalence of the dimethylamino protons. The stereochemistry at C-20 was determined as 'S' on the basis of the coupling constants



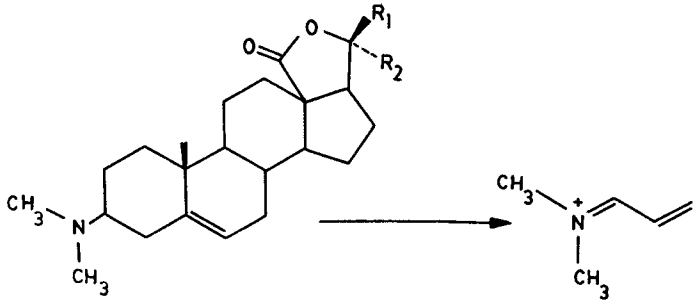
SCHEME 8



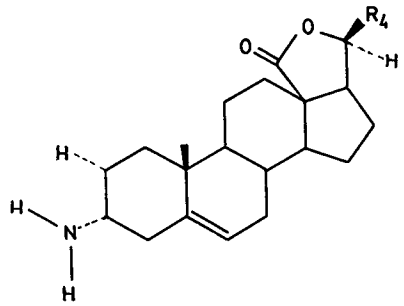
SCHEME 9

of the proton at C-20, which resonated as a doublet of quartets ($J_{20,21}$ = 6.5 Hz, $J_{20,17}$ = 4.5 Hz) (*34*).

Another plant of the Apocynaceae family, *Kibatalia ofitingensis* (Elm.) Woods has yielded paravallarine (**49**), 20-epiparavallarine (**58**), the corresponding *N*-methyl derivatives **59** and **60**, 2α-hydroxy-*N*-methyl-20-epiparavallarine (**61**), and gitingensine (**62**) (*35,36*). Both *N*-methylparavallarine (**59**) and 20-epi-*N*-methylparavallarine (**60**) gave bands in the IR spectrum at 2790 (*N,N*-dimethyl), 1750 (γ-lactone), 1670, 840, and 805 cm^{-1} (trisubstituted olefin). In the mass spectrum both gave peaks at *m/e* 357 (M^+), with the base peak at *m/e* 84 formed characteristically by fragmentation of ring A as presented in Scheme 10 (*35,37*). The NMR spectrum of both diastereoisomers showed singlets for the C-19 methyl protons at δ1.08, *N,N*-dimethyl protons at δ2.30, and a multiplet for H-6 at δ5.35. In *N*-methylparavallarine the 21-methyl protons resonated as a



59 $R_1 = CH_3$ $R_2 = H$
60 $R_1 = H$ $R_2 = CH_3$

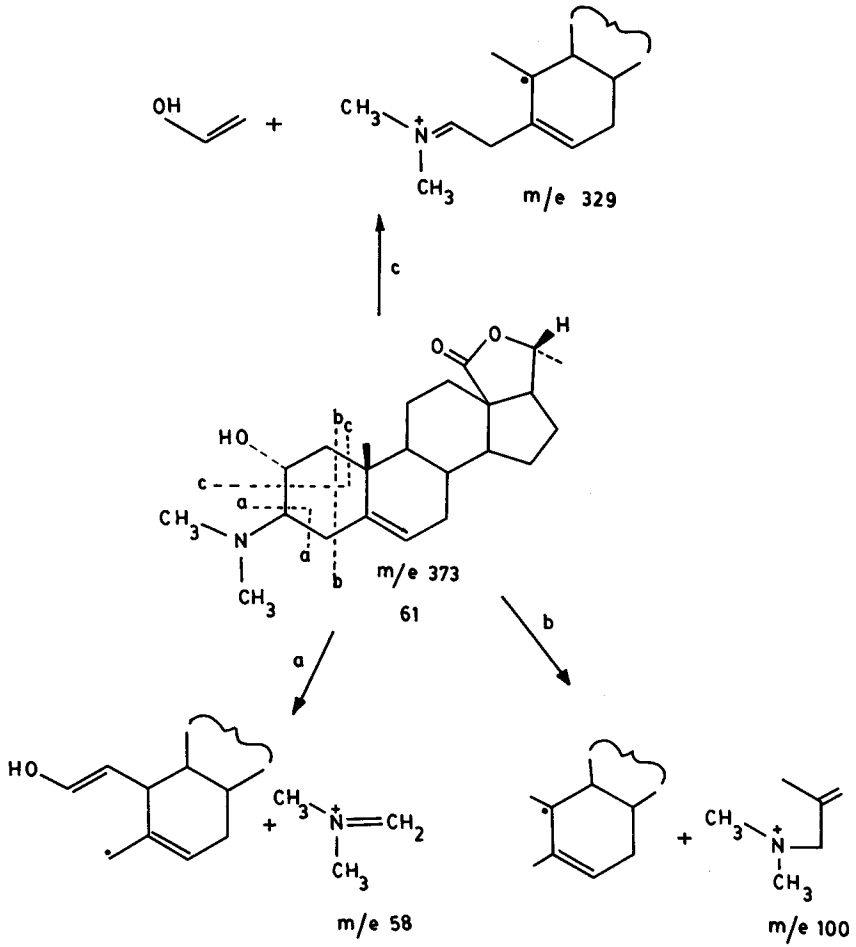


62

SCHEME 10

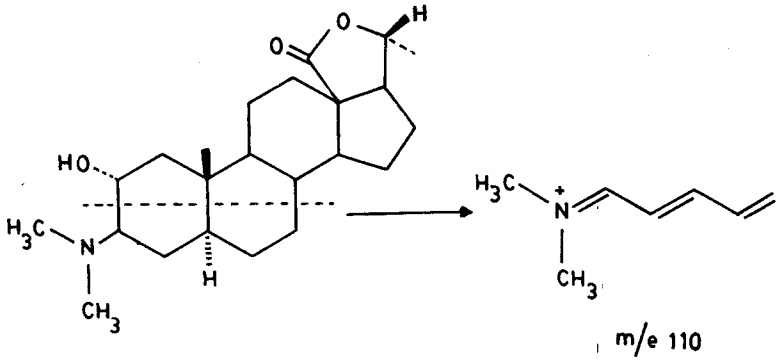
doublet at δ1.36 (J = 6.5 Hz) and H-20 as a multiplet at δ4.65 (J_1 = 6.5 Hz, J_2 = 4.5 Hz). The 21-methyl protons resonated as a doublet at δ1.38 (J = 6.5 Hz) in the 20-epimer, while H-20 appeared at δ4.35 (J_1 = 6.5 Hz, J_2 = 2.0 Hz), thus allowing differentiation of the two (*35*).

2α-Hydroxy-*N*-methyl-20-epiparavallarine was assigned structure **61** on the basis of spectroscopic evidence. Its ^{1}H-NMR afforded bands at 3300 (–OH), 2790 (*N,N*-dimethyl), 1750 (lactone C=O), 1670, 840, and 800 cm^{-1} (trisubstituted olefin) (*35*). The mass spectrum showed M$^+$ at *m/e* 373 along with fragments at *m/e* 58, 100, and 329 indicative of a 2-hydroxy-3-*N,N*-dimethylamino-5-pregnene derivative (Scheme 11) (*38*). Catalytic reduction of the double



SCHEME 11

bond gave the corresponding dihydroderivative which showed the base peak at *m/e* 110, characteristic for 2-hydroxy-*N,N*-dimethylamino-pregnane derivatives (Scheme 12). The ^{1}H-NMR spectrum was close to that reported for 20-epi-*N*-methylparavallarine (**60**), showing a three-proton singlet at δ1.13 for the 19-methyl, a six-proton singlet at δ2.33 for the dimethylamino function, a multiplet at δ5.35 for H-6, a doublet at δ1.39 (J = 6.5 Hz) for H-20, and a multiplet for the –OH group at δ3.40. The α-configuration of the hydroxyl group at C-2 and



SCHEME 12

the β-configuration for the dimethylamino group at C-3 was assigned on the basis of a comparison of the differences in positions of the bonded and free –OH vibrations in the IR spectrum of **61** with various 2-hydroxy-3-dimethylamino-pregnane derivatives (Table I) (*35*).

The stereochemistry of gitingensine (**62**) has been determined as '3R' and '20S' (*39*). The stereochemistry at C-3 was established by preparing the dimedone derivative of **62** and comparing the signs of its Cotton effects with the dimedone derivatives of 3α-amino and 3β-amino dimedone derivatives (*36*). Dimedone derivatives are optically active in an asymmetric environment and the sign of the Cotton effect reflects the configuration of the asymmetric center adjacent to the chromophore (*40*). The NMR spectrum also supported the 3α-configuration, since H-3 in the dimedone derivative of gitingensine resonated in the region δ3.50–3.81, indicating its β (equatorial) orientation [in an α (axial)

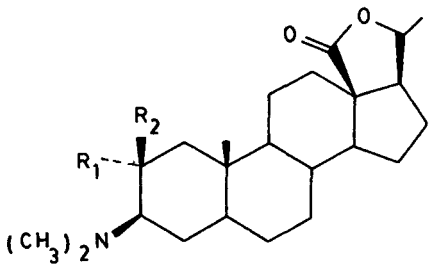
TABLE I
IR SPECTRA OF COMPOUNDS SHOWING FREE OR BONDED OH VIBRATIONS

				OH		
	OH	$N(CH_3)_2$	Series	νBonded	νFree	$\Delta\nu$
Cis isomer	2α	3α	5α-pregnane	3360	3630	270
	2β	3β	5α-pregnane	3456	3635	179
Trans isomer	2β	3α	5α-pregnane	3465	3633	168
	2α	3β	5α-pregnane	3493	3630	137
Kurchiiline	2α	3β	5-pregnene	3480	3635	155
(**33**)	2α	3β	5-pregnene	3496	3634	138

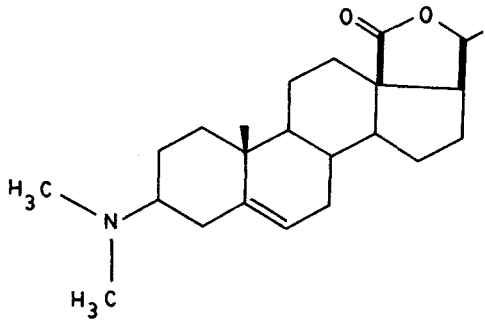
orientation, it was found to resonate in the region δ3.00–3.40]. The stereochemistry at C-20 was assigned as β on the basis of a positive Cotton effect observed at 217 nm and comparison with the Cotton effects of corresponding compounds with a known stereochemistry at C-20 (*36*).

Lanitine (**63**) (2α-hydroxy-*N*-methylparavallarine) and its 2β-isomer **64** have been isolated from the stem bark of *Kibatalia gitingensis* (*41*).

20-Epikibataline (**65**) was found to be the major constituent of the leaves of *Paravallaris microphylla* Pierre (*42*). A peak at *m/e* 84 in the mass spectrum corresponding to the ion $CH_2{=}CH{-}CH{=}N^+(CH_3)_2$ indicated the presence of a dimethylamino group at C-20. The '*R*' configuration of C-20 was proved by the NMR spectrum of 20-epikibataline, which exhibited a high field doublet at δ4.29 due to H-20. The molecular structure was confirmed by X-ray analysis.



63 $R_1 = OH, R_2 = H$
64 $R_1 = H, R_2 = OH$



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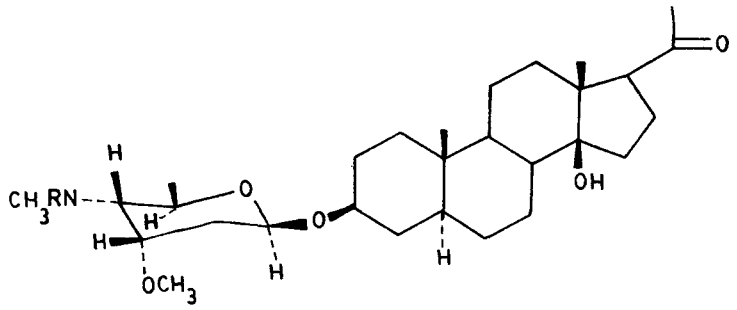


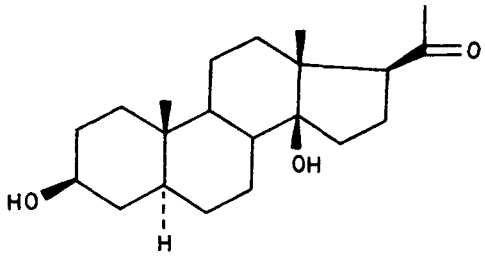
66 R = H

67 R = $COCH_3$

4. Amino-glyco-steroids from Apocynaceae

Amino-glyco-steroids represent a new class of glyco-alkaloids in which an amino-sugar is linked to a steroidal unit. When the amino-sugar is attached to a cardenolide, then the substances are known as amino-glyco-cardenolides.

The first amino-glyco-steroid, holacurtine, was isolated from the leaves of *Holarrhena curtisii* (*43*); its structure was shown to be **66** on the basis of its NMR, mass spectral data, and chemical degradation. Methanolysis of *N*-acetylholacurtine (**67**) produced the corresponding genin, holadiolone (**68**), and the amino-sugar, obtained as the β-methyl glycoside **69.** The structure of holadiolone (**68**) was established on the basis of spectral data and comparison with an authentic sample. The structure of the amino-sugar was elucidated on the basis of its NMR spectrum and confirmed by synthesis from β-methylglycoside of D-



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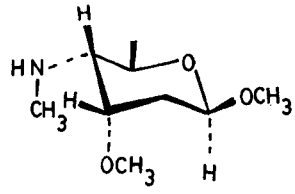
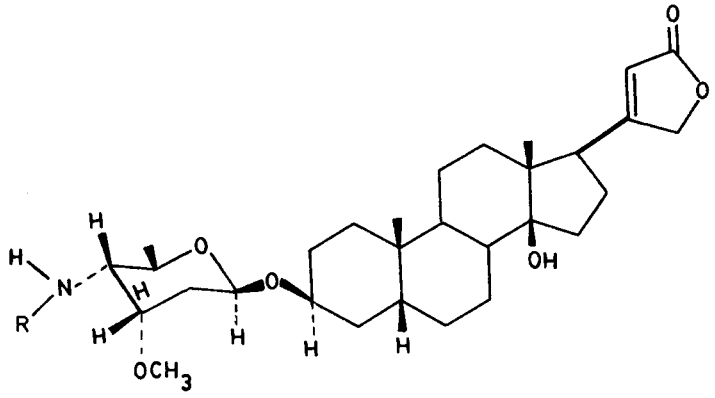


69

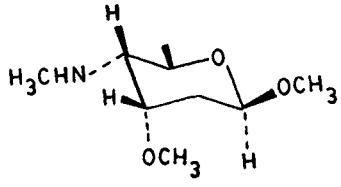
cymarose. The amino group at 4-position was introduced through reduction of the corresponding azide (*44*).

The first amino-glyco-cardenolide, mitiphylline (**70**), was isolated from the leaves of *H. mitis* (*45*). Methanolysis of mitiphylline in presence of acid gave digitoxigenin and the amino-sugar **71.** An absorption band at 1740 cm^{-1} in the IR spectrum of **70** revealed the presence of the lactone moiety. The ^{1}H-NMR of the steroid portion showed two three-proton singlets at δ0.87 and δ0.93. The C-21 methylene protons appeared as a multiplet at δ4.84. This downfield shift was attributed to the presence of oxygen of the lactone ring on that carbon. A triplet at δ5.83 was assigned to H-22 olefinic proton.



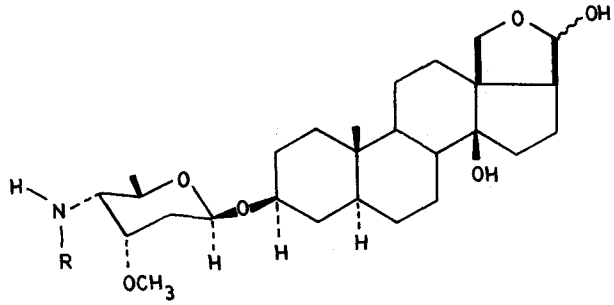
70 R = CH_3

81 R = H



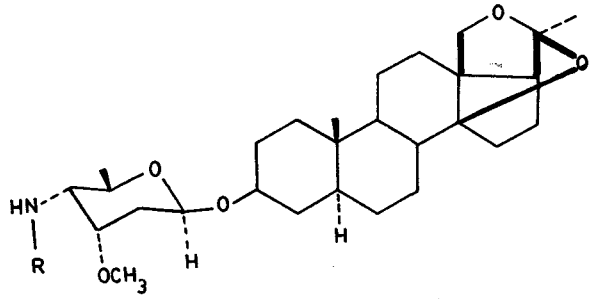
71

Two amino-glyco-steroids, holantosine-A (**72**) and holantosine-B (**73**) have been extracted from *H. antidysenterica* leaves (*46*). When treated with hydrogen chloride in methanol, they gave the β-methylglycoside of D-holosamine **74.** Acetylation of holantosine-A and holantosine-B produced the corresponding *N*-acetyl derivatives **75** and **76.**



72 R = H

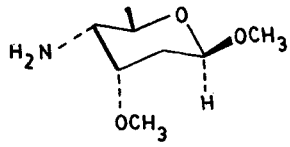
75 R = Ac



73 R = H

76 R = Ac

Another amino-glyco-steroid, holantosine-C (**77**) has been isolated from *H. antidysenterica* (*47*). Its structure was established by NMR studies of the *N*-acetylated derivative **78.** Two three-proton singlets were observed and assigned to the 19- and 21-methyl groups. Two doublets at δ3.53 and δ4.61 assigned to the C-18 methylene protons indicated the presence of an ether linkage.



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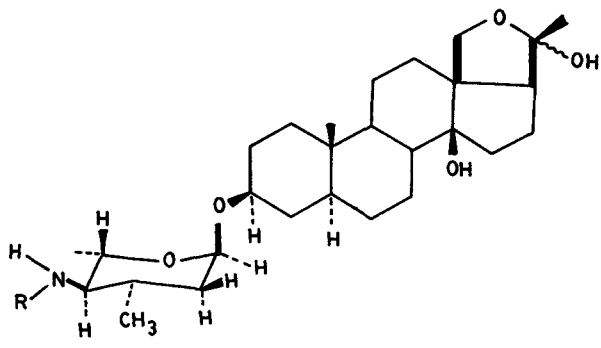
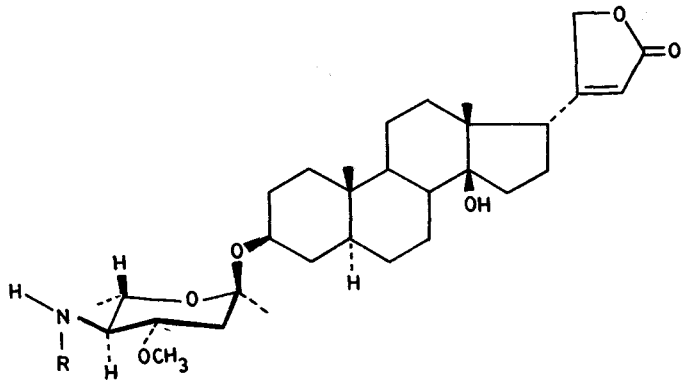


77 R = H

78 R = Ac

Holarosine-A (**79**), an amino-glyco-cardenolide, was also isolated from the leaves of *H. antidysenterica* (*47*). It is the L-glycoside and was extracted as *N*-acetylholarosine-A (*80*). The γ-lactone moiety was indicated by absorption at 1750 cm^{-1} in the IR spectrum and signals at δ4.78 (m, H-21) and δ5.9 (m, H-22) in the ^{1}H-NMR spectrum.

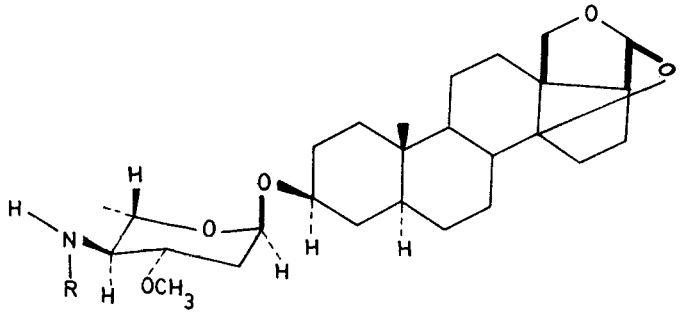


79 R = H

80 R = Ac

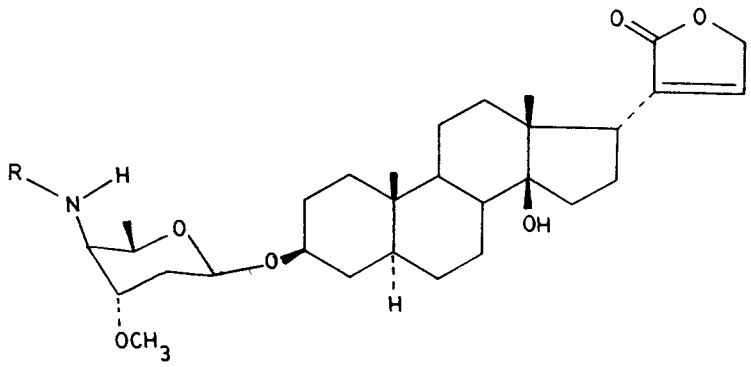
N-Demethylmitiphylline (**81**), extracted from the leaves of *H. mitis* (*48*), produced an NMR spectrum similar to that of mitiphilline. The only difference was the absence of a singlet at δ2.35 observed due to the *N*-methyl group in mitiphylline. The peak at *m/e* 87 in the mass spectrum of mitiphylline, corresponding to the ion $CH_3\overset{+}{N}H{-}CH{=}CH{-}OCH_3$, now appeared at *m/e* 73, which was in accordance with the ion $\overset{+}{N}H_2{-}CH{=}CH{-}OCH_3$ (*45*).

Holantosine-D (**82**) was also isolated from the leaves of *H. antidysenterica* (*47*). The alkaloid was acetylated to *N*-acetylholantosine-D (**83**) and its structure was determined by comparing its spectral data with that of *N*-acetylholantosines A and B (*46*).



82 R = H

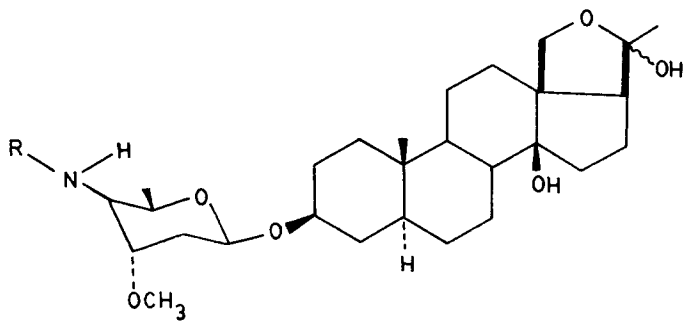
83 R = Ac



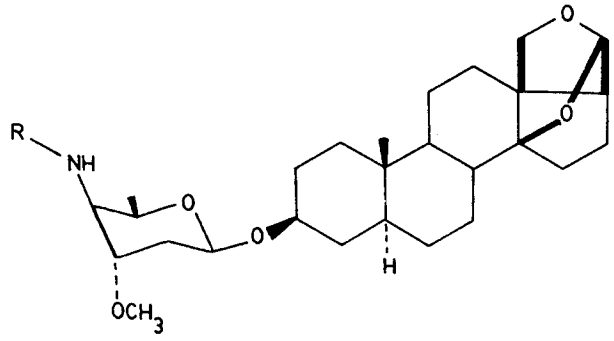
84 R = H

87 R = Ac

Amino-glyco-steroids holarosine-B (**84**), and holantosines E (**85**) and F (**86**) have been extracted from the leaves of *H. antidysenterica* (*49*). Holantosines E and F are the β-D-glycosides of an amino sugar, D-holacosamine, with holantogenin or anhydroholantogenin. Holantosine-E is isomeric to holantosine-A,



85 R = H
88 R = Ac

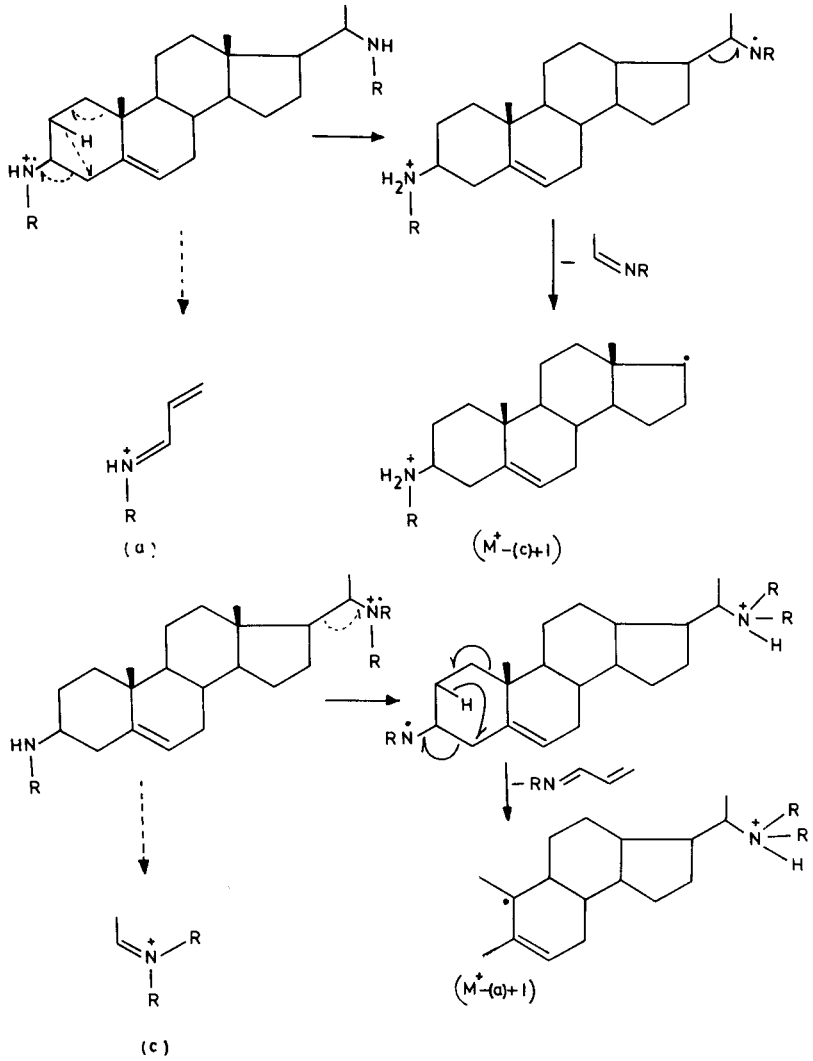


86 R = H
89 R = Ac

while holantosine-F is isomeric to holantosine-B (*46*). Spectroscopic studies of the *N*-acetylated products of holarosine-B (**87**), holantosine-E (**88**) and holantosine-F (**89**) led to the establishment of the structures of the parent bases.

5. Mass Spectrometry

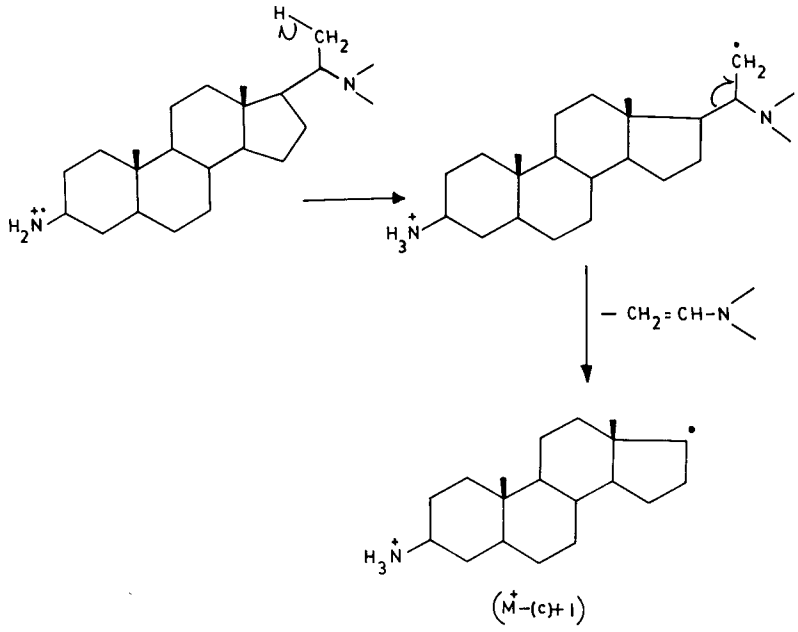
The fragmentation of steroidal diamines, which bear two nitrogen-containing functions in remote positions, was shown to be initiated by rearrangement of a hydrogen from one nitrogen to the other and produced the ions $M^+ - (c) + 1$ and $M^+ - (a) + 1$, in addition to the fragments (a) (characteristic of 3-amino steroids) and (c) (characteristic of 20-amino steroids) (*50*). The mechanism for their



SCHEME 13

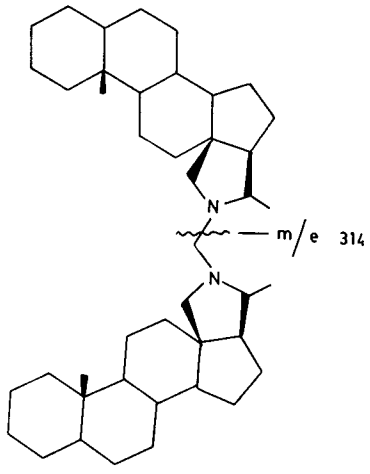
formation is given in Scheme 13. Peaks corresponding to these fragments are also observed in the mass spectra of the conanine series of alkaloids. In C-20 tertiary amines, hydrogen transfer takes place from the 21-methyl group leading to the ion corresponding to $M^+-(c)+1$ fragment (Scheme 14).

The mass spectral fragmentation pattern of the dimer **90,** a reduction product of the cynamide of conamine, has been investigated (*51*). The fragment at *m/e* 314 was obtained by heterolytic cleavage of the C—N bond.



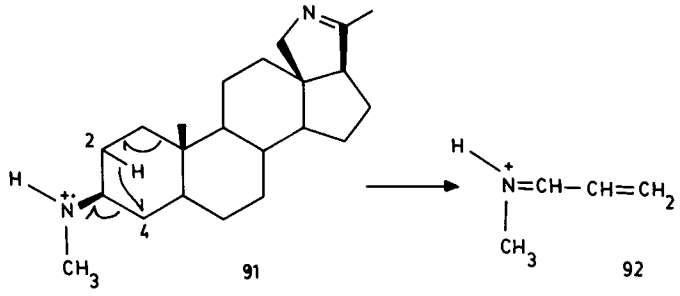
SCHEME 14

The mechanism of formation of ion **92,** characteristic of 3-amino-steroids, was determined by [2-^{2}H]- and [4-^{2}H]-labeling experiments with 3β-*N*-demeth-



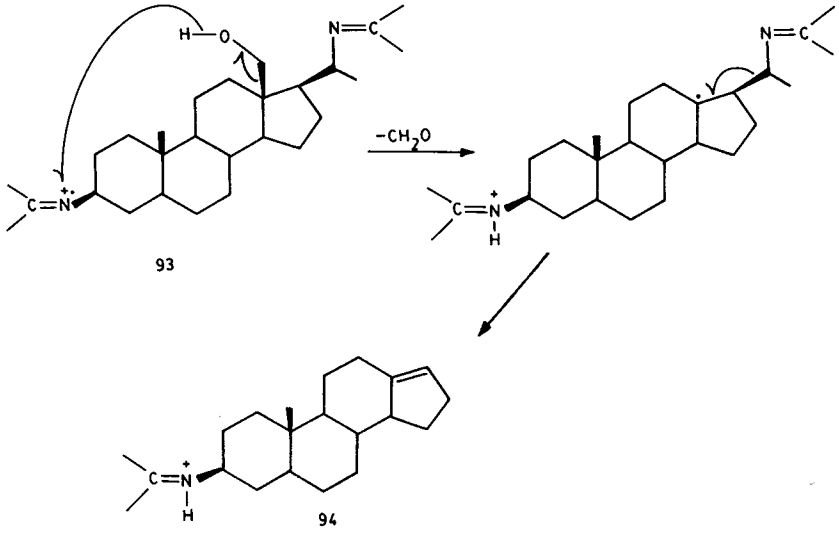
90

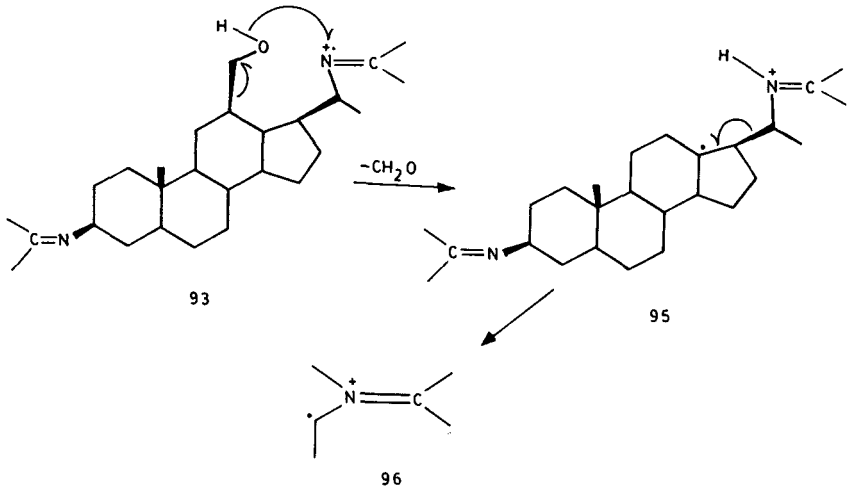
yl-5α-20(*N*)-conene (**91**) (*52*). It was demonstrated that hydrogen transfer occurred from C-2 to C-4, leading to the ion **92** (Scheme 15).



SCHEME 15

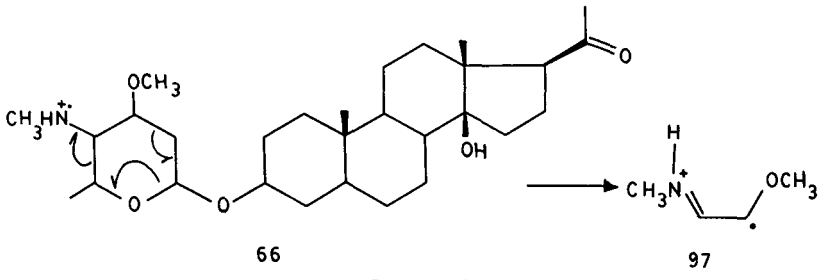
The mass fragmentation of steroidal amines **93** possessing a hydroxyl group at C-18 is initiated by transfer of the hydroxylic hydrogen to the nitrogen of the amino group, resulting in the formation of ions **94, 95,** and **96** corresponding to the *m/e* 300, *m/e* M^+-30, and *m/e* 85 fragments as illustrated in Scheme 16 (*53*).



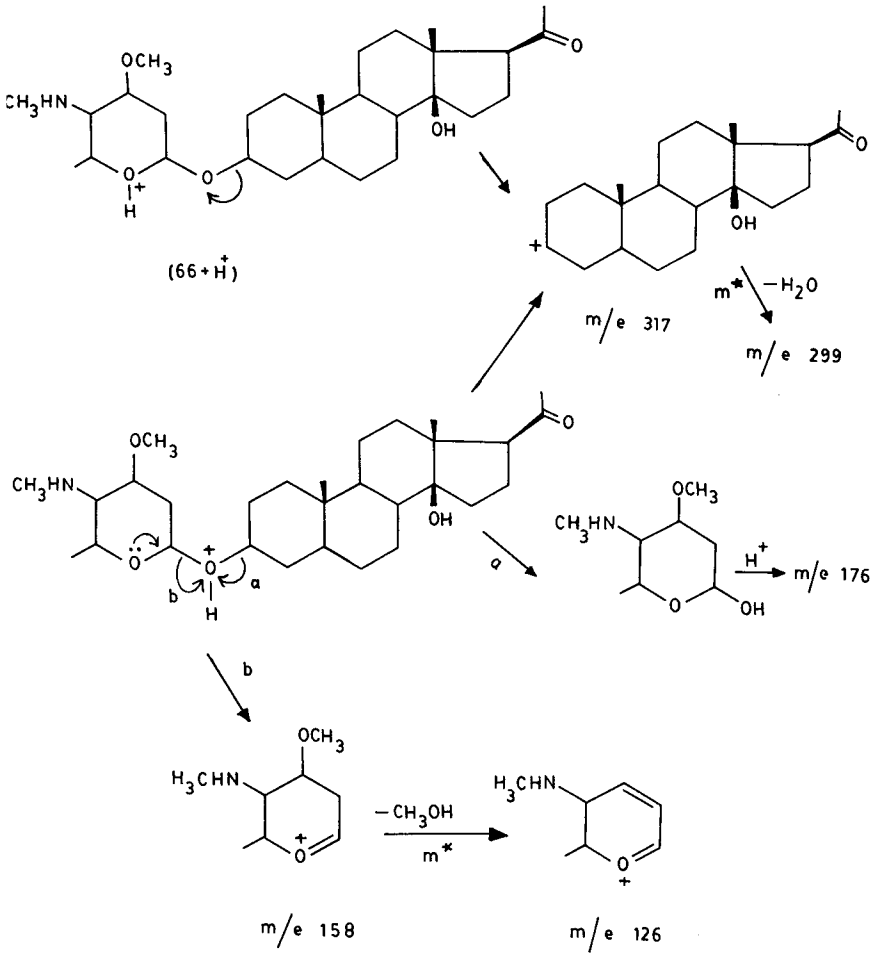


SCHEME 16

Electron-impact and chemical-ionization mass spectroscopic techniques were applied to holacurtine (**66**) (*54*). The base peak was observed at *m/e* 87, corresponding to the ion **97,** suggesting the close proximity of the ether and amino groups (Scheme 17). The chemical-ionization mass spectrum showed fragments at *m/e* 317, 299, 176, 158, and 126; plausible mechanisms for their formation are shown in Scheme 18.

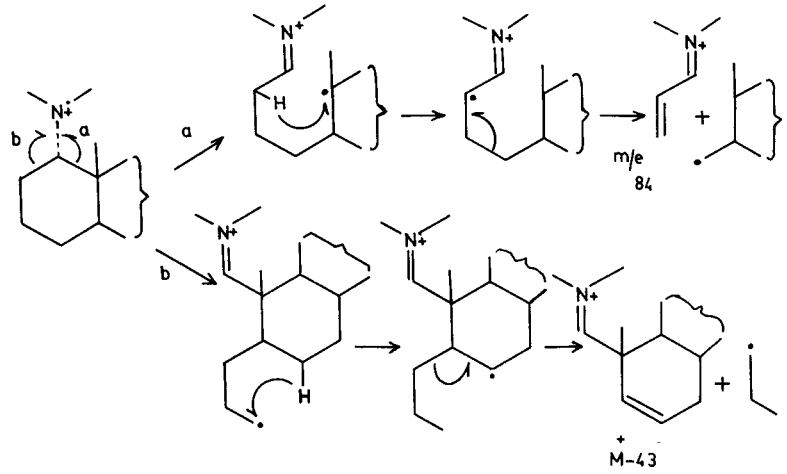


SCHEME 17



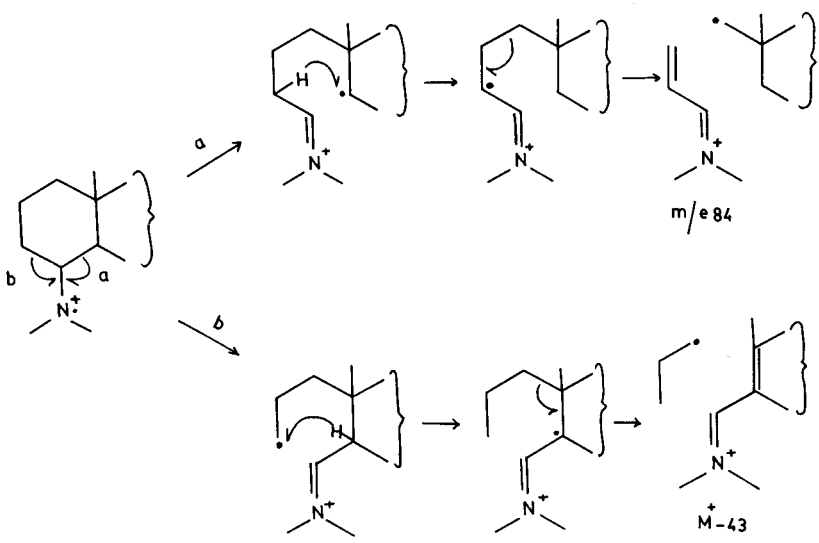
SCHEME 18

A comparison of the fragmentation pattern of conanine with heteroconanine derivatives showed that the relative intensity of fragments at *m/e* 71 and M^+-15 was different in the two cases. In heteroconanine derivatives the M^+-15 peak, corresponding to the loss of 21-methyl group, was more intense as compared to the peak at *m/e* 71, attributed to the fragmentation of pyrrolidine ring; in conanine derivatives the two fragments were of equal intensity, suggesting that both processes occurred to a comparable extent (55).



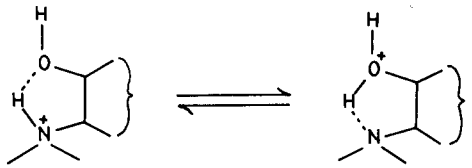
SCHEME 19

Mass spectra of deuterated 1- and 4-dimethylamino steroids, showing peaks at *m/e* 85 and M^+-44 (which appeared at *m/e* 84 and M^+-43 in nondeuterated compounds), indicated that a hydrogen–deuterium exchange occurred during the fragmentation process (*56*). Mechanisms proposed for the formation of these ions are presented in Schemes 19 and 20.



SCHEME 20

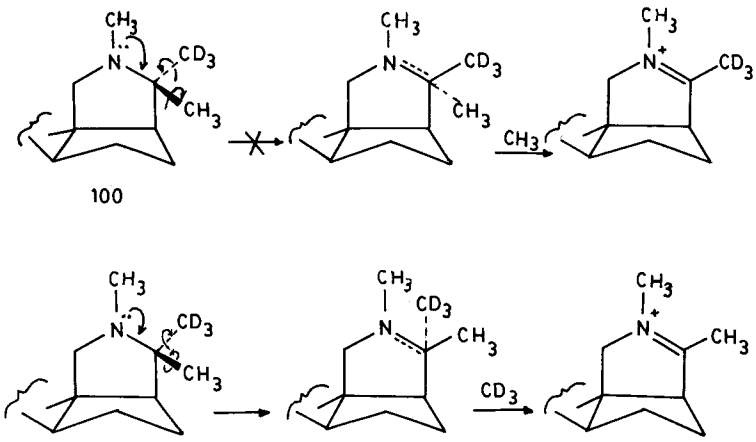
The configuration and conformation of steroidal amino alcohols may be ascertained by chemical ionization mass spectrometry. Studies of the chemical ionization mass spectra of several steroidal amino alcohols have shown that compounds which exhibit free OH bands in their IR spectra exhibit base peaks at *m/e* M^+-1 and an intense peak at MH^+-18, corresponding to the loss of water from the MH^+ ion. No peak was observed at *m/e* (MH^+-18) in the mass spectra of compounds showing absorptions due to bonded OH group in the IR spectra. Formation of the stable complex (**98** ⇌ **99**) could explain this difference in the fragmentation pattern, since the complex **99** is not prone to MH^+-18 fragmentation due to hydrogen bonding (Scheme 21) (*57*).



98 **99**

SCHEME 21

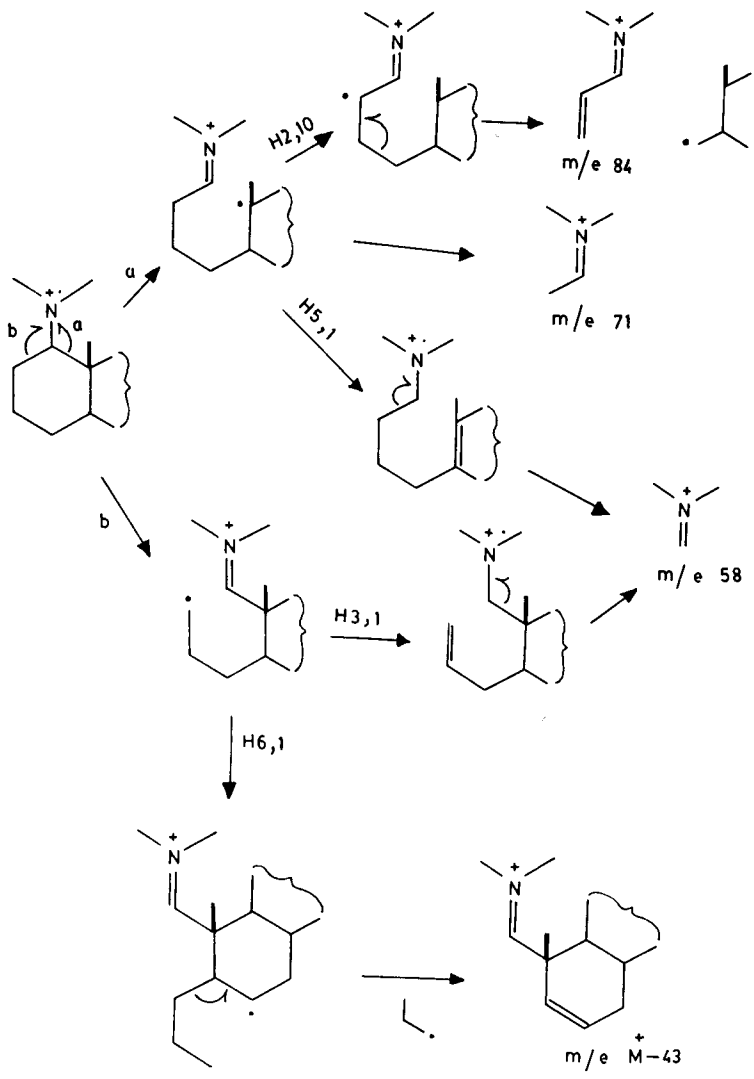
The chemical-ionization mass spectra of 20-methylconanine (**100**), in which the 20-methyl group had been deuterated, showed that it is the 20α-methyl group which is lost during fragmentation, giving rise to the M^+-15 fragment. This



100

SCHEME 22

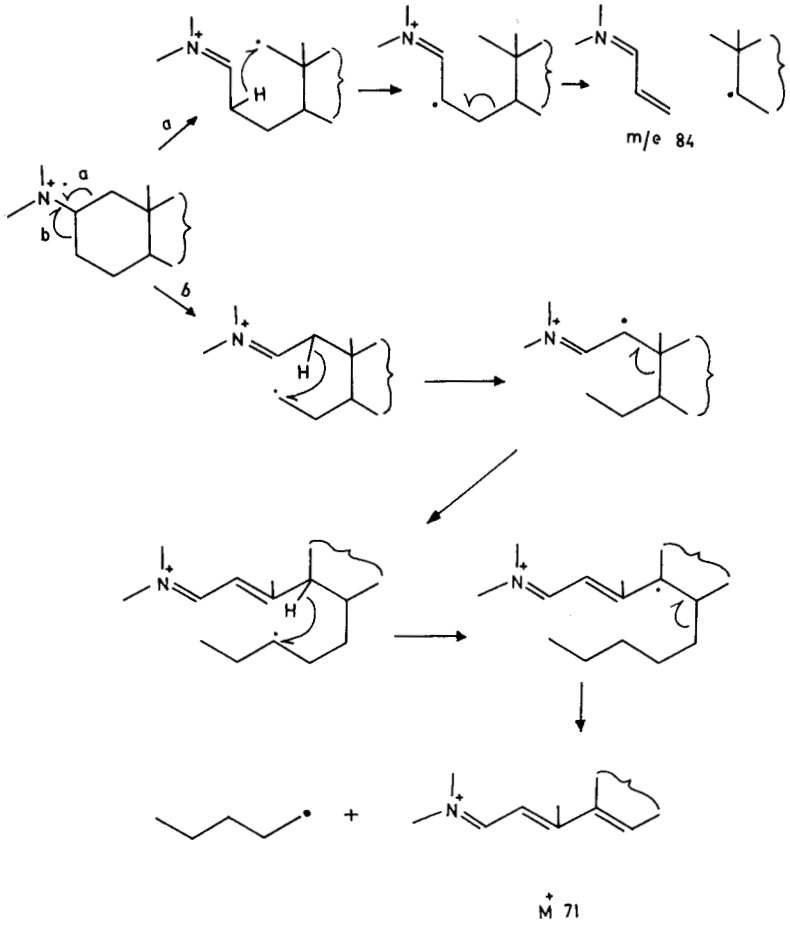
stereoselectivity can be rationalized in terms of the energy of transition states drawn for α- or β-fragmentation (*58*). As shown in Scheme 22, removal of the 21-methyl group results in the transformation of C-20 from a tetragonal to a trigonal carbon. The transition state requiring removal of α-methyl group is energetically more favorable as compared to that involving the loss of the β-methyl group.



SCHEME 23

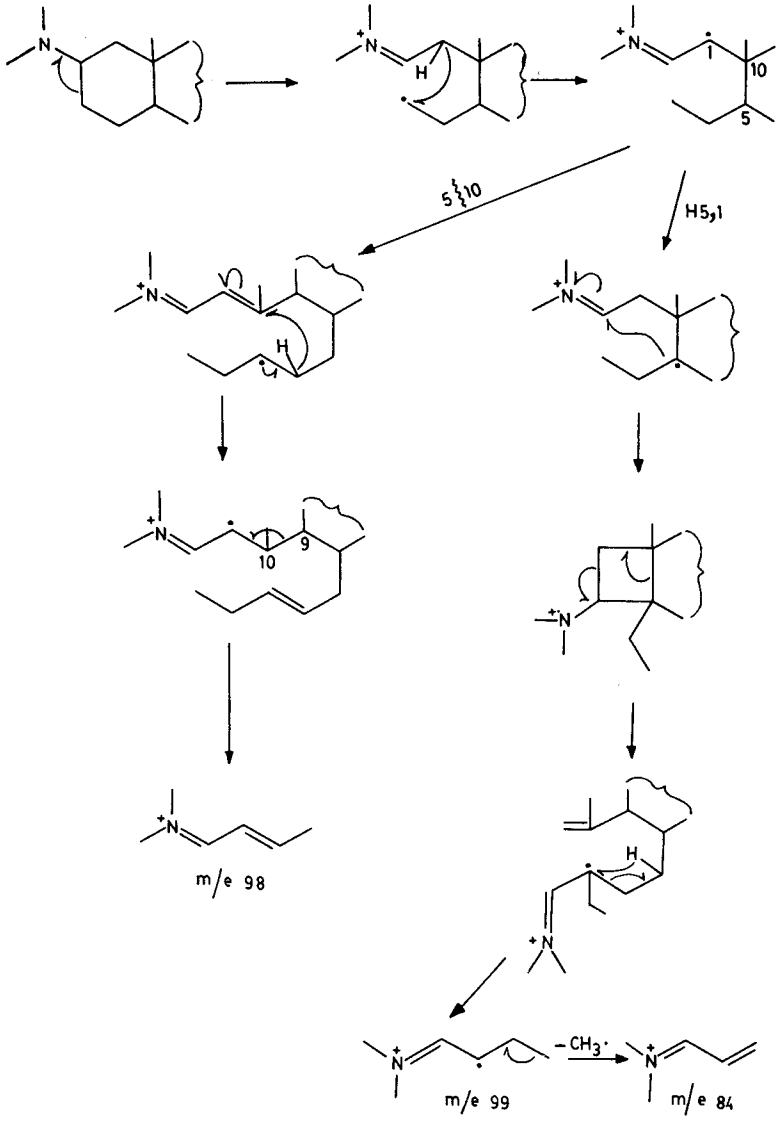
Mass spectral studies of 1-amino steroids have shown that the configuration of the amino group does not affect the fragmentation pattern of these steroids (*59*). The formation of various mass fragments of 1-amino steroids is shown in Scheme 23.

A comparative study of the mass fragmentation of 2-dimethylaminosteroids with that of 2β-dimethylamino-3-α-hydroxy-pregnane helped in the determination of the mechanism for the formation of the principal fragment ions (*60*). The mass spectrum of 2-aminosteroids showed a characteristic fragment at *m/e* M^+-71 with the base peak at *m/e* 84. It was proposed that for the formation of the ion at *m/e* 84, the C-1 to C-2 bond is first broken and hydrogen transfer then takes place from C-3 to C-1 while formation of ion at M^+-71 requires the initial rupture of C-2 to C-3 bond, as illustrated in Scheme 24. Deuterium labeling at



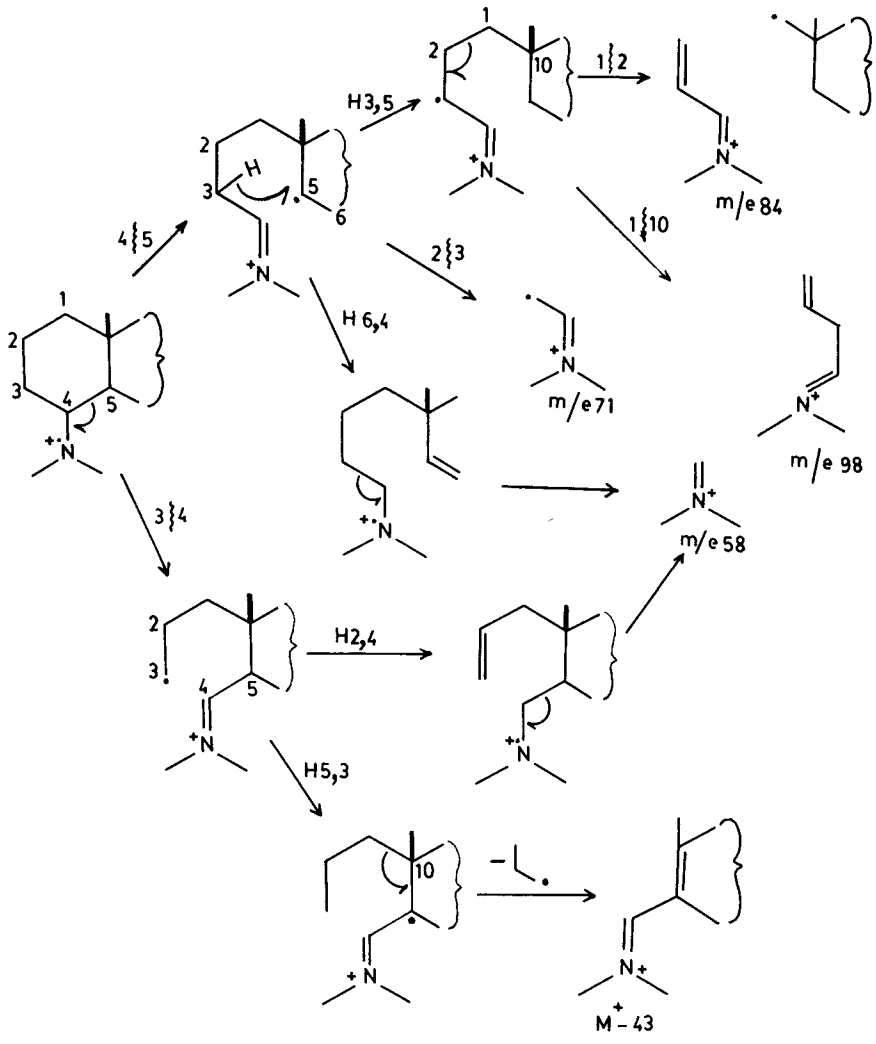
SCHEME 24

C-3 and C-1 gave rise to the base peak at *m/e* 85 which supported the proposed mechanism. The peaks observed at *m/e* 100 (84 + 16) and M^+-(71 + 16) in the mass spectrum of 2β-amino-3α-hydroxypregnane were also consistent with these mechanisms. However, the two other peaks at *m/e* 98 and 99, characteristic of 2-aminosteroids, appeared to involve the initial rupture of the C-2 to C-3 bond as shown in Scheme 25.



SCHEME 25

4-Aminosteroids show characteristic peaks at *m/e* 98, 84, 71, 58, and (M^+--43) corresponding to the ions shown in Scheme 26. Occurrence of intramolecular H-D scrambling in analogues deuterated at positions 3 and 5 has been attributed to the loss of a primary radical in the fragmentation process which would possess a low rate constant (*61*).

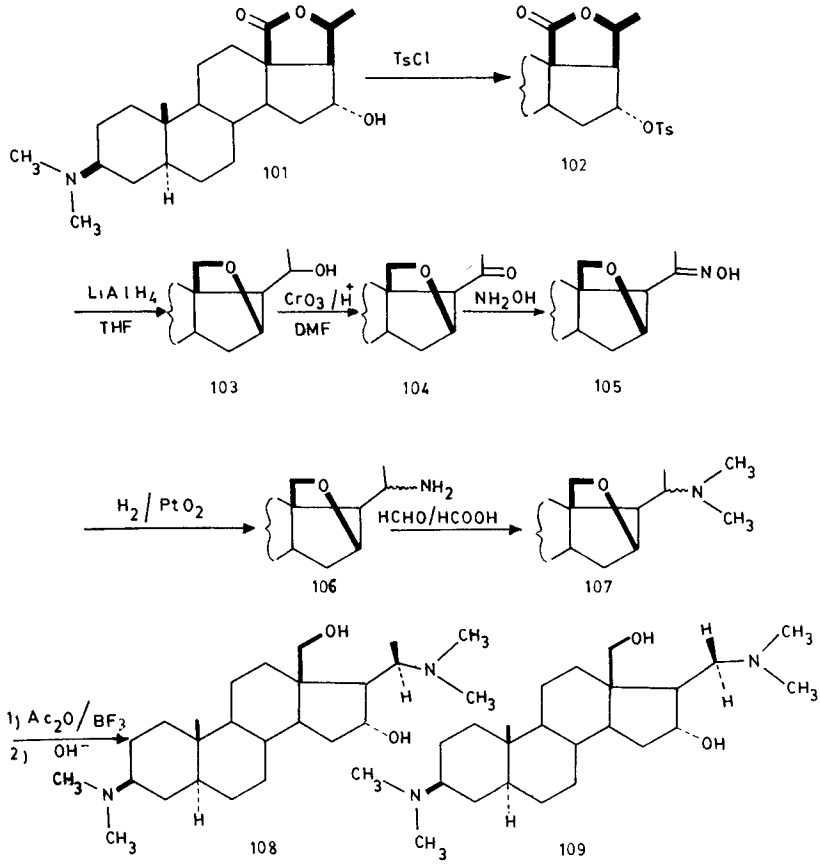


SCHEME 26

B. Syntheses and Chemical Transformations

1. The Conanine Group

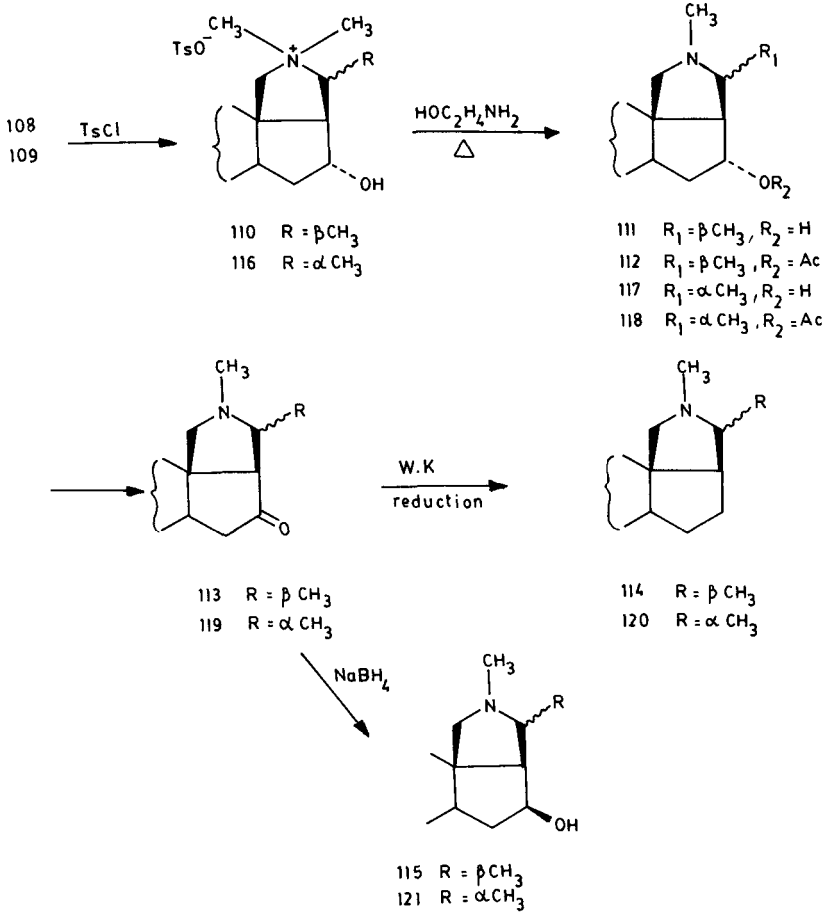
A synthetic approach to derivatives of dihydroconessine and dihydroheteroconessine series oxygenated at C-16 has been reported starting from *N*-methyldihydroparavallaridine (**101**) (*62*). The tosylate (**102**) of *N*-methyldihydroparavallaridine was reduced with LAH to give the 20-hydroxy derivative **103.** Oxidation of **103** yielded the corresponding 20-oxo derivative **104** which was converted to the oxime **105.** Catalytic reduction of the oxime **105** gave a mixture of (20*R*)- and (20*S*)-amino compounds **106.** These compounds were *N*-methylated to **107** and then reacted with acetic anhydride–boron trifluoride and saponified,



SCHEME 27

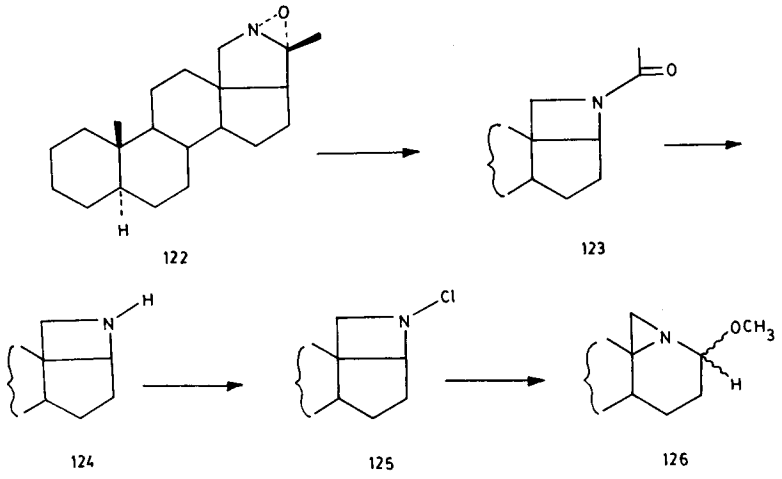
yielding the (20*S*)- and (20*R*)-16α, 18-dihydroxy-*N*,*N*′-dimethylamino derivatives **108** and **109** respectively (Scheme 27). Tosylation and intramolecular cyclization resulted in the formation of ammonium compounds **110** which was converted into 16α-hydroxy-dihydroconessine (**111**), and **116**, which was converted into 16α-hydroxy-dihydroheteroconessine (**117**), by heating with ethanolamine. The ketones **113** and **119,** prepared from the 16α-hydroxy derivatives **111** and **117** by Jones oxidation, were reduced by the Wolff–Kishner procedure to the compounds **114** and **120.** On the other hand, reduction of the ketones **113** and **119** with $NaBH_4$ afforded the 16α-hydroxy derivatives **115** and **121,** respectively (Scheme 28).

Conessine (**9**) has been converted to the oxaziran **122,** which can be photochemically transformed to the *N*-acetyl azetidine **123** (*63*). The reaction in-

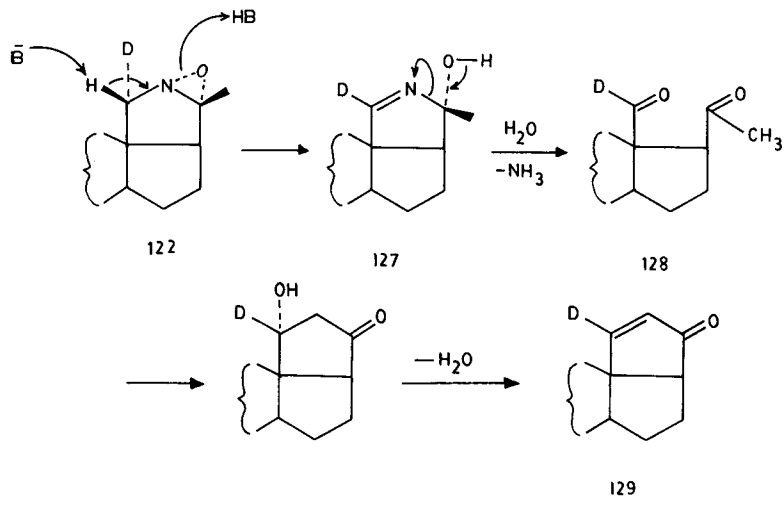


SCHEME 28

volves the known photoisomerization of nitrones to oxazirans (*64*). The latter was hydrolyzed to **124** and chlorinated to the *N*-chloroderivative **125.** Reaction of **125** with sodium methoxide yielded the rearranged compound 18 *N*-cyclo-17-methoxy-17a-aza-D-homo-5α-androstane (**126**) (Scheme 29). Conversion of the α-oxaziran **122** to the conjugated ketone **128** through the formation of the hydroxy imine **127** in alkaline medium has also been reported (*65,66*). The reaction involves the generation of the dicarbonyl compound **129** followed by cyclization

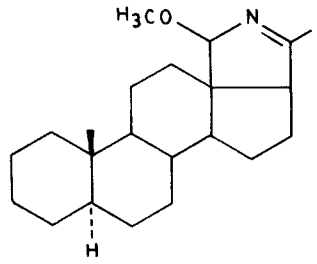


SCHEME 29



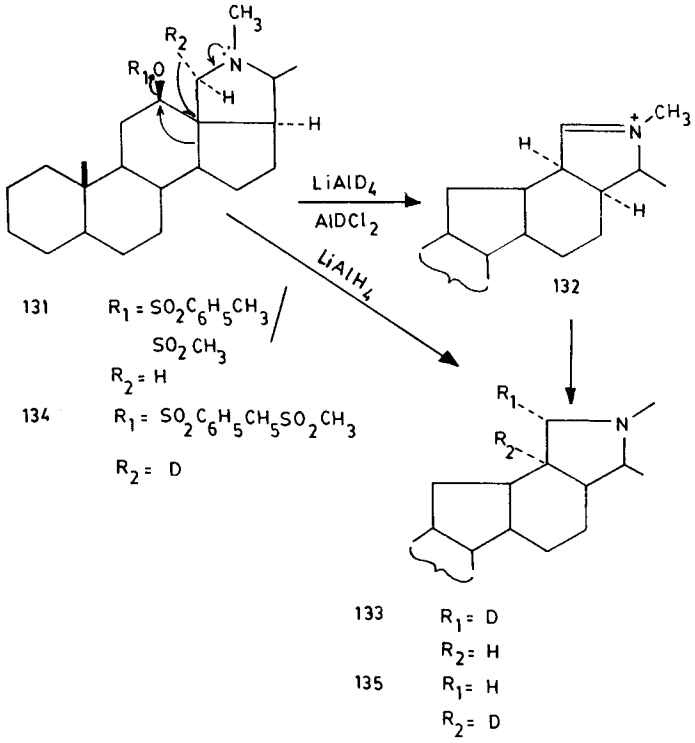
SCHEME 30

(Scheme 30). When refluxed in methanol, **127** gave **130,** which is an intermediate for the synthesis of steroids substituted at C-18 and C-20 (*65*).



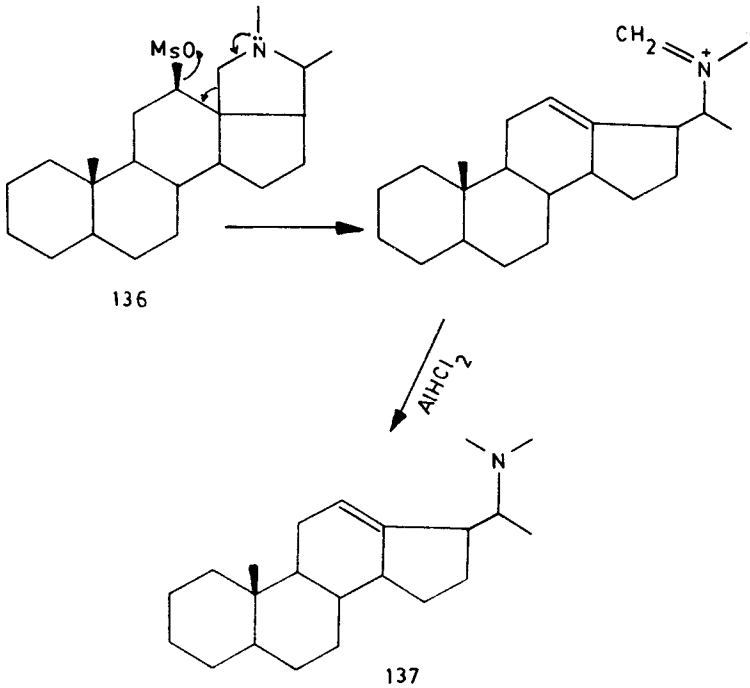
130

Rearrangement of the sulfonic esters **131** of 12β-hydroxy-5α-conanine to *C*-nor-D-homoconanine (**133**) in the presence of LAH and $AlHCl_2$ has been studied



SCHEME 31

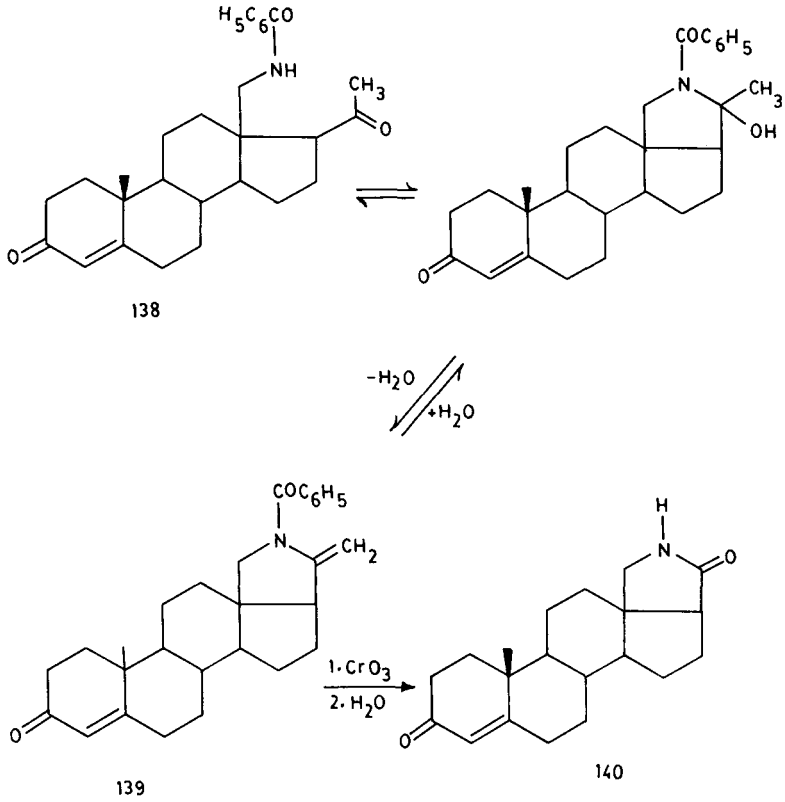
(*67*). In the presence of deuterated reagents, *C*-nor-D-homoconanine showed incorporation of one deuterium atom at 18α-position, suggesting that the rearrangement occurred by a hydride transfer from C-18α to C-13, forming an immonium ion **132,** which was then reduced stereospecifically to give **133.** Conversion of the 18-deuterated compound **134** into **135** with LAH supported this mechanism for the rearrangement (Scheme 31). Reinvestigation of the mechanism using deuterated reducing agents has established the stereochemistry at positions 12 and 13 of **133** (*68*). Degradation experiments on the rearranged product provided further support towards its structure (*69*).



SCHEME 32

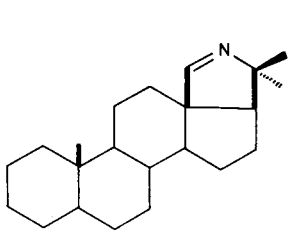
Grob fragmentation and reduction of the mesylate of 12α-hydroxyconanine (**136**) with $AlHCl_2$ in the presence of ether led to the compound **137.** The mechanism is shown in Scheme 32 (*70*).

Oxidation of 18-benzoylaminoprogesterone (**138**) with chromium trioxide has been investigated (*71*). Cyclization of **138** followed by dehydration with benzoyl chloride led to the enamide **139,** which was oxidized with chromium trioxide to give the lactam **140** of 18-amino-3-oxo-4-etianic acid (Scheme 33).

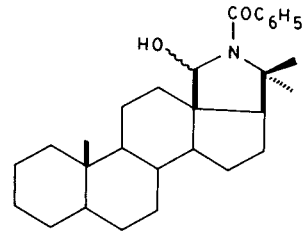


SCHEME 33

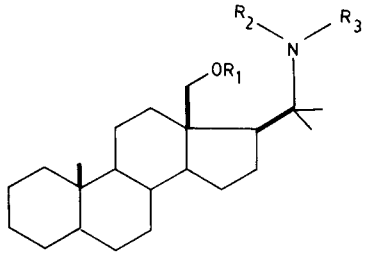
The methoxylated imine **130** has been methylated at C-20 to give the imine **141** by reaction with methyl magnesium iodide (*72*). Reduction of the 18-hydroxy benzamide **142,** prepared from the imine **141** with $NaBH_4$, yielded the alcohol **143.** This was converted to the cyclic ether **144** in acidic medium. Alkaline hydrolysis of **143** to **145** and *N*-methylation yielded the 20-dimethylamine derivative **146.** This reaction was reinvestigated and it was found that the epimeric 18-hydroxy benzamides **142** were in equilibrium (*73*). It was suggested that epimerization could occur through the ring-closed immonium ion or through the open aldehyde form (Scheme 34). The 18β-H isomer was shown to be the most stable one by NMR studies.



141



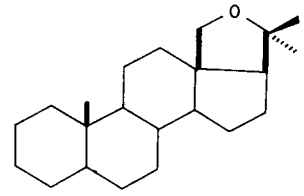
142



143 $R_1 = R_2 = H, R_3 = COC_6H_5$

145 $R_1 = R_2 = R_3 = H$

146 $R_1 = H \quad R_2 = R_3 = CH_3$



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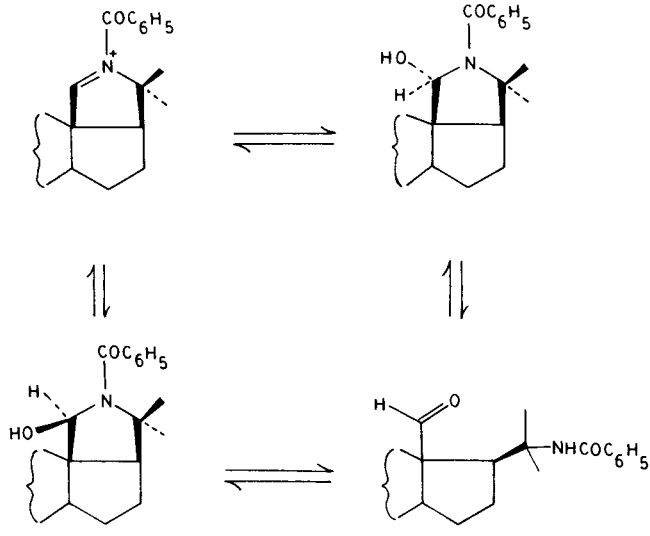
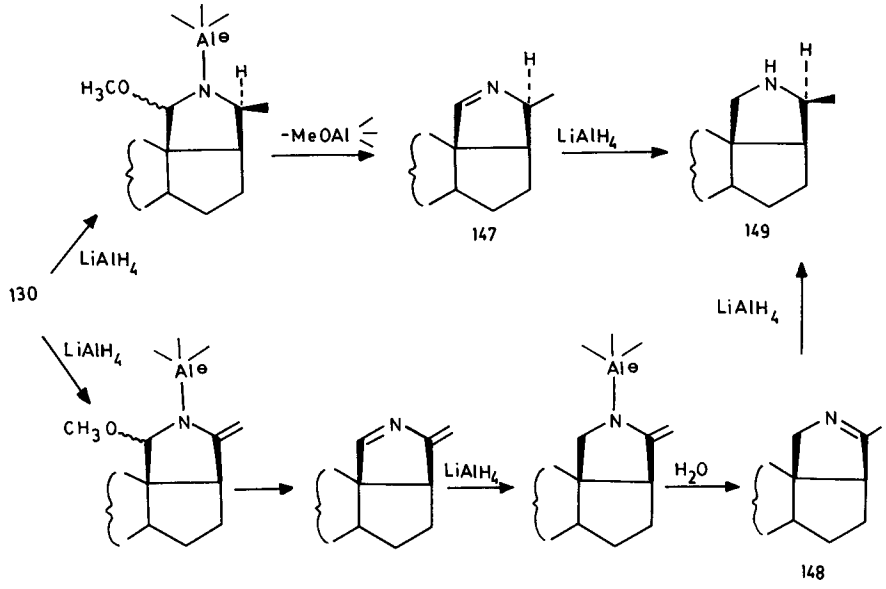


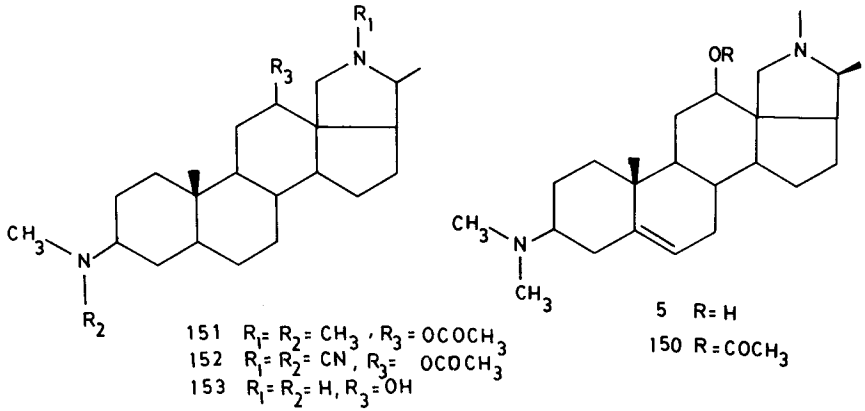
SCHEME 34

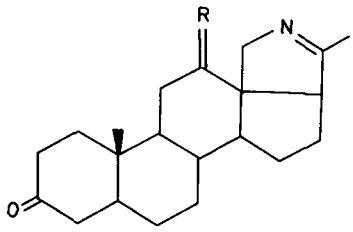
The stereospecific reduction of the methoxylated imine **130** with LAH or $NaBH_4$ gave the isomeric imines **147** and **148** together with *N*-demethylconanine (**149**), the yields of the three products being dependent on the time of the reaction (*74*). The mechanism suggested for their formation is presented in Scheme 35.



SCHEME 35

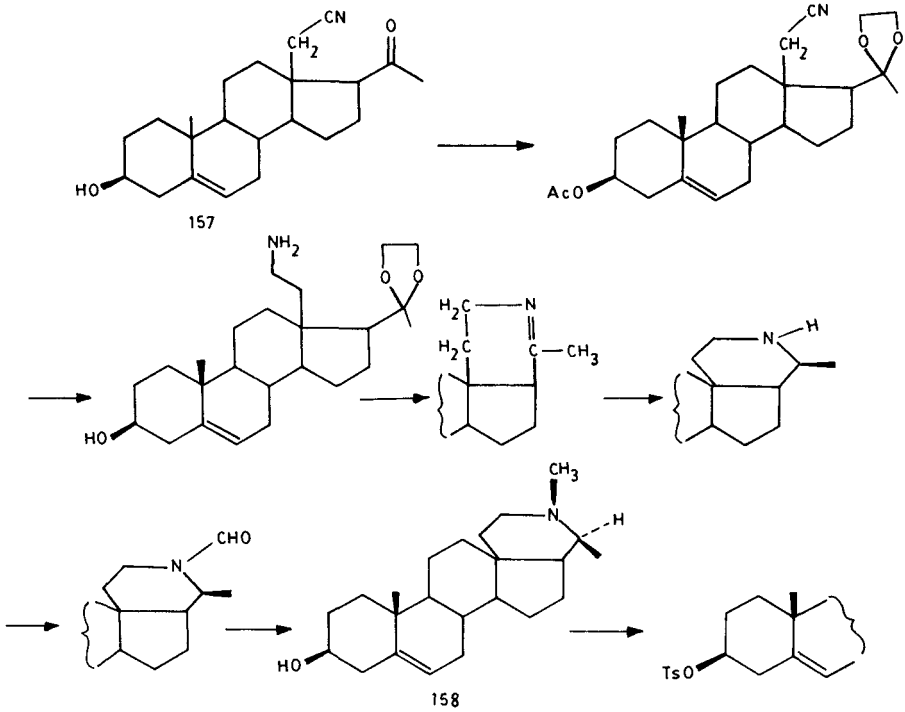
The alkaloid holarrhenine (**5**) has been transformed into the 12β-hydroxy (**154**), 12-keto (**155**), and 12α-hydroxy (**156**) derivatives of *N*-demethyl-5α,20(*N*)-conen-3-one (*75*). Catalytic reduction of *O*-acetylholarrhenine



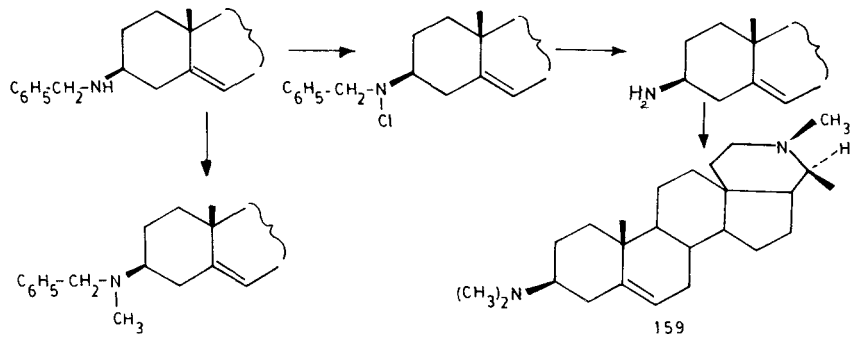


154 R = αOH, βH
155 R = O
156 R = βOH, αH

(**150**) to the dihydro compound **151,** followed by reaction with cyanogen bromide, yielded the dicyano derivative **152.** This was hydrolyzed to **153** and subjected to Ruschig deamination to give 12β-hydroxy-*N*-demethyl-5α,20-(*N*)-conen-3-one (**154**). Oxidation of **154** with chromium trioxide yielded the 12-keto derivative **155,** which was stereospecifically reduced to the 12α-hydroxy derivative **156** by Elk's procedure.



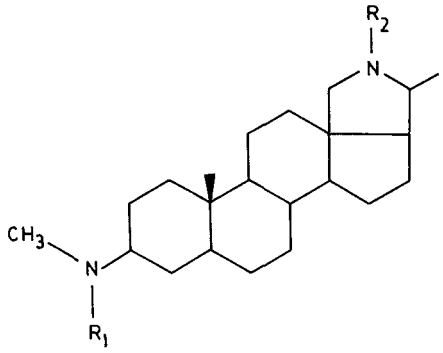
SCHEME 36 (*continued*)



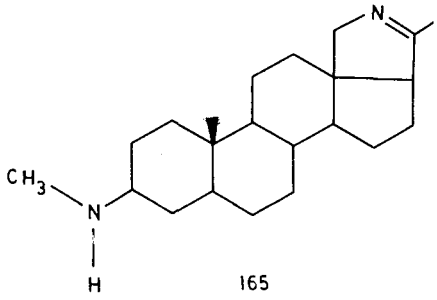
SCHEME 36 *(continued)*

The syntheses of 18-homolatifoline (**158**) and 18-homoconessine (**159**) from 18-cyanopregnenolone (**157**) have been described. The reaction sequence is outlined in Scheme 36 (*76*).

Dihydroisoconessimine (**160**) has been converted to the imine **165** in a number of steps (*77*). *N*-Formylation of **160** to **161** and reaction with cyanogen bromide led to the *N*-cyano compound **162.** Reaction with alkaline hydrogen peroxide gave **163,** which on treatment with nitrous acid afforded the secondary amine **164.** This was then chlorinated at the C-20 nitrogen and reacted with sodium methoxide to give the imine **165.**

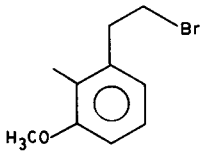


160 $R_1 = H$, $R_2 = CH_3$
161 $R_1 = CHO$, $R_2 = CH_3$
162 $R_1 = CHO$, $R_2 = CN$
163 $R_1 = CHO$, $R_2 = CONH_2$
164 $R_1 = CHO$, $R_2 = H$



165

The tetracyclic aminophenol **176,** a potentially important intermediate for the synthesis of conessine, has been prepared as follows (*78*). 2-(2-Methyl-3-methoxy-phenyl)ethyl bromide (**166**) was condensed with the potassium enolate of dimethyl cyclopentanone-2,3-dicarboxylate (**167**) to give the keto ester **168** which was cyclized to the tricyclic ester **169.** Stereospecific catalytic reduction of **169** produced the ester **170** having a *trans* ring junction. The acid ester **171,** prepared from **170** by partial hydrolysis, was converted to the diazoketone **172** and then reduced with hydroiodic acid to give the keto ester **173.** Leuckart reaction with *N*-methylformamide then led stereospecifically to the pyrrolidone **174.** Reduction of the lactam **174** with LAH gave the tetracyclic pyrrolidine **175,** demethoxylation of which in hydrobromic acid–acetic acid yielded the tetracyclic phenolic amine **176,** corresponding to the BCDE rings of conessine.



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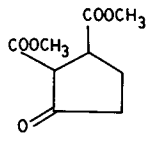

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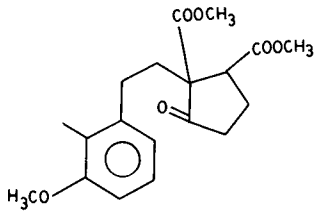

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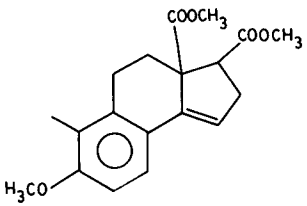

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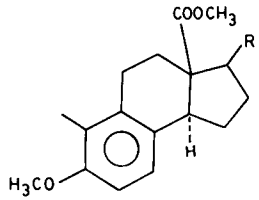
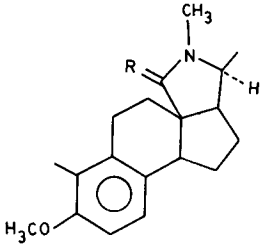
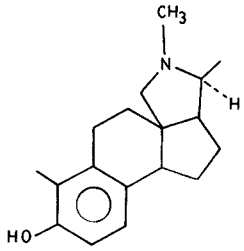


170 R = $COOCH_3$
171 R = COOH
172 R = $COCHN_2$
173 R = $COCH_3$

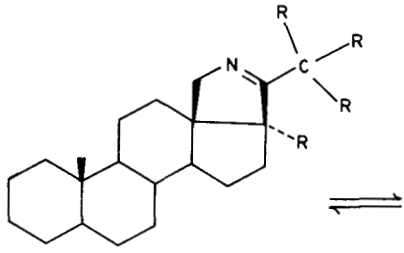


174 R = O
175 R = H_2

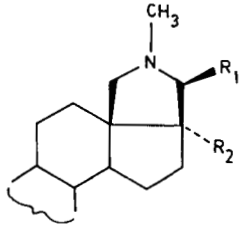


176

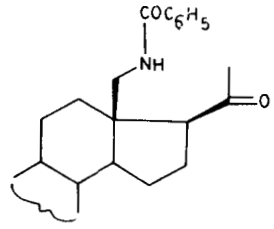
Selective incorporation of deuterium on carbons 17 and 21 of 5α,20(*N*)-conene (**177**) was made possible due to imine–enamine equilibrium in the pyrrolidine ring, resulting in the preparation of [21-$^2H_3$]-conanine (**179**) and [17-^{2}H] conanine (**184**) (*79*). Reaction of 5α,20(*N*)-conene (**177**) with deuteromethanol yielded **178** which was reduced with $NaBH_4$ and *N*-methylated to give [21-$^2H_3$] conanine (**179**). Deuteration of the ketone **180,** prepared from 5α,20(*N*)-conene (**177**), led to some epimerization at position 17, and reaction of the epimeric ketones **181** and **182** with deuterated acid yielded the [17α-^{2}H]-imine **183.** Reduction and *N*-methylation of **183** gave the [17α-^{2}H]-conanine (**184**).



177 R = H
178 R = D
183 R = H or D



179 R_1 = CD_3, R_2 = H
184 R_1 = CH_3, R_2 = H



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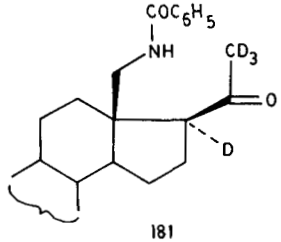

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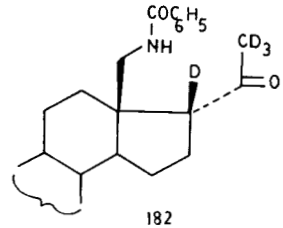
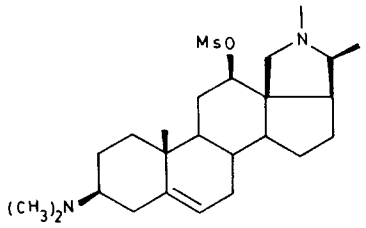


182

A modification of Uffer's experiment led to a significant improvement in the yields of the resulting rearranged product from holarrhenine mesylate (*80*). Complete rearrangement of holarrhenine mesylate (**185**) to *C*-nor-D-homo derivative **186** was observed when the reaction was performed in anhydrous alcohol using an excess of $NaBH_4$ instead of LAH. Labeling experiments strongly supported the mechanism suggested earlier for rearrangement to the *C*-nor-D-homo-derivative (*68*).



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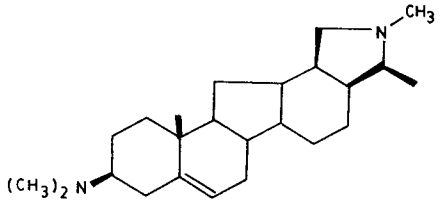
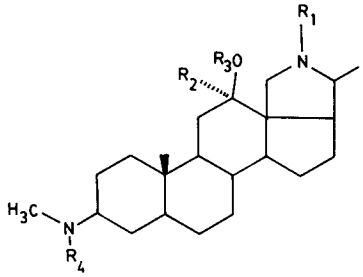


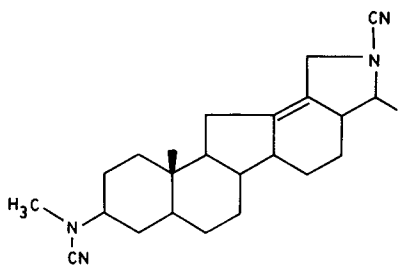
186

Rearrangement of 12β-mesyloxy-*N,N'*-dicyanodihydroconimine to *C*-nor-D-homoderivative was observed by the action of collidine (*81*). 12β-Mesyloxy-*N,N'*-dicyanodihydroconimine (**188**) prepared from dihydroholarrhenine (**187**) underwent rearrangement in the presence of collidine to give the olefins (**189**) and (**190**).



187 $R_1 = R_4 = CH_3, R_2 = R_3 = H$

188 $R_1 = R_4 = CN, R_2 = H, R_3 = Ms$



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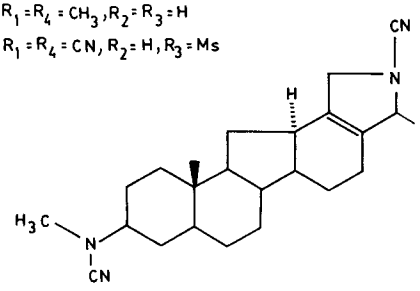

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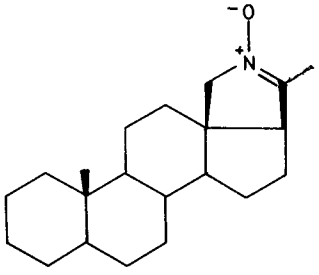

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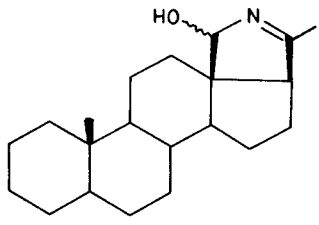
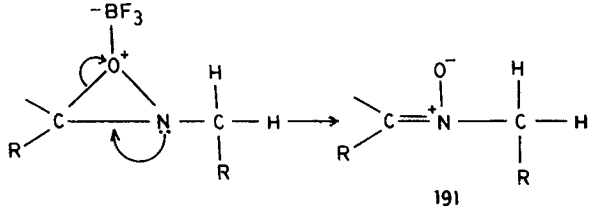


192

The heterocyclic oxaziridine **122** may be isomerized in an acidic medium to the nitrone **191** or to the iminocarbinol **192** (*82*). In the presence of a Lewis acid **122** yielded **191** (Scheme 37) while treatment of **122** with a protonic acid gave

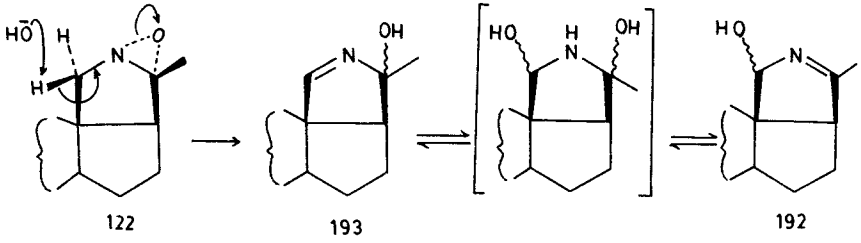


191

SCHEME 37

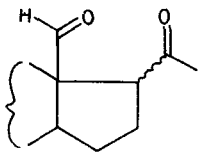
192 through the formation of **193** (Scheme 38). Hydrolytic cleavage of the pyrrolidine ring gave the keto aldehydes **194,** epimeric at C-17 (*83*).

The difference in the reactivity of the imino group towards peracids due to the presence and nature of the functional group at position 12 has been studied in



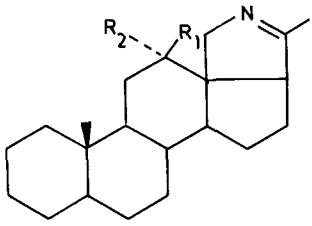
122 193 192

SCHEME 38



194

derivatives of 5α,20(*N*)-conene (**177**) (*84*). Treatment of C-12 substituted derivatives **195** of 5α,20(*N*)-conene with *p*-nitroperbenzoic acid yielded a mixture of the nitrone **196,** and the (20*S*)- and (20*R*)-oxazirans **197** and **198** respectively, while the nonsubstituted derivative yielded only the (20*R*)-oxaziran **198** under similar conditions.



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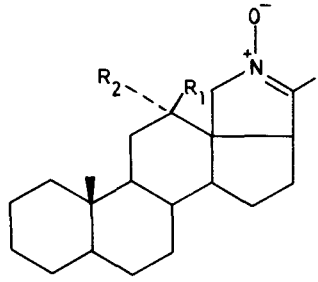

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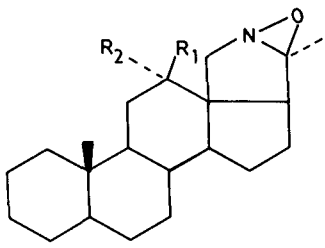

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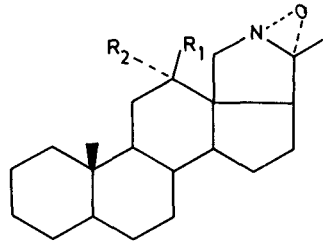


198

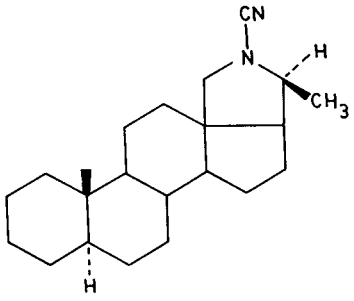
$R_1 = H, R_2 = H$

$R_1 = OH, R_2 = H$

$R_1 = OAc, R_2 = H$

$R_1 = <O\square O>, R_2 = <O\square O>$

$R_1 = O, R_2 = O$



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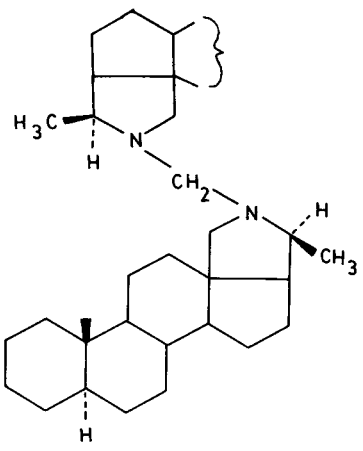

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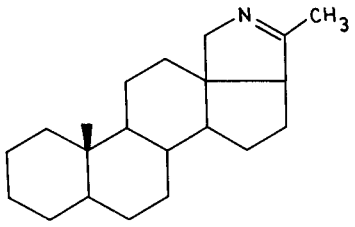
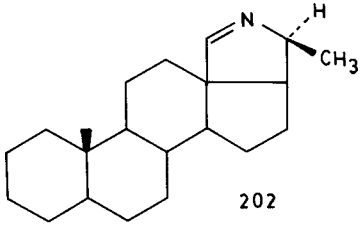


201



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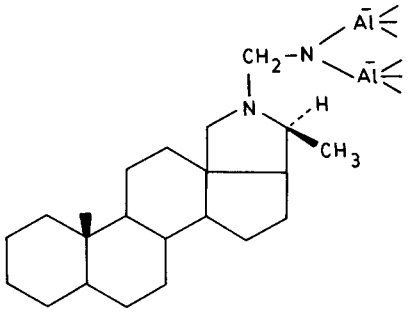
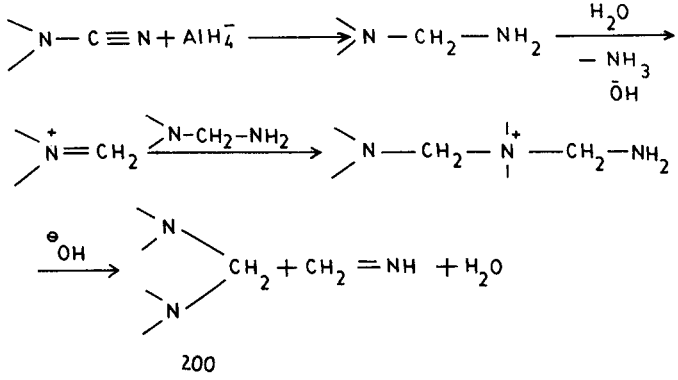


203

Reduction of *N*-cyano-*N*-demethylconanine with LAH has been reinvestigated (*85*). When *N*-cyano-*N*-demethylconanine (**199**) was reduced with LAH under nitrogen at room temperature, the methylenic *gem*-diamine **200** was obtained together with the isomeric imines **201** and **202.** The suggested mechanism for the

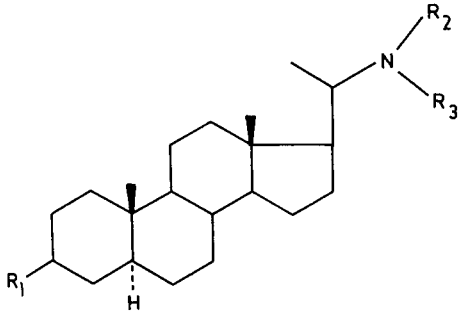


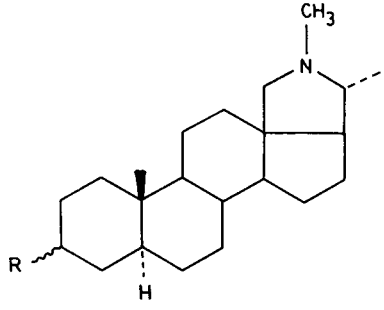
200

SCHEME 39

formation of **200** is shown in Scheme 39. The formation of imines **201** and **202** could be explained by the formation of the intermediate **203.**

The syntheses of heteroconanine (**210**) and conanine (**179**) have been achieved by two reaction sequences starting from the amino steroids epimeric at position 20 (*86*). The 20β-amino compound **204** was converted into the corresponding carbamate **205** by reaction with ethyl chloroformate and then reduced with LAH to give the *N*-methyl derivative **206.** Chlorination of **206** to **207** and intramolecular cyclization by the Hofmann–Loeffler reaction led to heteroconan-3β-ol (**208**). This was then tosylated to **209** and reduced with LAH to produce





204 R_1 = OH, R_2 = R_3 = H, 20 β

205 R_1 = OH, R_2 = $CO_2C_2H_5$, R_3 = H, 20 β

206 R_1 = OH, R_2 = CH_3, R_3 = H, 20 β

207 R_1 = OH, R_2 = CH_3, R_3 = Cl, 20 β

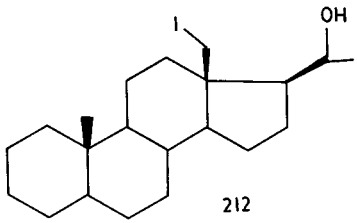
211 R_1 = OH, R_2 = H, R_3 = H, 20α

208 R = βOH

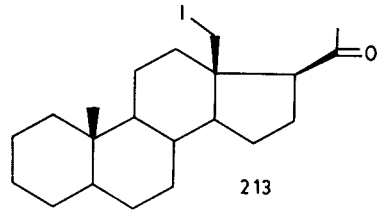
209 R = βOTS

210 R = H

heteroconanine (**210**). An identical reaction sequence with the 20α-amine **211** finally yielded conanine (**179**).



212



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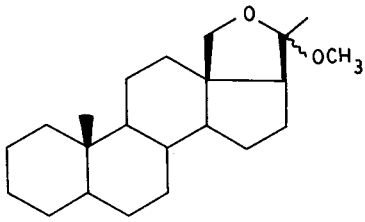
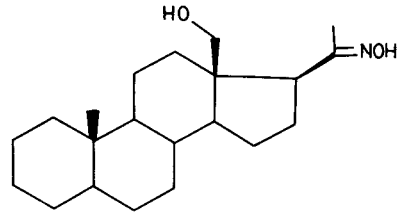


214



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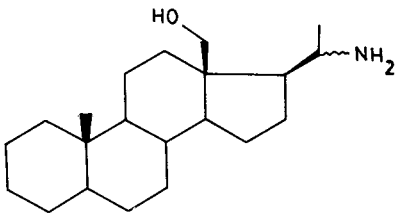

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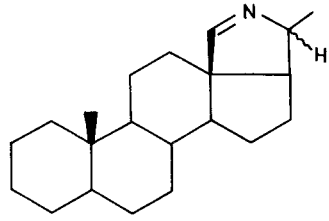
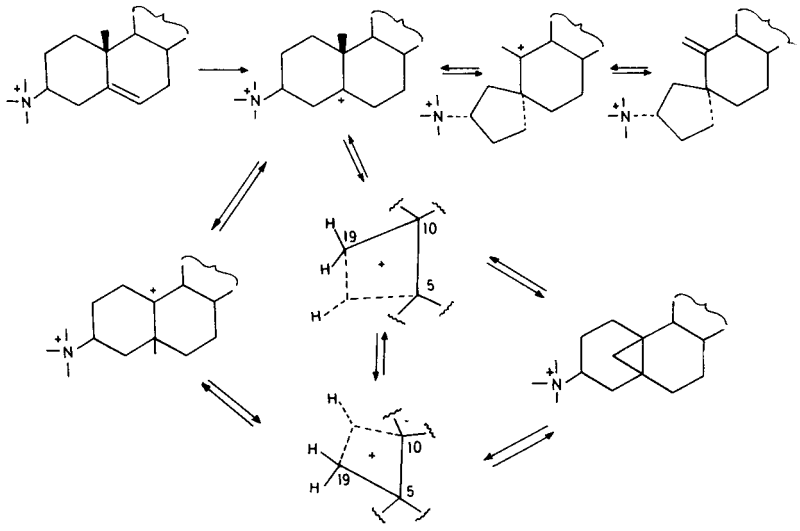


217

N-demethylconenines (**217**), epimeric at C-20, have also been prepared from the (20*R*)-18-iodo compound (*87*). Oxidation of **212** prepared from the (20*R*)-hydroxy compound by reaction with $Pb(OAc)_4$—iodine to the iodoketone **213,** and subsequent reaction with methanolic silver acetate, yielded the acetal **214.** The acetal was cleaved on reaction with hydroxylamine to give the oxime-alcohol **215,** which was reduced to the amino alcohols **216** and then oxidized to the corresponding (20*S*)- and (20*R*)-*N*-demethylconenines **217.**

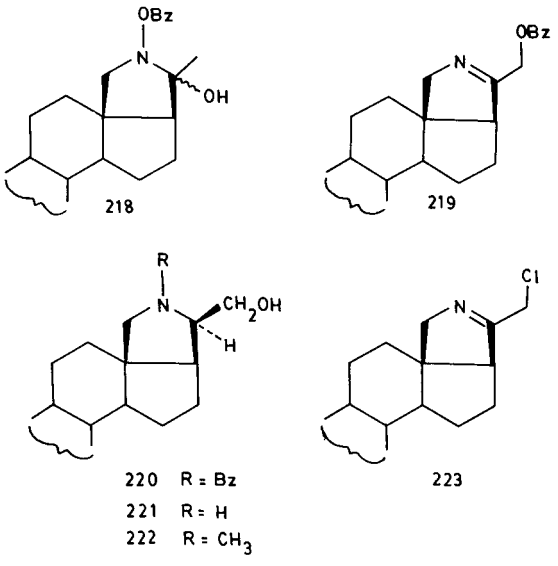
Skeletal isomerization of 3β-amino-steroids (namely, methylholaphylline and conessine) was observed in D_2SO_4 with deuteration of the 19-methyl group. In the case of 3α-amino steroids, however, deuteration of the 19-methyl group did not take place. The mechanisms proposed for deuteration of the 19-methyl group are presented in Scheme 40 (*88*).

The steroidal nitrone **191** showed differences in reactivity towards benzoyl

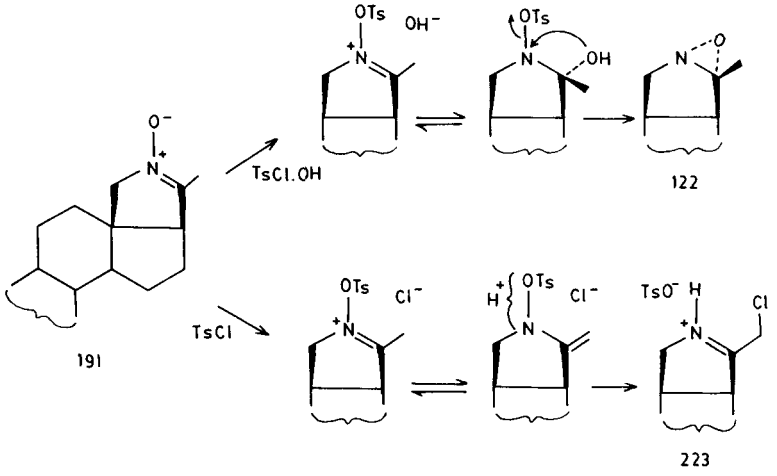


SCHEME 40

chloride and toluene-*p*-sulfonyl chloride (*89,90*). Reaction of **191** with benzoyl chloride at room temperature yielded a mixture of the benzamido alcohols **218** epimeric at position 20. These were converted to the imine **219,** which was reduced to **220.** Hydrolysis to **221** and methylation yielded the corresponding *N*-

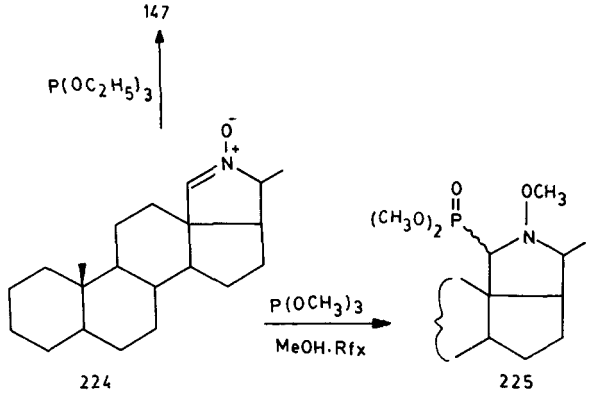


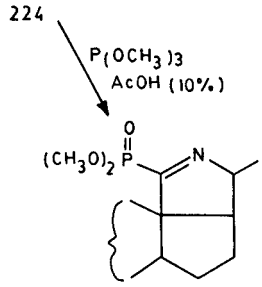
methyl derivative **222.** On the other hand, the nitrone **191** afforded the oxaziridine **122** on reaction with toluene-*p*-sulphonic acid. In the absence of base, the chloro compound **223** was obtained from **191,** which was converted to **219** on reaction with sodium benzoate in HMPTA. Formation of oxaziridine **122** and the chloro compound **223** have been suggested to proceed by the mechanism shown in Scheme 41



SCHEME 41

The reaction of the nitrone **224** with alkyl phosphite has been studied (*91,92*). When the nitrone **224** was refluxed in methanol with trimethyl phosphite, a mixture of isomers **225** epimeric at C-18 was obtained. Similar reaction in 10% acetic acid produced the imine **226.** (Scheme 42). With triethyl phosphite the nitrone **224** yielded the deoxygenated product **147** together with products analogous to **225** and **226.**

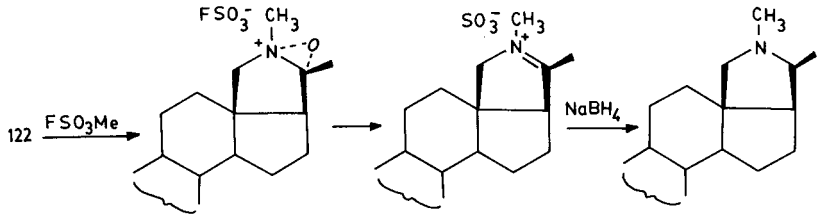




226

SCHEME 42

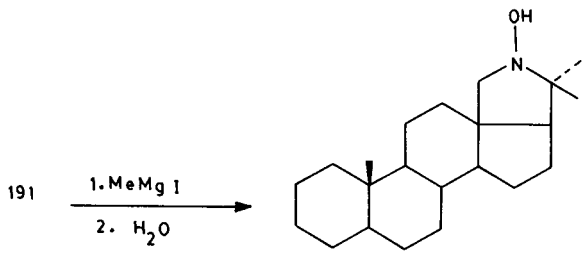
On treatment with methylfluorosulfonate, the oxaziridine **122** was converted into the oxaziridinium salt **227,** which was unstable in solution and gave **228.** Reduction of **228** with $NaBH_4$ yielded **229** (Scheme 43) (*93*).



227 228 229

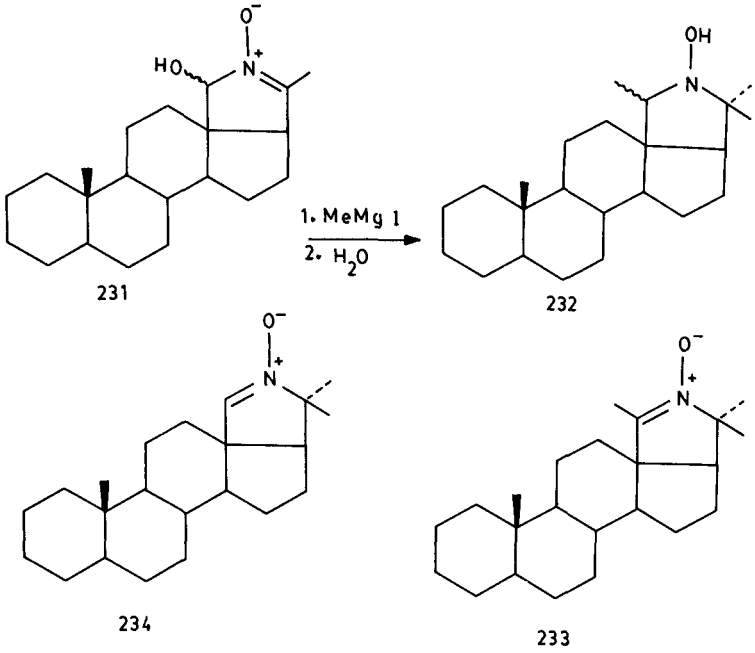
SCHEME 43

The effect of Grignard reagent on steroidal nitrones and α-hydroxy nitrones has been studied (*94*). The nitrone **191,** on reaction with methyl magnesium iodide yielded the compound **230** (Scheme 44). Similar reaction with α-hydroxy



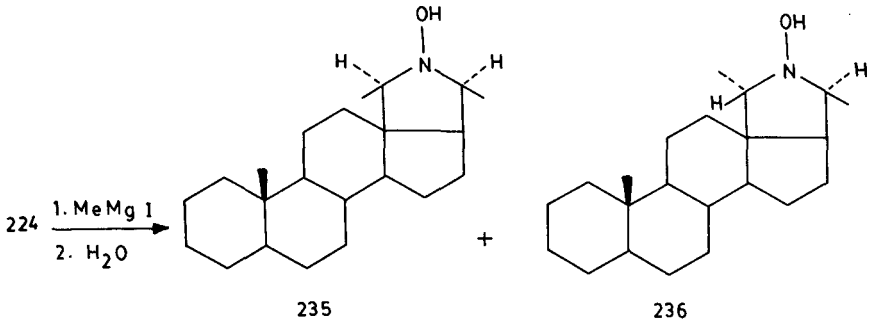
230

SCHEME 44



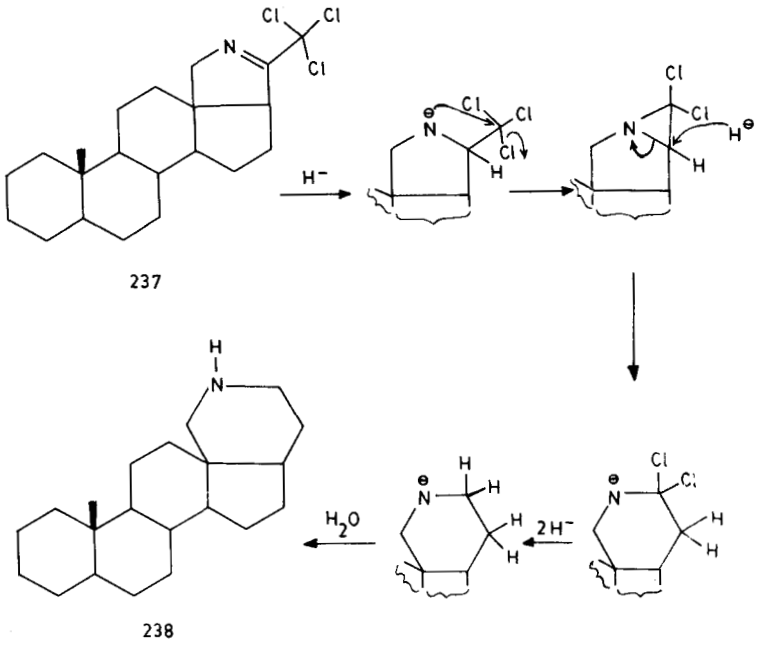
SCHEME 45

nitrone **231** led to the 18α,20-dimethylhydroxylamines **232,** 18,20-dimethyl-18(*N*)-nitrone **233,** and the 20-methyl-18(*N*)-nitrone **234.** The latter was reduced with $NaBH_4$ to **230.** Hydroxylamines **232** were also formed from the nitrone **234** (Scheme 45). Treatment of **234** with an excess of methyl magnesium iodide, however, did not result in the introduction of a second methyl group at C-18. The 20-hydroxy-18(*N*)-nitrone **224** was converted into the nitriles **235** and **236** by reaction with methyl magnesium iodide (Scheme 46).



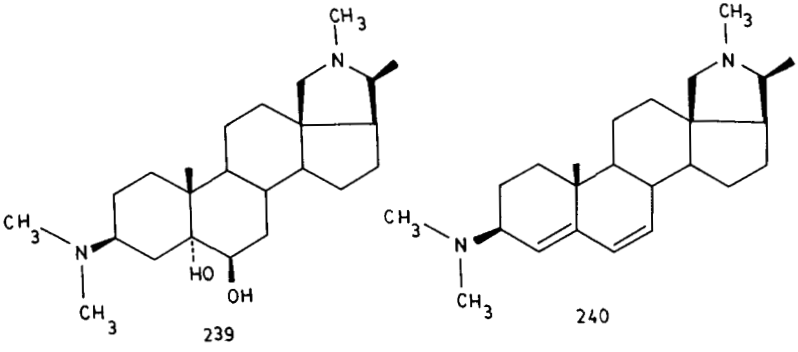
SCHEME 46

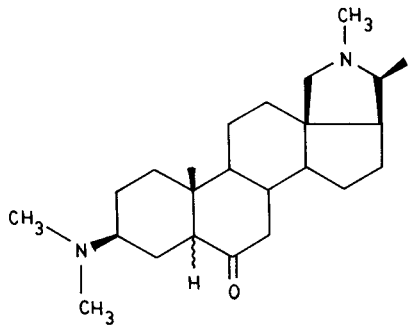
Enlargement of ring E (pyrrolidine ring) by reduction of the trichloroimine **237** with LAH to give *N*-demethyl-21-nor-*E*-homo-5α-conanine (**238**) has been reported (*95*). The proposed mechanism of formation is shown in Scheme 47.



SCHEME 47

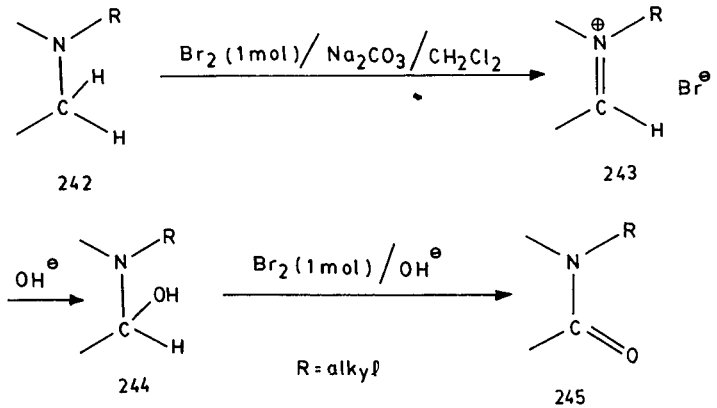
Dehydration of dihydroxyconessine **239** with H_2SO_4 yielded two compounds, 3β-dimethylaminoconan-4,6-diene (**240**) and 3β-dimethylaminoconan-6-one (**241**) (*96*). Preparation of **239** has been achieved by reaction of conessine (**9**) with nitrous acid (*97*).



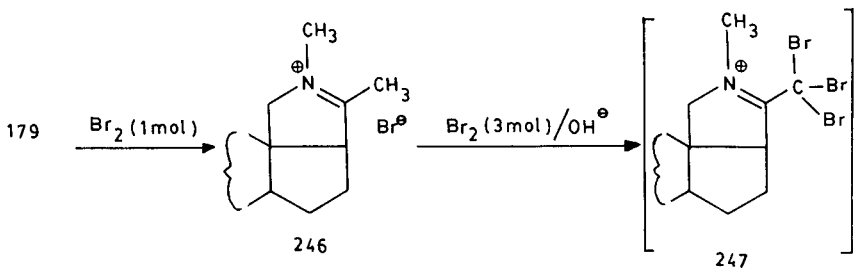


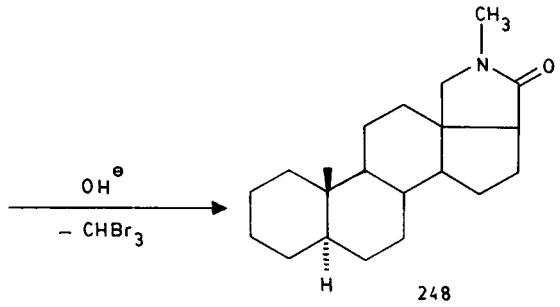
241

Selective oxidation of cyclic tertiary amines **242** having hydrogen atoms α to nitrogen with bromine or NBS in basic medium resulted in the formation of lactams **245** (*98*). The oxidation probably proceeds through the intermediate formation of the iminium salt **243** and the α-hydroxyamine **244** (Scheme 48). In the oxidation of conanine (**179**) the iminium salt **246** may be perbrominated at



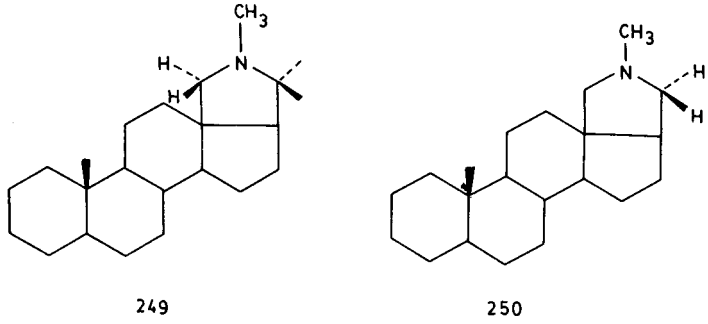
SCHEME 48



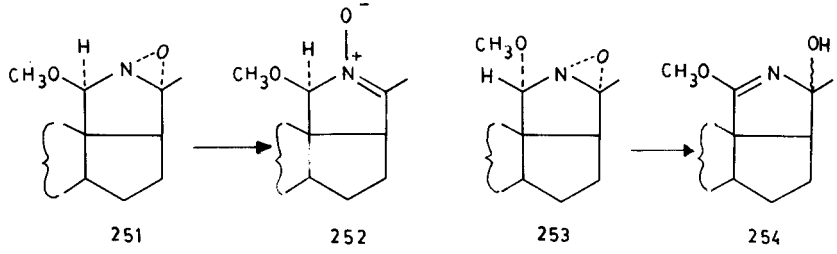


SCHEME 49

position 21 to give **247,** which then undergoes elimination of tribromoethane to give the lactam **248** (Scheme 49). Oxidation of 20-methylconanine **249** and 21-norconanine **250** has been similarly affected (*99*).



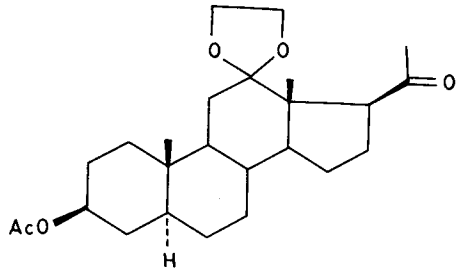
Isomerization of the steroidal oxaziran into the imino-carbinols or nitrones in the presence of acid or base has been investigated (*100*). Compound **251,** bearing *cis*-oriented H-18 and oxaziran ring, was isomerized exclusively to the nitrone **252.** Isomerization of **253** possessing 18,H *trans* to the oxaziran ring, however, yielded the C-20 epimeric imino-carbinols **254** (Scheme 50).



SCHEME 50

The Hofmann–Loeffler reaction has been utilized for the cyclization of 2α-methylamino-pregnane derivatives bearing an oxygen function in the neighborhood of the reaction site to the corresponding conanine derivatives (*101*). Dihydroholarrhenine (**187**) has been synthesized from **255** by this procedure.

The immonium ion **256** and the corresponding enamine **257** were converted into **122, 258,** and **259** with *p*-nitroperbenzoic acid (*102*).



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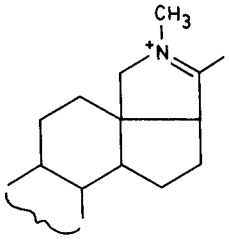
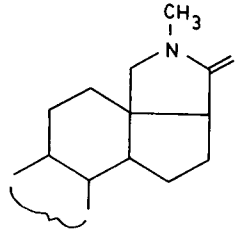


256



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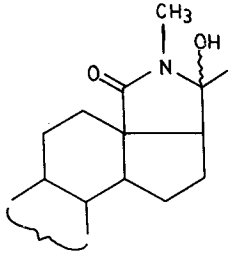
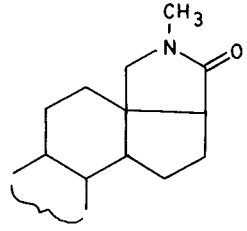
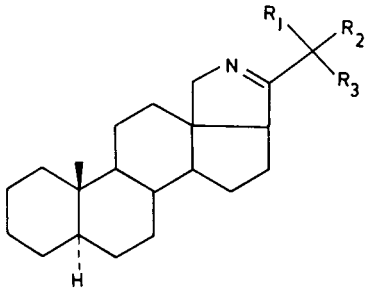


258



259

Halogenation of *N*-desmethyl-20(N)-5α-conanine (**148**) led to the monochlorinated steroidal imine **260,** the dichlorinated steroidal imine **261,** and the trichlorinated steroidal imine **237.** Similarly, the corresponding di and tribromo derivatives **262** and **263** could be prepared (*103*). Treatment of **148** with sodium hypochlorite in the presence of base produced the oxaziran **122** together with the halogenated imines, indicating the existence of an N-halogenated intermediate **264.**

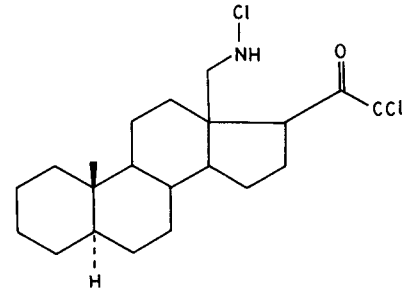


260 R_1 = Cl, R_2 = R_3 = H

261 R_1 = R_2 = Cl, R_3 = H

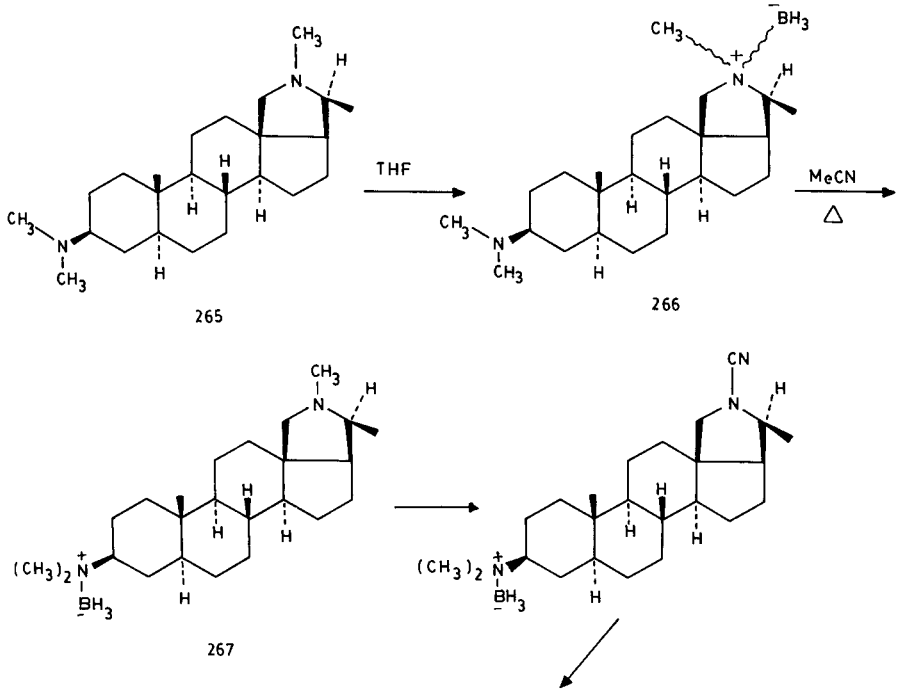
262 R_1 = R_2 = Br, R_3 = H

263 R_1 = R_2 = R_3 = Br



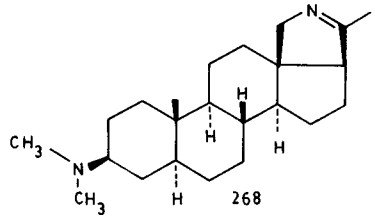
264

Reaction of conanine derivatives with borane yielded the borane complexes, which behaved as protecting groups for the tertiary amine function (*104*). Dihydroconessimine **268** was prepared from dihydroconessine (**265**) through the



265 266

267

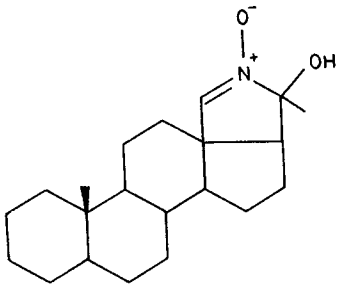


268

SCHEME 51

monoborane intermediate **267.** The latter was obtained by selective deprotection of the bisborane complex **266** (Scheme 51).

Isomerization of α-hydroxynitrones **269** to **270** has been reported in the presence of toluene-*p*-sulfonic acid (*105*). Stereospecific reduction of **270** with $NaBH_4$ yielded the (20*S*) hydroxylamine **271.** The nitrone **272** was obtained on incomplete reduction. The isomeric α-hydroxynitrones **269** and **270** reacted with methanolic sulfuric acid to give the unstable methoxy nitrone **273** which gave the dimeric compound **274** on crystallization. Deuteration experiments have shown the existence of an equilibrium between the immonium compound **270** and the enamine **275** in an alkaline medium (*106*).



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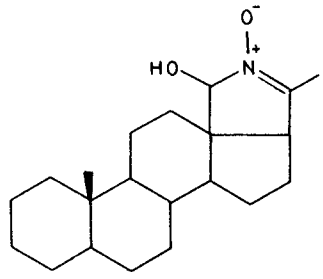

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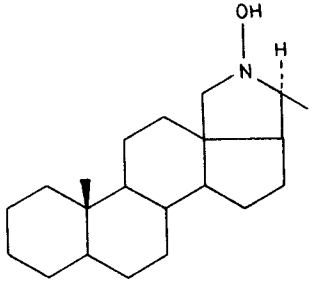

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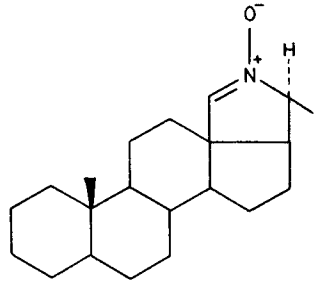

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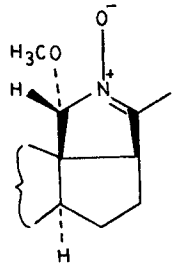

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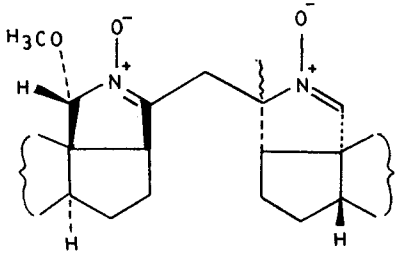

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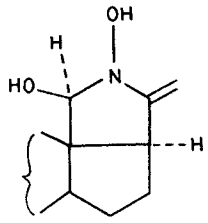

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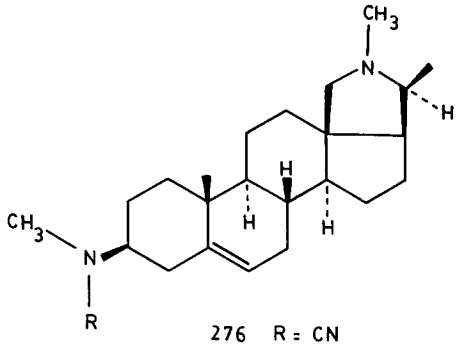
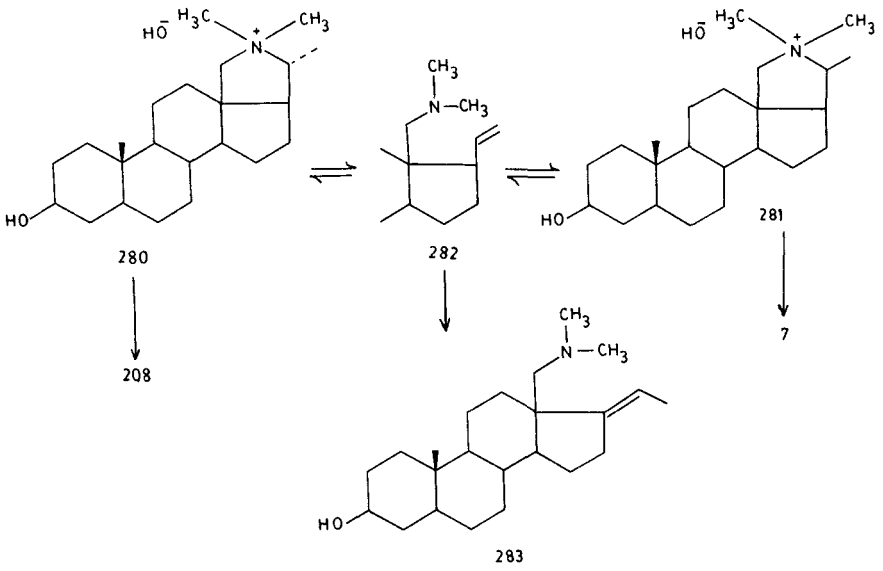
N-Cyano-*N*-desmethyl derivative **276** prepared from conessine (*107*) yielded the acid amide **277** on careful hydrolysis, diamine **278** on reduction, and guanido derivative **279** on treatment with ammonia (*108*).



276 R = CN
277 R = $CONH_2$
278 R = CH_2NH_2
279 R = $C(NH_2)=NH$

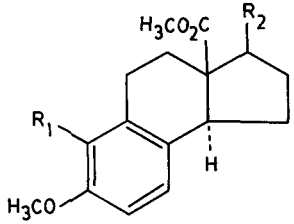
Reaction of heteroconanol methiodide (**280**), conanol methiodide (**281**), or 18-dimethylamino-5α-pregn-20-en-3β-ol (**282**) with a large excess of potassium hydroxide in hot ethylene glycol led to heteroconanol (**208**), conanol (**7**), and a



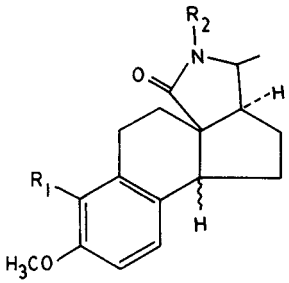
SCHEME 52

new product **283** which proved to be an isomer of **282** (Scheme 52). It was assumed that the Hofmann elimination product **282** is involved as an equilibrium intermediate in each of the three reactions (*109*).

An approach towards the synthesis of *dl*-conessine resulted in the preparation of tetracyclic intermediates **294** and **295** from the tricyclic diesters **284** and **285,**



284	$R_1 = CH_3$, $R_2 = CO_2CH_3$
285	$R_1 = H$, $R_2 = CO_2CH_3$
286	$R_1 = CH_3$, $R_2 = CO_2H$
287	$R_1 = H$, $R_2 = CO_2H$
288	$R_1 = CH_3$, $R_2 = COCH_3$
289	$R_1 = H$, $R_2 = COCH_3$

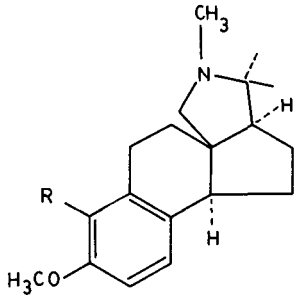


290 $R_1 = CH_3, R_2 = H$

291 $R_1 = R_2 = H$

292 $R_1 = R_2 = CH_3$

293 $R_1 = H, R_2 = CH_3$



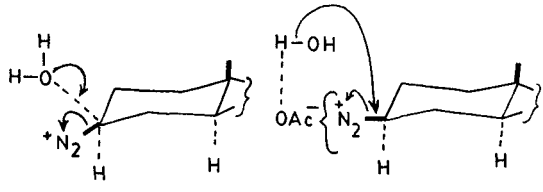
294 $R = CH_3$

295 $R = H$

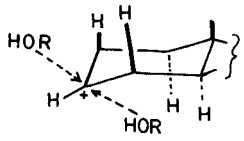
thus constructing the heterocyclic ring E of conessine (*110*). Partial hydrolysis of **284** and **285** led to the corresponding acid esters **286** and **287.** Using acid chlorides and diazoketones, **286** was converted to the ketone **288** and **287** was converted to ketone **289.** Leuckart reaction on **288** and **289** respectively yielded the corresponding epimeric lactams **290** and **291.** *N*-Methylation of the lactams **290** and **291** to **292** and **293,** and subsequent reduction, gave the tetracyclic amines **294** and **295,** corresponding to the BCDE ring system of conessine. This provided another route for the construction of ring E of conessine.

2. The Pregnane Group

A reinvestigation of the deamination reaction in steroidal systems has shown that, contrary to earlier reports (*111,112*), deamination of 3β-amino-5α-cholestane to the corresponding 2β-alcohol with nitrous acid proceeds with predominant retention of configuration at C-3, accompanied by some elimination (*113*). The proposed mechanism for retention may involve either a pyramidal S_N2 transition state **296** or an internal S_Ni substitution of the solvated diazonium ion pair **297.** The 3α-alcohol, which is the minor inversion product, may arise either



296 297



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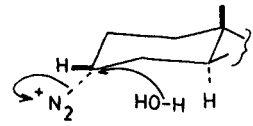

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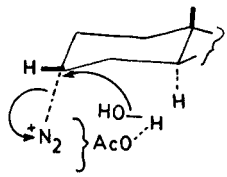
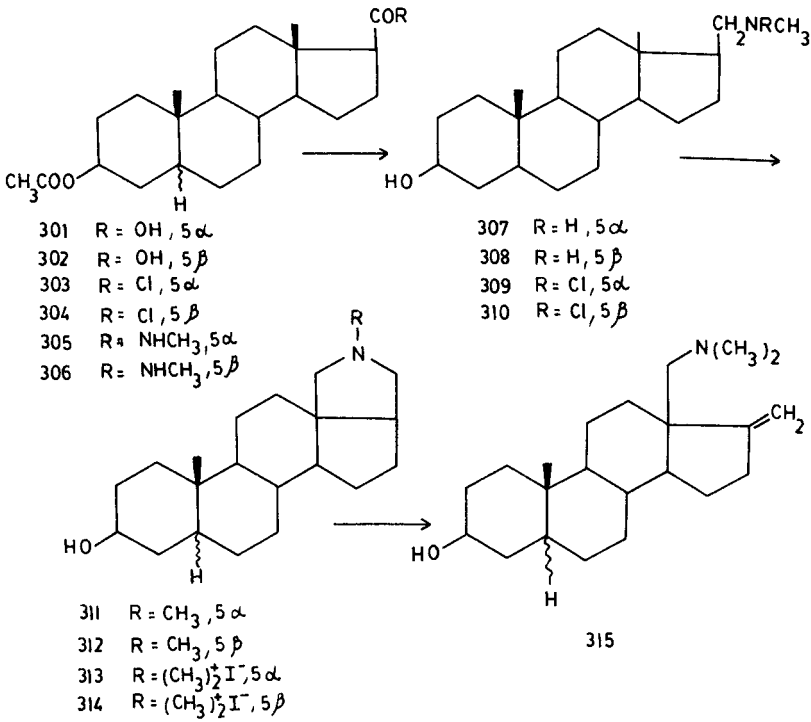


300

through the involvement of a linear S_N2 transition state of the solvated diazonium ion, or through the intermediacy of the carbonium ion **298.** Similarly, the formation of 3α-alcohol from 3α-amino-5α-cholestane may involve either a pyramidal S_N2 transition state **299** or an internal S_Ni substitution of the solvated diazonium ion pair **300.**

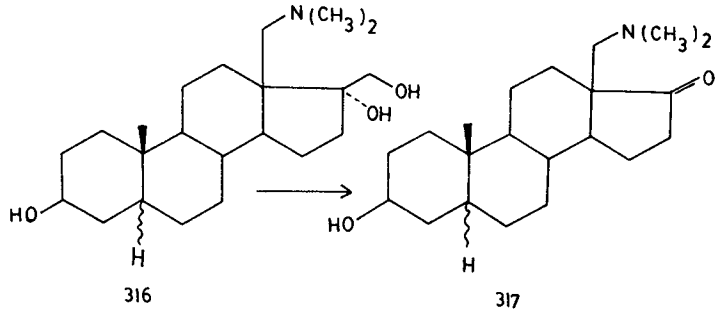


301 R = OH, 5α
302 R = OH, 5β
303 R = Cl, 5α
304 R = Cl, 5β
305 R = $NHCH_3$, 5α
306 R = $NHCH_3$, 5β

307 R = H, 5α
308 R = H, 5β
309 R = Cl, 5α
310 R = Cl, 5β

311 R = CH_3, 5α
312 R = CH_3, 5β
313 R = $(CH_3)_2^+I^-$, 5α
314 R = $(CH_3)_2^+I^-$, 5β

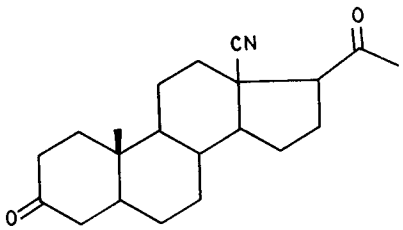
315



SCHEME 53

18-Dimethylamino-3β-hydroxy-androst-17-ones (**317**) epimeric at C-5 were prepared from the corresponding 3β-acetoxy etianic acid (*114*). The epimeric *N*-methyl amides **305** and **306,** prepared from 3β-acetoxy-5α-etianic acid (**301**) and its 5β-isomer **302** through the corresponding acid chlorides **303** and **304,** were reduced with sodium aluminium hydride to give the monomethylamines **307** and **308** respectively. Conversion of the C-5 epimeric amines **307** and **308** to the corresponding *N*-chloroderivatives **309** and **310** followed by a modified Loeffler–Freytag reaction in trifluoroacetic acid (*115*) yielded the norconanines **311** and **312,** which were quaternized with methyl iodide to the quaternary salts **313** and **314.** Degradation of the quaternized norconanines **313** and **314** to the olefins **315** epimeric at C-5 was accomplished with potassium *t*-butoxide in dimethylformamide. The olefins **315** were treated with performic acid to give the hydroxy derivatives **316.** The C-5 epimeric dimethylaminoketones **317** were prepared by periodic acid cleavage of the hydroxy compounds **316** (Scheme 53).

Preparation of 18-nor-13β-cyanotestosterone (**322**) from 18-nor-13β-cyano-5α- pregnan-3,20-dione (**318**) has been accomplished using Bayer–Villiger reaction (*116*). Reaction of **318** with trifluoroacetic acid yielded the corresponding 17β-acetoxy compound **319.** This was converted to the 2-bromo derivative by



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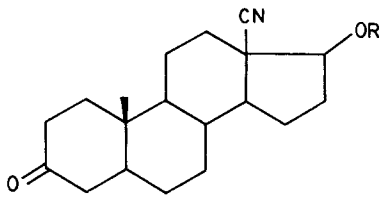


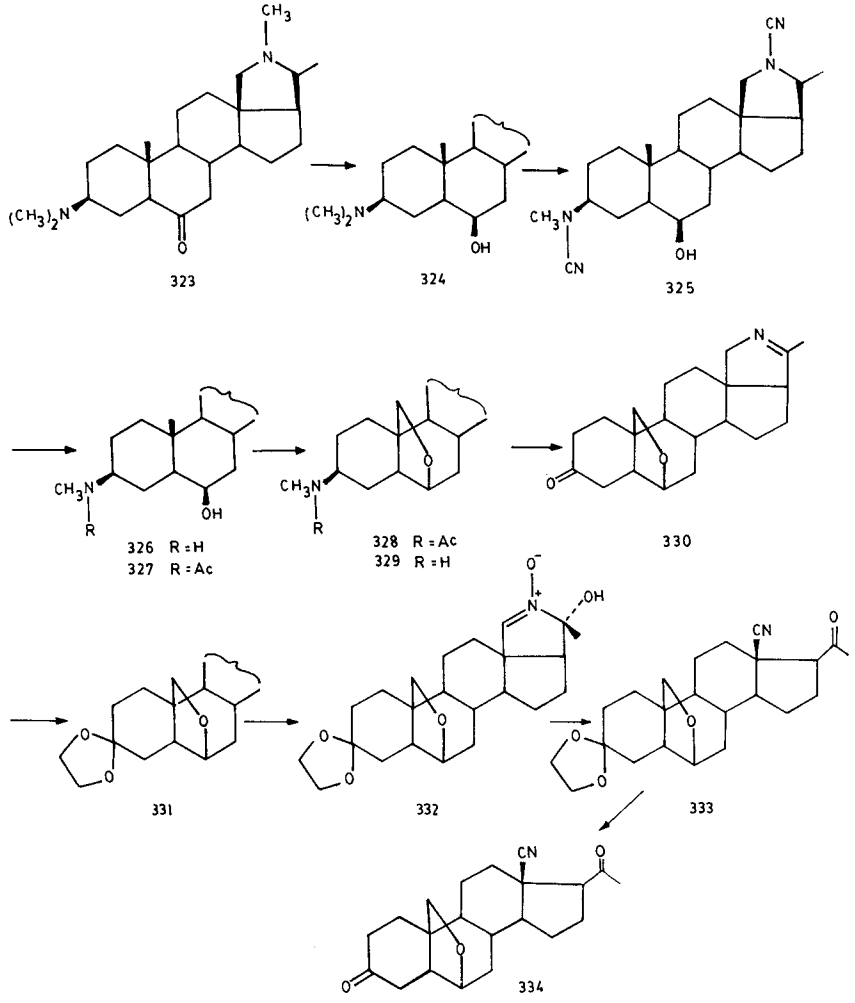
319 R= Ac

320 R= Ac, 1,2- dehydro

321 R= Ac, 4,5- dehydro

322 R= H, 4,5- dehydro

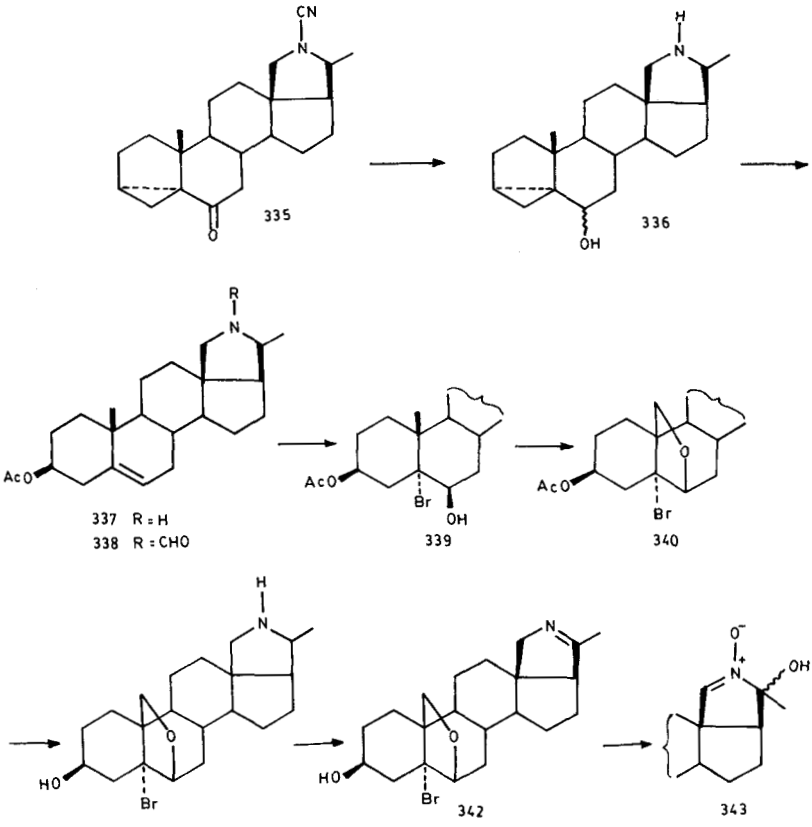
reaction with phenyltrimethylammonium bromide and dehydrohalogenated to the olefin **320.** *The 4(5)-double bond was introduced by conversion of* **319** *to the* 2,4-dibromo derivative which was dehydrohalogenated and subsequently reduced with zinc to give 18-nor-17β-acetoxy-13β-cyano-4-androstene- 3-one (**321**). Hydrolysis of **321** gave 18-nor-13β-cyanotestosterone (**322**). A similar method has been used previously for the preparation of derivatives of 18-nor-13β-cyano-5α-androstane (*117*).

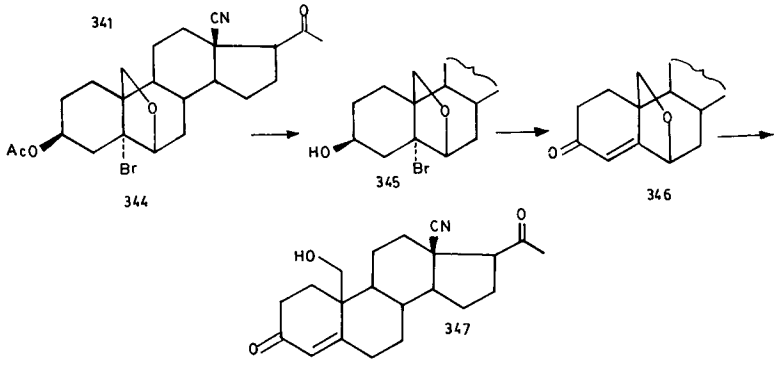


SCHEME 54

The partial synthesis of 6β,19-oxo-18-nor-13β-cyano-5α-pregnane-3,20-dione (**334**) has been achieved (*118*) with 6-ketodihydroconessine (**323**) as the starting compound. Reduction of **323** to the 6β-hydroxy derivative **324,** and reaction with cyanogen bromide, led to the *N,N'*-dicyano derivative **325.** This was hydrolyzed to **326** and acetylated to the corresponding *N*-acetyl derivative **327.** Reaction of **327** with $Pb(OAc)_4$ led to an oxidative intramolecular radical cyclization. Subsequent reduction with $NaBH_4$ yielded the 6β,19-oxo-derivative **328.** Deacetylation of **328** to **329** and Ruschig deamination yielded the imine **330.** The corresponding ketal **331** was converted into the α-hydroxy nitrone **332** by oxidation with *p*-nitroperbenzoic acid and then treated with $POCl_3$ to give the 13β-cyano derivative **333.** Hydrolysis of **333** led to the formation of 6β,19-oxo-18-nor-13β-cyano-5α-pregnane-3,20-dione (**334**) (Scheme 54).

N-Demethyl-*N*-cyano-3α,5α-cyclo-6-conanone (**335**) prepared from conessine (**9**) (*119*) may be converted to 18-nor-13β-cyano-19-hydroxyprogesterone (**347**) by functionalization of the C-19 methyl group and degradation of the

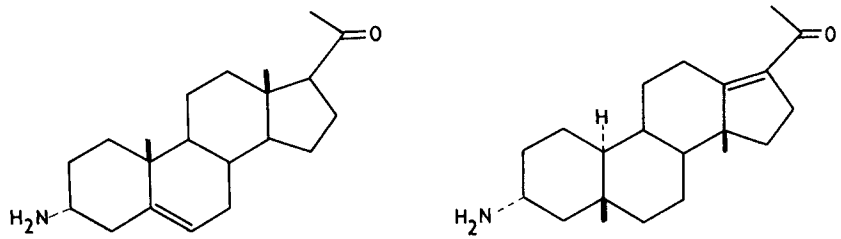


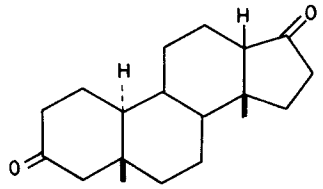


SCHEME 55

pyrrolidine ring (*120*). LAH reduction of the C-6 keto group of **335** followed by hydrolysis of the N-cyano group yielded the corresponding 6-hydroxy compound **336,** which on reaction with acetic acid and sulfuric acid resulted in the ring opening of the cyclopropane latifoline (**338**); formation of the bromohydrin **339,** and oxidative cyclization with $Pb(OAc)_4$ yielded the 19-oxo-compound **340.** Hydrolysis of **340** to the secondary amine **341** and Ruschig reaction led to the imine **342,** which was then oxidized with *p*-nitroperbenzoic acid to the α-hydroxy nitrone **343.** The ketonitrile **344,** obtained on treatment of **343** with acetic anhydride, was hydrolyzed to the corresponding 3-hydroxy compound **345** and oxidized with Jones reagent to 18-nor-6β,19-oxo-13β-cyano-4-pregnan-3,20-dione (**346**). Reductive ring opening with zinc led to the 18-nor-13β-cyano-19-hydroxyprogesterone (**347**) (Scheme 55).

Rearrangement of the 3α- and 3β-amino steroids in acidic medium has been investigated (*121–123*). Treatment of holamine (**348**) with sulfuric acid gave isoholamine (**349**) having the α-configuration at C-10. The structure of **349** was confirmed by its conversion to *A*-nor-D-homo-5β,14α-androstane-3,17-dione





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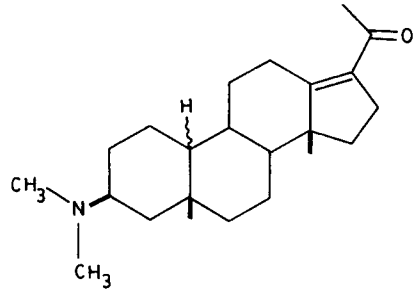

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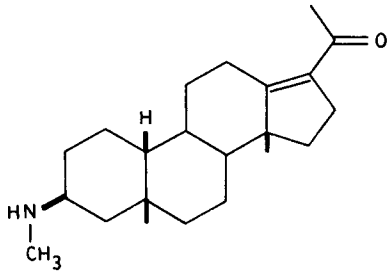
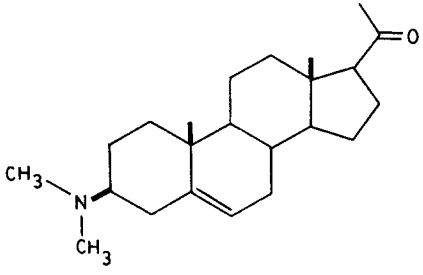


352

(**350**). Methylholaphylline (**24**) under similar conditions gave a mixture of epimeric ketones (**351**), the 10β-ketone being the major compound. The presence of a 3β-dimethylamino group seemed to be responsible for the formation of the 10β-isomer as the predominant product. Similarly, the ketone **352** was obtained from holaphylline (**25**). In order to study the mechanism for this rearrangement, 8β-$^{2}H_{1}$-holamine and 8β-$^{2}H_{1}$-methylholaphylline were prepared and treated with H_2SO_4 (*124*). The formation of rearranged products **349** and **351** was accompanied by high loss of deuterium, suggesting that the rearrangement pathway involves formation of the olefin-possessing 8,9-double bond followed by reprotonation at C-9.



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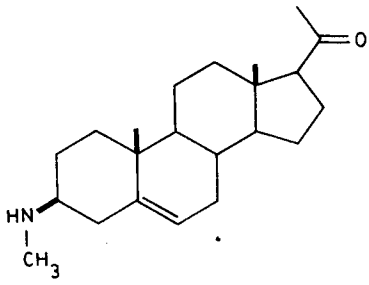

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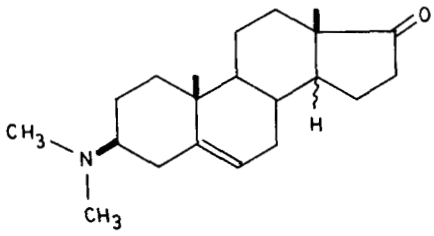
The distribution of label in polydeuterated isoholamine, formed on rearrangement of holamine in D_2SO_4 has been determined by high-field NMR studies and has led to a proposal for the mechanism of the backbone rearrangement of amino steroids (*125*). It was considered that the formation of a carbonium ion at C-5 is followed by migration of the C-19 methyl group from C-10 to C-5. Shifting of the charge from C-10 to C-14 may proceed by 1,2-hydrogen shifts or by a protonation-deprotonation mechanism (*123*).

3β-Dimethylamino-5β-methyl-14-oestr-8-en-17-one (**354**) was obtained from the 14α- and 14β-isomers (**353**) of 3β-dimethylaminoandrost-5-ene-17-one on reaction with H_2SO_4 (*126*). It was suggested that the C-14 proton also takes part in the rearrangement but the more thermodynamically stable olefin with 8(9)-double bond is finally obtained. Under similar conditions, 3α-methylami-



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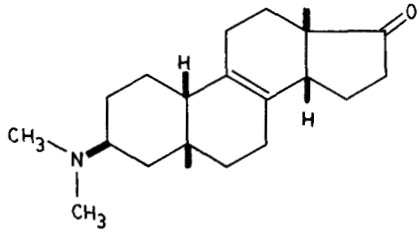

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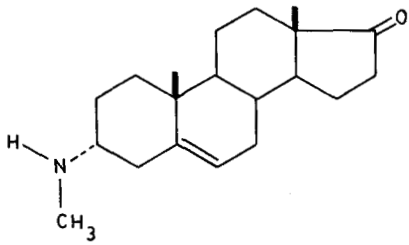

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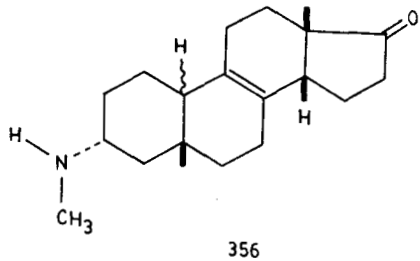

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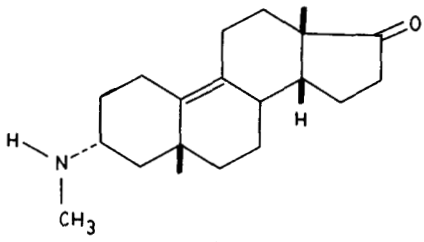

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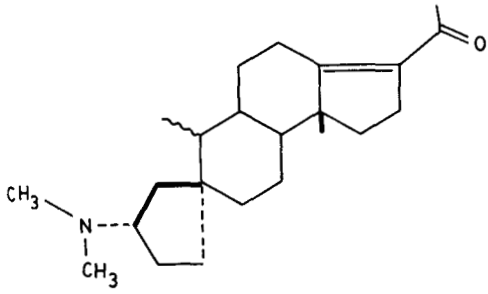

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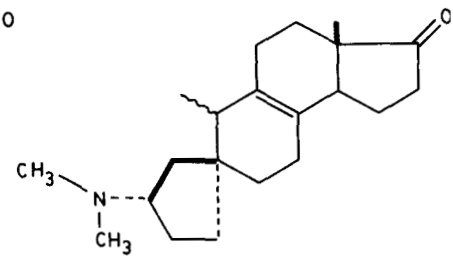
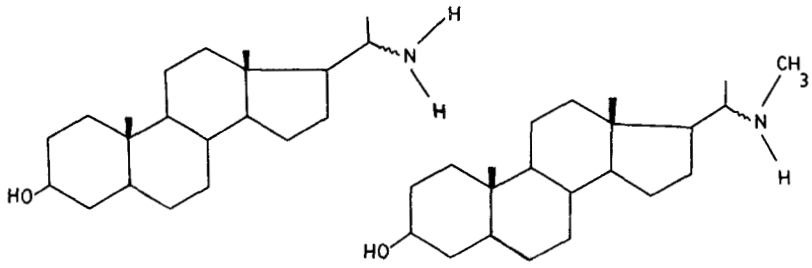


359

noandrost-5-en-17-one (**355**) produced an equilibrium mixture of 10α and 10β compounds (**356**). It was assumed that the intermediate **357** led to the formation of C-10 epimers. From these results it was concluded that the final products of a rearrangement could not be predicted without considering the nature, position, and configuration of the substituents present in a molecule. A number of similar rearrangements with various steroidal amines has also been reported (*127–129*).

Isomerization of methylholaphylline (**24**) and 3β-dimethylaminoandrost-5-en-17-one (**353**) to **358** and **359** was observed to occur in sulfuric acid acetic acid (*126,127*).



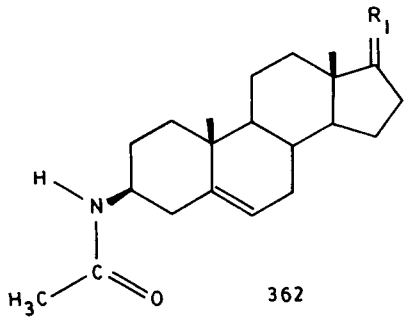
360

361

Methylation of holafebrine (**360**) to irehine (**361**) by reaction with formaldehyde in methanol, followed by reduction with $NaBH_4$, was found to be more effective than the classical Eschweiler–Clarke method (*130*).

O-Methylation of secondary amides **362** to the imidates **363** with methyl fluorosulfonate has been described (*131*). Reduction of the imidates **363** with sodium amalgum afforded the corresponding primary amines **364.**

The regioselective reductive amination of steroidal ketones with sodium borocyanohydride has been investigated (*132*). 5α-Pregnane-3,20-dione (**365**) was converted to the steroidal alkaloids funtumine (**366**) and *N*-methylfun-



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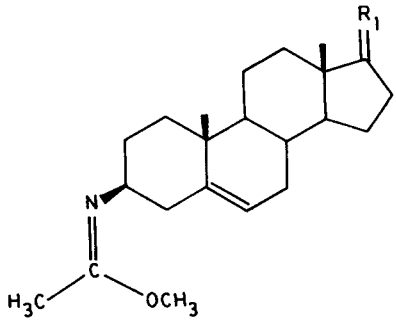

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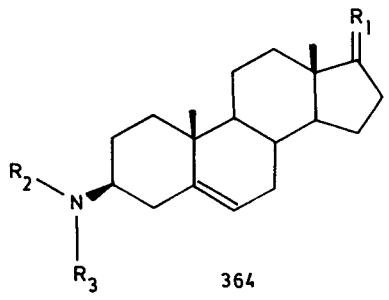
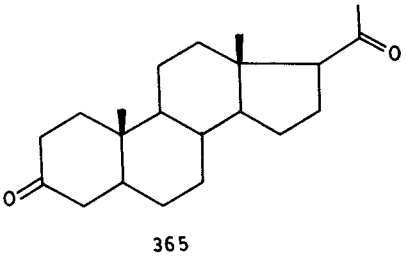


364

$R_1 = \alpha H, \beta C_8H_{17}$
$\alpha H, \beta COCH_3$
O or H_2

362 $R_2 = H, R_3 = C_2H_5$
363 $R_2 = R_3 = H$
364 $R_2 = R_3 = CH_3$



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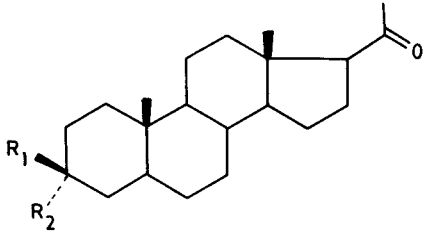
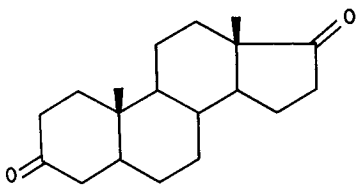
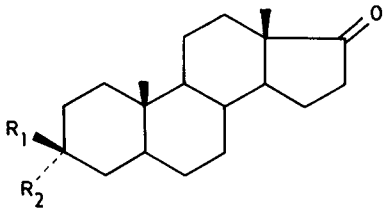


366 $R_1 = H, R_2 = NH_2$
367 $R_1 = H, R_2 = NHCH_3$
368 $R_1 = H, R_2 = N(CH_3)2$

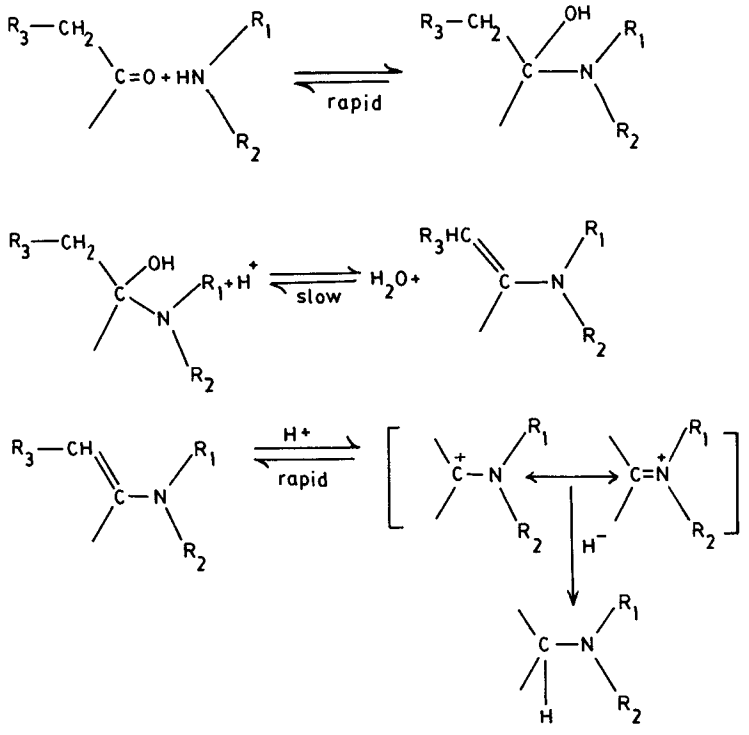


369

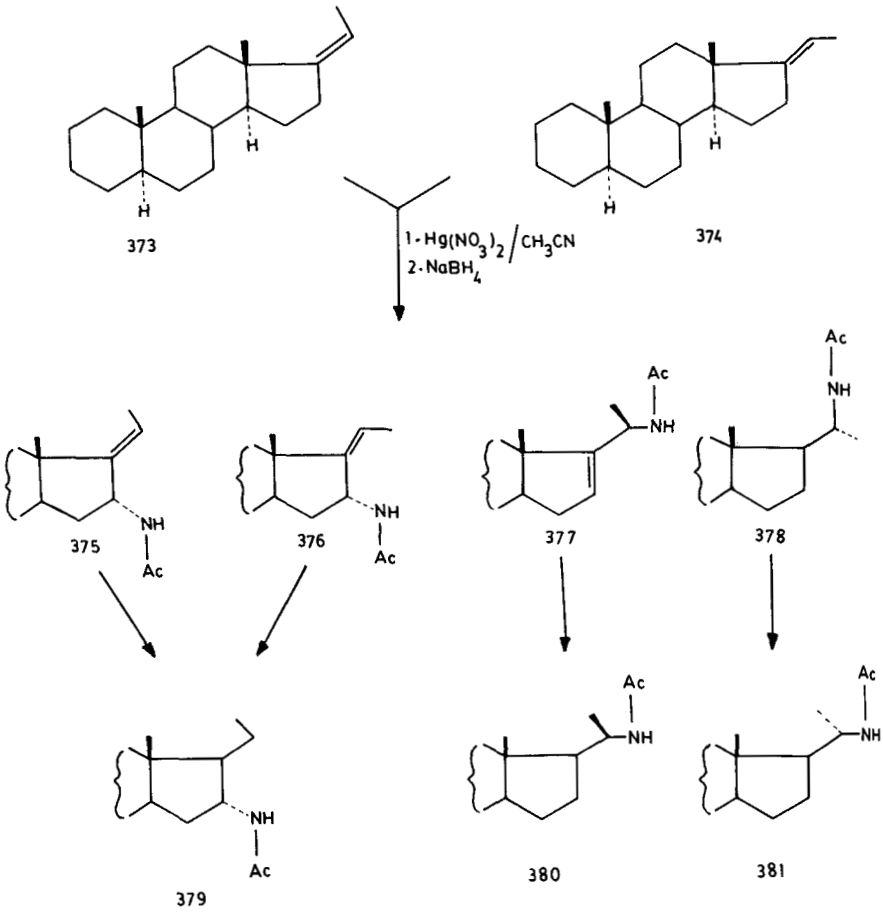


370 $R_1 = H, R_2 = NH_2$
371 $R_1 = H, R_2 = NHCH_3$
372 $R_1 = H, R_2 = N(CH_3)_2$

tumine (**367**), together with **368,** by reductive amination with $NaBH_3CN$ and the corresponding aminating reagent ($NH_4^+AcO^-$, $CH_3NH_3^+Cl^-$, or $(CH_3)_2NH_2^+Cl^-$). A similar reaction with 5α-androstane-3,17-dione (**369**) gave the 3-amino derivatives **370, 371,** and **372** (Scheme 56).



SCHEME 56

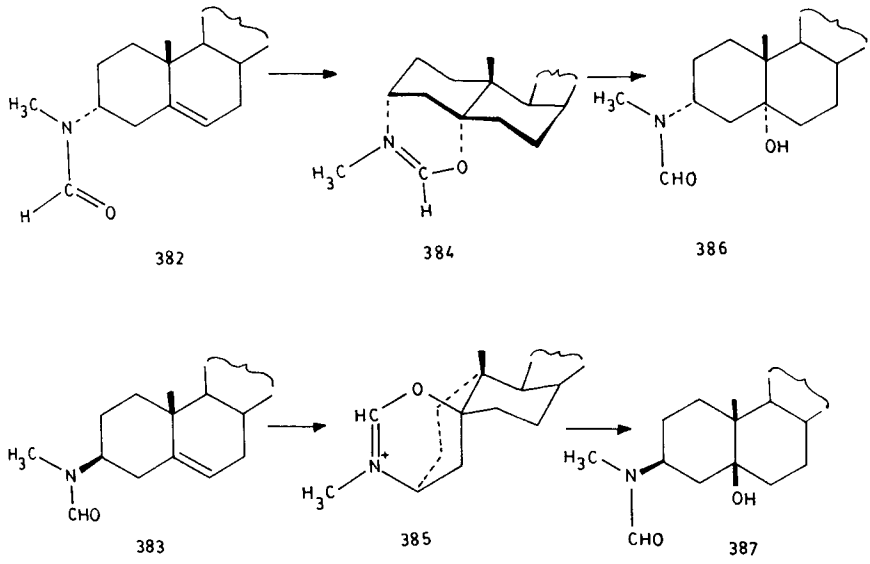


SCHEME 57

The Z and E isomers (**373**) and (**374**) of 5α-pregn-17-ene have been transformed to the allylic amides **375–378** by reaction with acetonitrile in the presence of mercuric nitrate, followed by reduction with sodium borohydride (*133*). Catalytic reduction of **375** and **376** led to the amide **379,** whereas **377** and **378** gave the (20*S*)- and (20*R*)-acetamido-5α-pregnanes (**380**) and (**381**), respectively (Scheme 57).

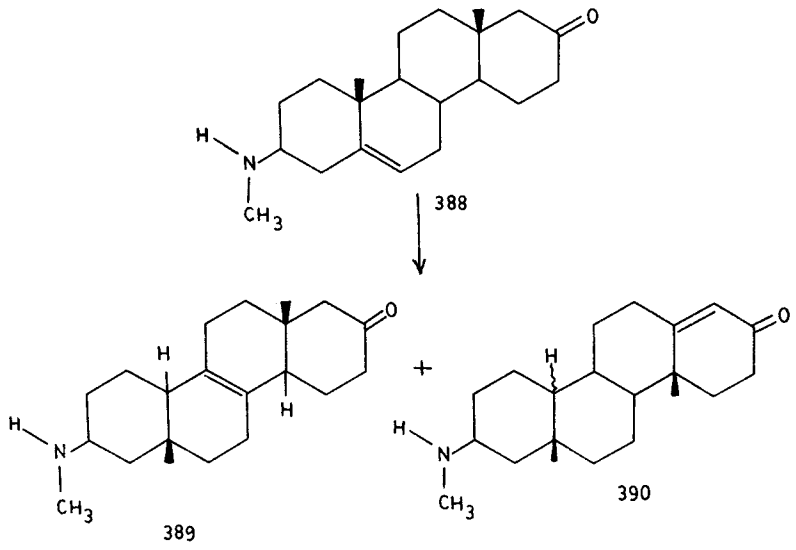
The 3α- and 3β-amido-5(6)-dehydro steroids **382** and **383** undergo stereoselective hydroxylation at position 5 in the presence of trifluoroacetic acid to give the corresponding hydroxy compounds **386** and **387** through the dihydrooxazinium intermediates **384** and **385** respectively (Scheme 58) (*134*).

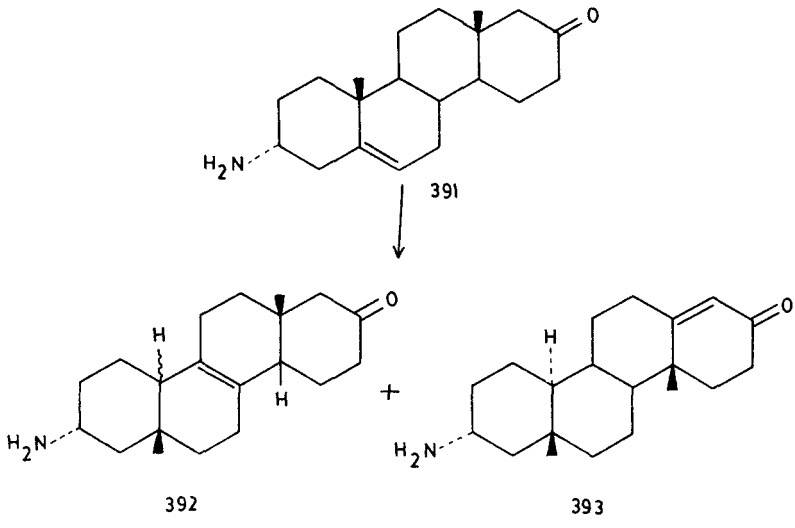
Backbone rearrangement of 3β-methylamino-D-homoandrost-5-en-17-one



SCHEME 58

(**388**) in sulfuric acid at 0°C yields the isomers **389** and **390.** On the other hand, 3 α-amino-D-homoandrost-5-en-17-one (**391**) was isomerized to **392** and **393,** as represented in Scheme 59 (*135*).

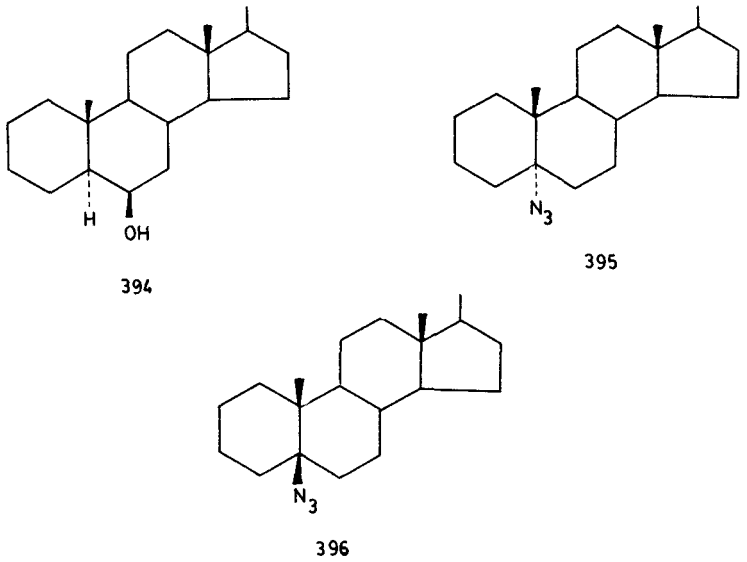


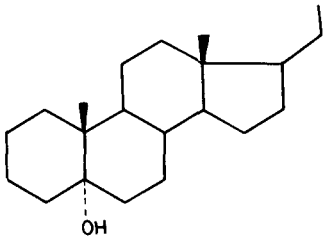


SCHEME 59

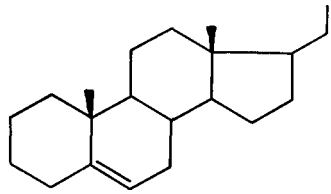
Steroidal tertiary alcohols or olefins have been converted into the 5-, 13-, and 14-azido derivatives by reaction with N_3H/BF_3-etherate in benzene (*136,137*).

Reaction of 6β-hydroxy-5α-pregnane (**394**) with HN_3/BF_3 in ether yielded a mixture of the 5α-azido-pregnane **395** and 5β-azidopregnane (**396**). These epimeric azidopregnanes were also prepared from the 5α- and 5β-hydroxypreg-





397



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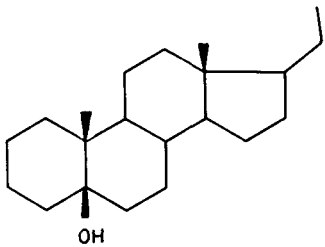

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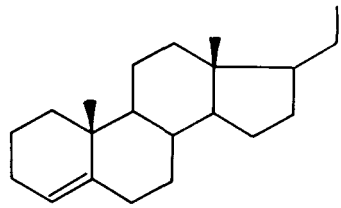
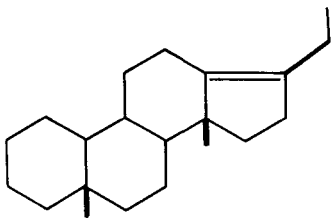


400



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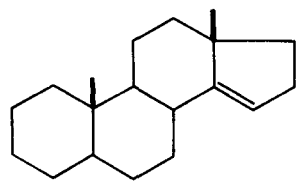

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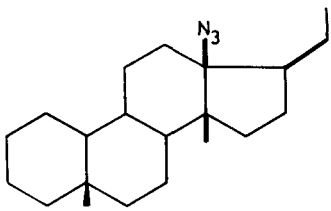

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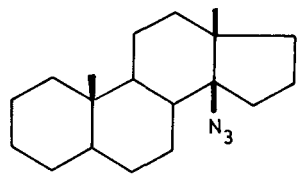
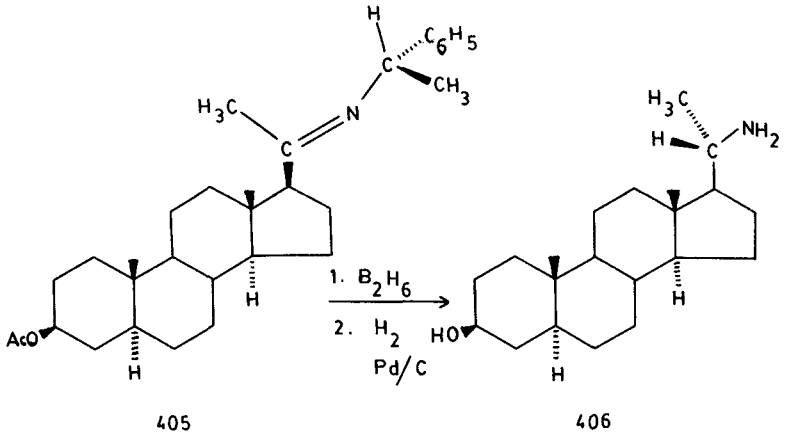


404

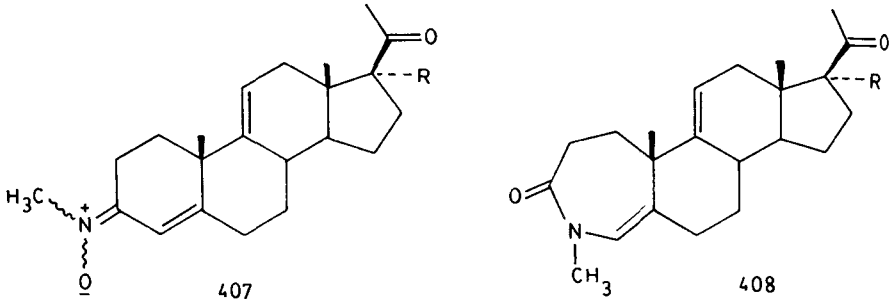
nanes (**397**) and (**398**) through the formation of olefins **399** and **400.** Similar reaction with 18,19-dinor-5β,14β-dimethyl-pregn-13(17)-ene (**401**) and androst-14-ene (**402**) yielded the 13β-azido derivative **403** and 14β-azidoandrostane (**404**), both stereoselectively and regioselectively. The azides could be reduced to give the corresponding amino derivatives.



405 406

SCHEME 60

Stereospecific synthesis of 20α-aminosteroids has been accomplished by reduction of an imine containing a chiral center on nitrogen (*138*). Reduction of the imine **405,** prepared from (−)-(*S*)-α-phenylethylamine, with diborane and subsequent debenzylation, gave funtuphyllamine-A **406** as the only product (Scheme 60).

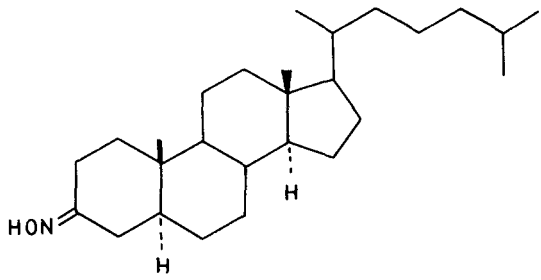


407 408

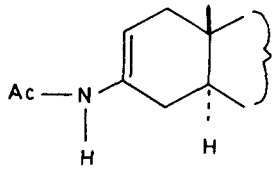
R = H or OAc

Ketonic nitrones undergo a novel rearrangement with toluene-*p*-sulfonyl chloride to give *N*-alkyl lactams (*139*). Reaction of the nitrones **407** with toluene-*p*-sulphonyl chloride yielded the lactams **408.** This procedure provides a convenient alternative to the Beckman rearrangement. Both syn- and anti-isomers led to the same lactam.

A new general method for the synthesis of enamides from ketoximes has been described (*140*). Reaction of 5α-cholestan-3-one oxime **409** with acetic anhy-



409



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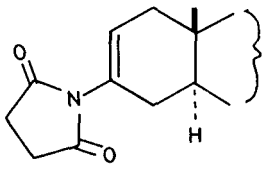
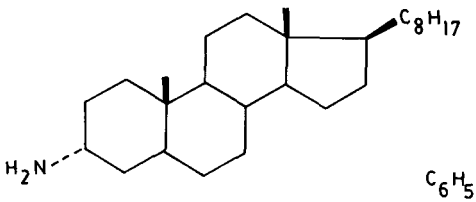
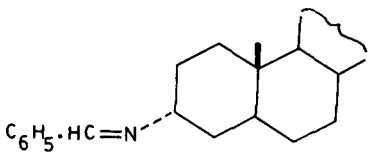


411

dride and pyridine yielded the enamide **410,** but with succinic anhydride in pyridine **411** was obtained. α-Acetoxylation may be affected on reaction with $Pb(OAc)_4$, and the method has been used for the introduction of functional groups in the side chain of 17α,21-diacetoxy-20-oxopregnane (*141*).



412



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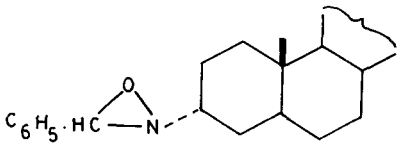
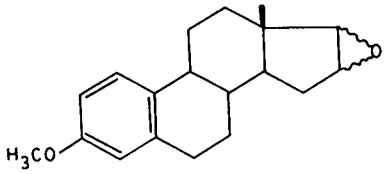
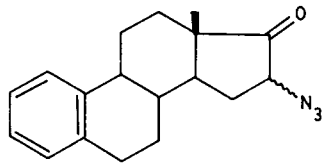


414

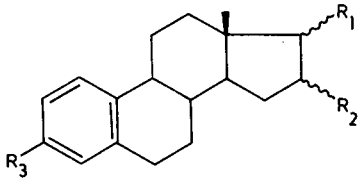
The oxidative deamination of amines to ketones through oxaziridines has been investigated (*142*). Condensation of amines such as **412** with 2-pyridine-carboxaldehyde gave the corresponding imines **413.** Oxidation of **413** with *m*-chloroperbenzoic acid provided a mixture of E and Z isomers of oxaziridines **414** which, in the presence of base, undergo ring opening to the corresponding ketones.



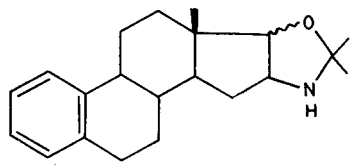
415 16β-H, 17β-H
416 16α-H, 17α-H



419 16α-H
421 16β-H

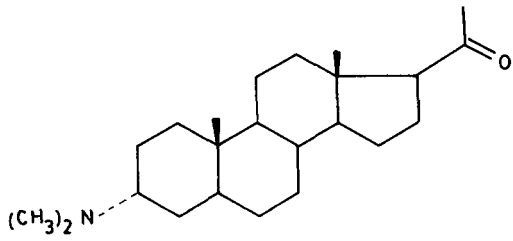


417 R_1 = αOH, R_2 = βN_3, R_3 = OCH_3
418 R_1 = βOH, R_2 = αN_3, R_3 = OCH_3
420 R_1 = βOH, R_2 = βN_3, R_3 = H
422 R_1 = αOH, R_2 = αN_3, R_3 = H
423 R_1 = αOH, R_2 = βNH_2, R_3 = OCH_3
424 R_1 = βOH, R_2 = αNH_2, R_3 = OCH_3
425 R_1 = βOH, R_2 = βNH_2, R_3 = H
426 R_1 = αOH, R_2 = αNH_2, R_3 = H



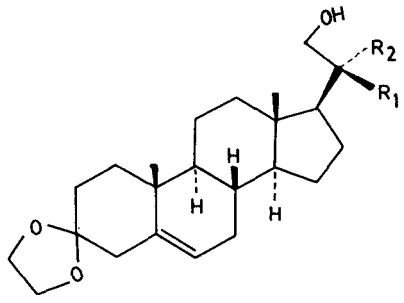
427 16α-H, 17α-H
428 16β-H, 17β-H

The synthesis of four epimeric amino alcohols (**423–426**) of oestra-1,3,5(10)-trien-3-methyl ether has been achieved by the following series of reactions (*143*). The epoxide ring in 16α,17α-epoxide **415** was cleaved by reaction with sodium azide to give the 16β,17α-azido alcohol **417.** The analogous reaction of the 16β, 17β-epoxide **416** yielded the 16α,17β-azido alcohol **418.** The 16β,17β-azido alcohol **420** was prepared by sodium borohydride reduction of the 16β-azido-17-ketone **419,** whereas reduction of 16α-azido-17-ketone **421** with lithium borohydride yielded the 16α,17α-azido alcohol **422.** The azido alcohols were reduced with hydrazine hydrate/Raney nickel to give the corresponding amino alcohols **423, 424, 425,** and **426.** The *cis*-amino alcohol **425** yielded the corresponding oxazolidine **427** on reaction with acetone; **426** yielded **428.**



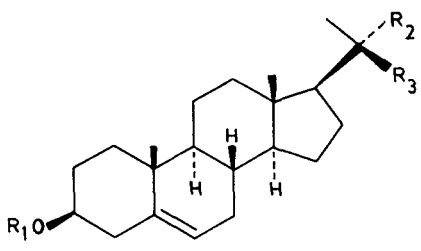
429

Selective *N*-dimethylation of primary amines by formylation and subsequent reduction with sodium cyanoborohydride has been reported (*144*). Funtumine (**366**) was converted into *N,N'*-dimethylfuntumine (**429**) by reaction with formaldehyde and sodium cyanoborohydride.

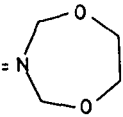


430 $R_1 = NH_2, R_2 = H$
431 $R_1 = H, R_2 = NH_2$
432 $R_1 = OH, R_2 = H$
433 $R_1 = H, R_2 = OH$

Reaction of the epimeric 20-aminopregnane derivatives **430** and **431** with nitrous acid led to the corresponding diols **432** and **433,** with retention of configuration (*145*).

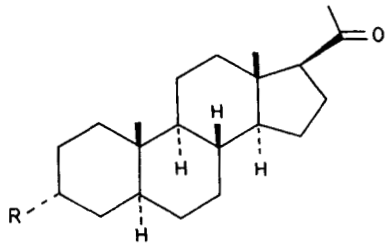


434 $R_1 = R_2 = H, R_3 = NH_2$

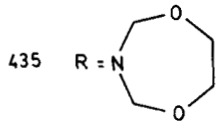


436 $R_1 = R_2 = H, R_3 =$

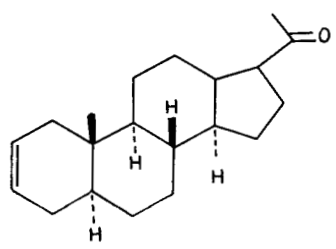
438 $R_1 = Ac, R_2 = H, R_3 = N(CH_3)_2$
439 $R_1 = Ac, R_2 = N(CH_3)_2, R_3 = H$



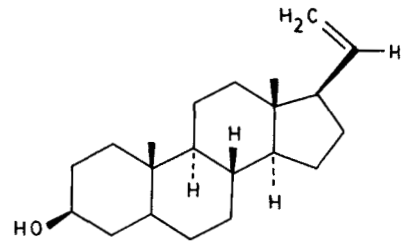
366 R = NH_2
429 R = $N(CH_3)_2$



435



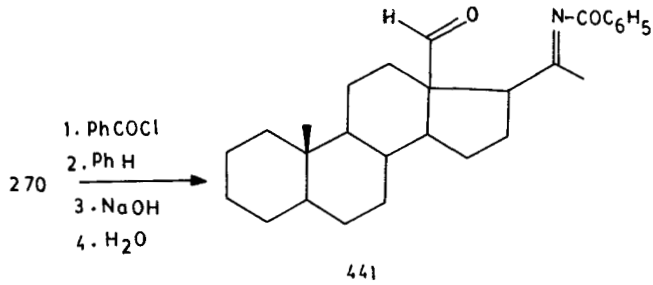
437



440

Funtumine (**366**) and epiholafebrine (**434**) react with formaldehyde and ethane-1,2-diol in benzene to afford the corresponding dioxazepines **435** and **436** (*146*).

Treatment of *N,N'*-dimethylfuntumine (**429**) with 2,2,2-trichloroethylchloroformate led predominantly to the deamination product **437,** rather than the expected N-demethylation product (*147*). Similar reaction with the epimeric 20-dimethylaminopregnane derivatives **438** and **439** produced the olefin **440** in high yields.

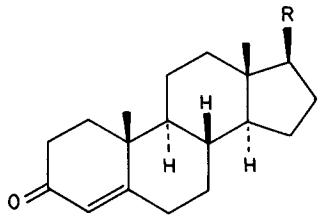


270

441

SCHEME 61

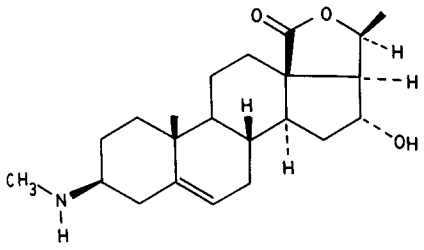
Pregnane derivatives bearing different functions on C-18 and C-20 have been prepared from the hydroxynitrones **269** and **270** by reaction with sodium borohydride and acid chlorides (*148*). In particular, benzoylation of hydroxynitrone **270** to **441** enabled selective oxidation and reduction of the free aldehyde at C-18 (Scheme 61).



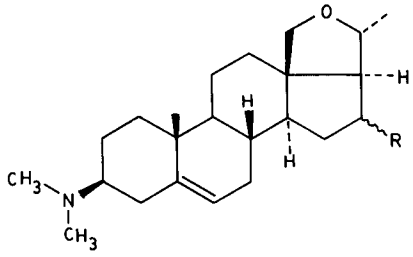
442 R = $CHCH_3NHCO_2CH_3$
443 R = $CHCH_3NH_2$
444 R = $COCH_3$

The aminopregnane **443,** obtained from **442** by acid hydrolysis, was condensed with 3,5-di-*t*-butyl-*o*-quinone to give an anil which was hydrolyzed to progesterone (**444**) (*149*). Aminopregnane derivatives have also been prepared by degradation of solasodine (*150*).

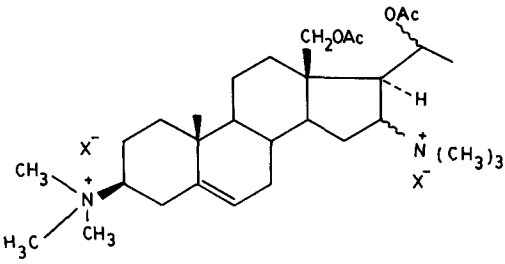
The quaternary ammonium salt **449** has been prepared from paravallaridine (**51**) (*151*). Epoxypregnenol (**445**), prepared from paravallaridine by successive N-methylation, reduction, and recyclization, underwent oxidation and then reduction to give **446.** This was then tosylated, substituted by azide ion, and reduced to afford **447.** Treatment of the *N*-dimethyl derivative **448** with acetic anhydride and subsequent quaternization yielded **449.**



51



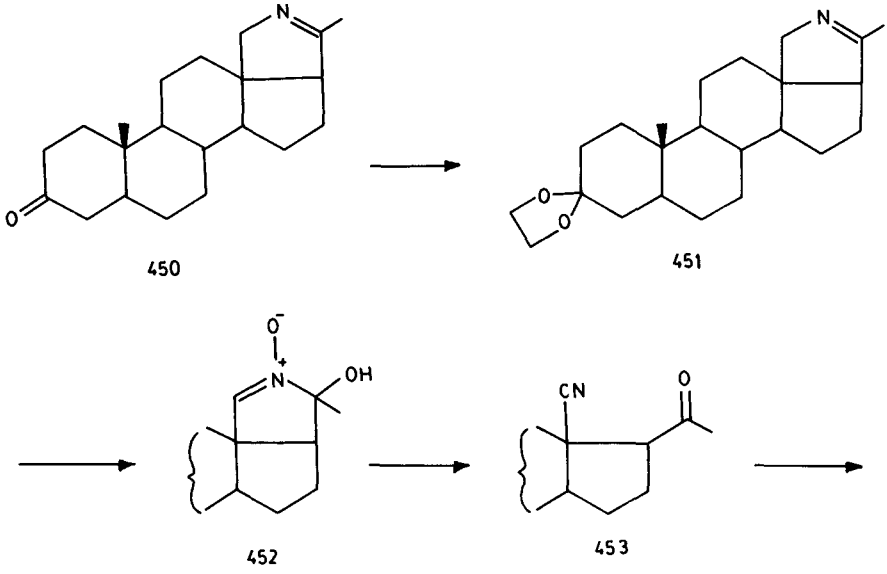
445 R = α OH
446 R = β OH
447 R = α NH_2
448 R = $N(CH_3)_2$

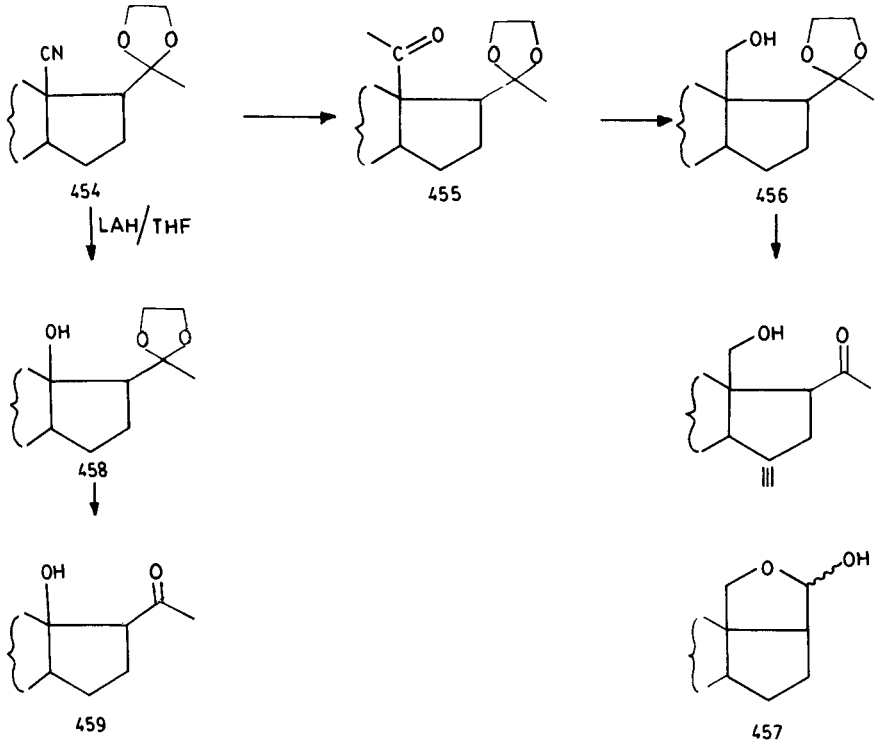


449

3. The Paravallarine Group

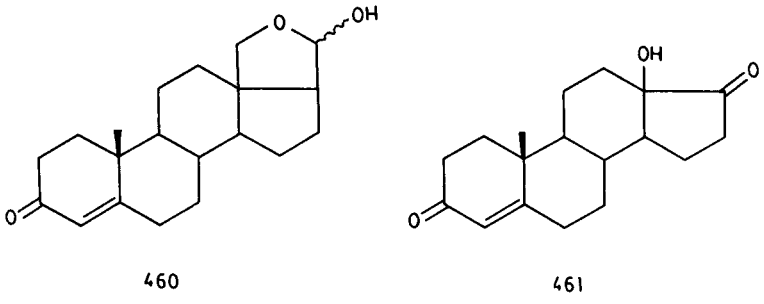
The synthesis of 18-hydroxy-4,5-dihydroprogesterone (**457**) and 18-nor-13β-hydroxy-4,5-dihydroprogesterone (**459**) has been reported (*152*). The ketal **451** of *N*-demethyl-4,20(*N*)-conene-3-one (**450**) was converted into the α-hydroxy nitrone **452** by reaction with *p*-nitroperbenzoic acid. Treatment of α-hydroxy-nitrone **452** with phosphorus oxychloride yielded the 18-cyano derivative **453,** which was then converted into the diketal **454.** Subsequent reduction of the diketal **454** with LAH gave the corresponding aldehyde **455,** which was reduced to the primary alcohol **456** with $NaBH_4$. Reaction of the primary alcohol **456** with acetic acid led to the formation of 18-hydroxy-4,5-dihydroprogesterone (**457**). On the other hand, reduction of **454** with LAH in THF interestingly

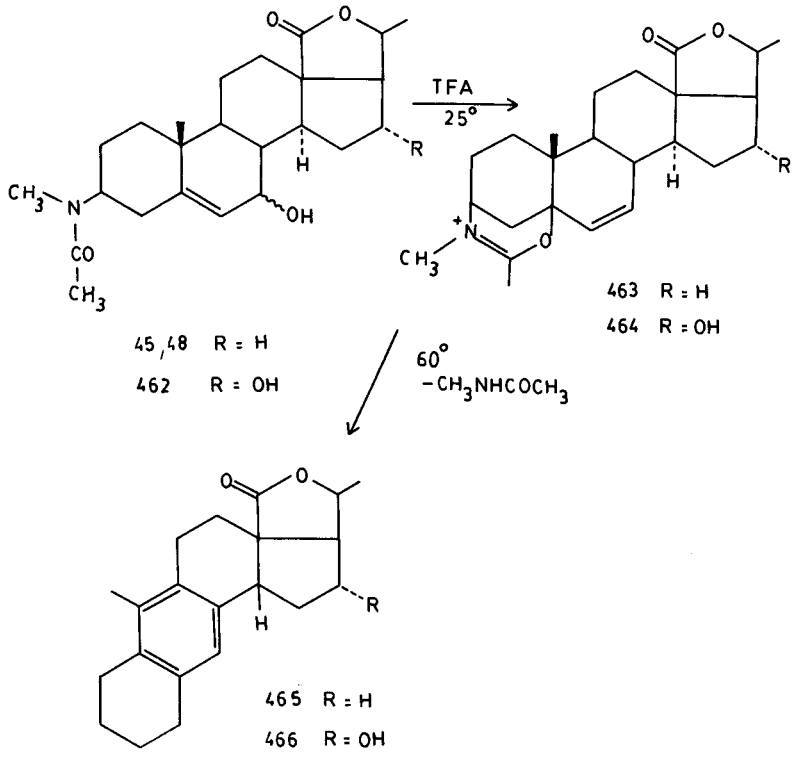




SCHEME 62

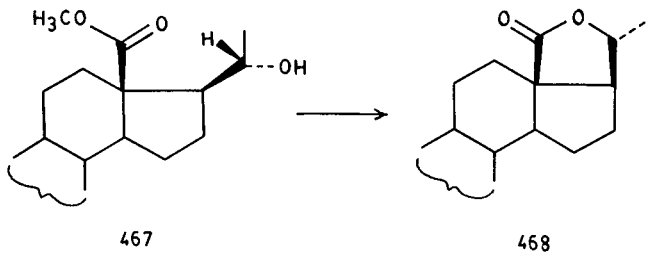
resulted in the replacement of the cyano group by a hydroxyl group and afforded the corresponding tertiary alcohol **458.** This was converted into 18-nor-13β-hydroxy-4,5-dihydroprogesterone (**459**) in the presence of acetic acid (Scheme 62). An identical procedure was used for the preparation of 18-hydroxy-progesterone (**460**) and 18-nor-13β-hydroxyprogesterone (**461**) (*153*).



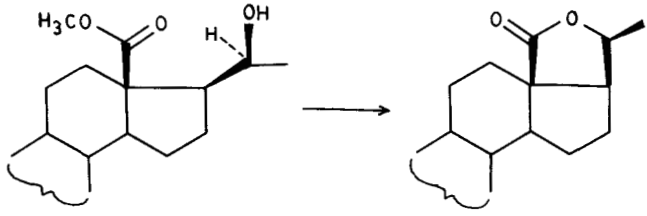


SCHEME 63

The anthrasteroid derivatives **465** and **466** have also been prepared from 7α- and 7β-hydroxy-*N*-acetyl-paravallarine (**45** and **48**) and 7α- and 7β-hydroxy-paravallaridine (**462**) by rearrangement in the presence of trifluoroacetic acid through the formation of dihydroxazinium intermediates **463** and **464** respectively (Scheme 63) (*154*).



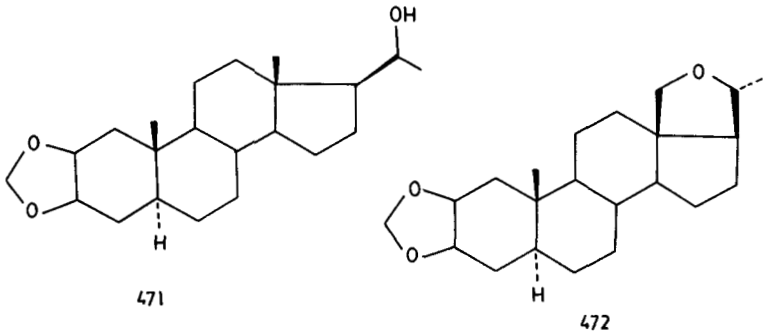
467 468



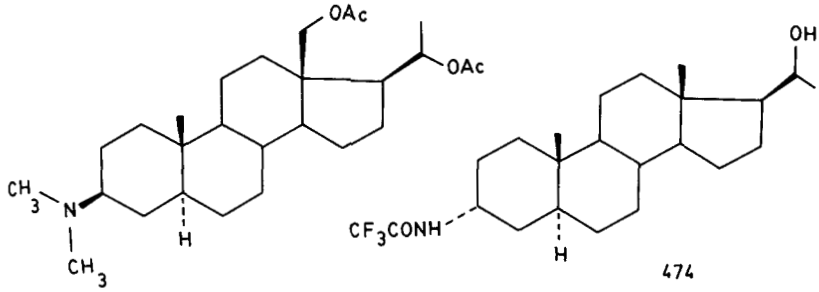
469 470

SCHEME 64

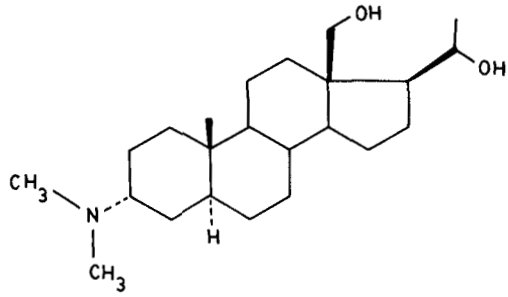
Stereoselective conversion of the 18-methoxycarbonyl steroids to the corresponding lactones has been reported (*155*). The reaction proceeds by intramolecular cyclization, with inversion of configuration at C-20 on treatment with toluene-*p*-sulphonyl chloride, the (20*S*)-alcohol **467** giving the (20*R*)-lactone **468,** whereas the (20*S*)-alcohol **469** gives the (20*S*)-lactone **470.** Similar cyclization reactions have been used for the synthesis of (20*S*)- and (20*R*)-18(*N*) steroidal pyrrolines and conessidine (Scheme 64) (*156,157*).



471 472



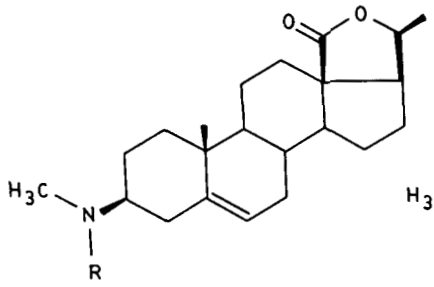
473 474



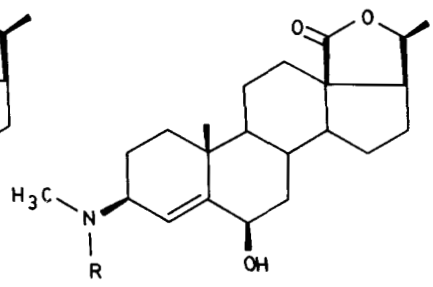
475

The 20β-hydroxy cyclic ethylene ketal **471** was transformed into the (20*S*)-3β-dimethylamino-18,20-diacetoxy compound **473** in six steps, the key step being iodination of **471** with HOI and intramolecular cyclization to **472** (*158*). Similar reaction with *N*-(trifluoroacetyl)-isofuntumidine (**474**) has been used to prepare (20*S*)-3α-dimethylamino-18,20-diol (**475**) (*159*).

A modified Treibs reaction has been used for the allylic substitution of steroidal olefins under mild conditions (*160*). Thus with mercury(II) trifluorace-



476



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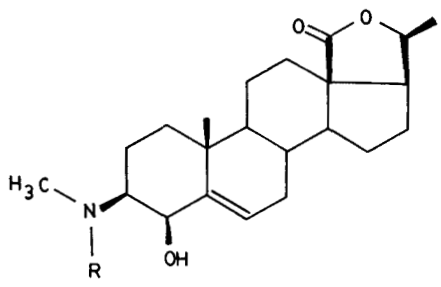


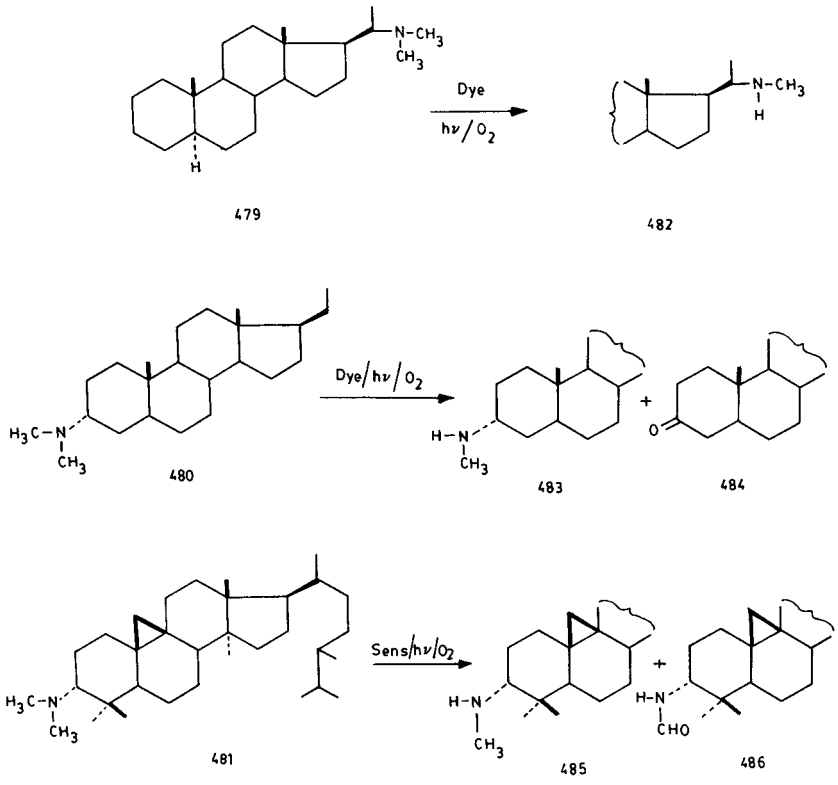
478

R = H, CH_3, $COCH_3$ or $COCF_3$

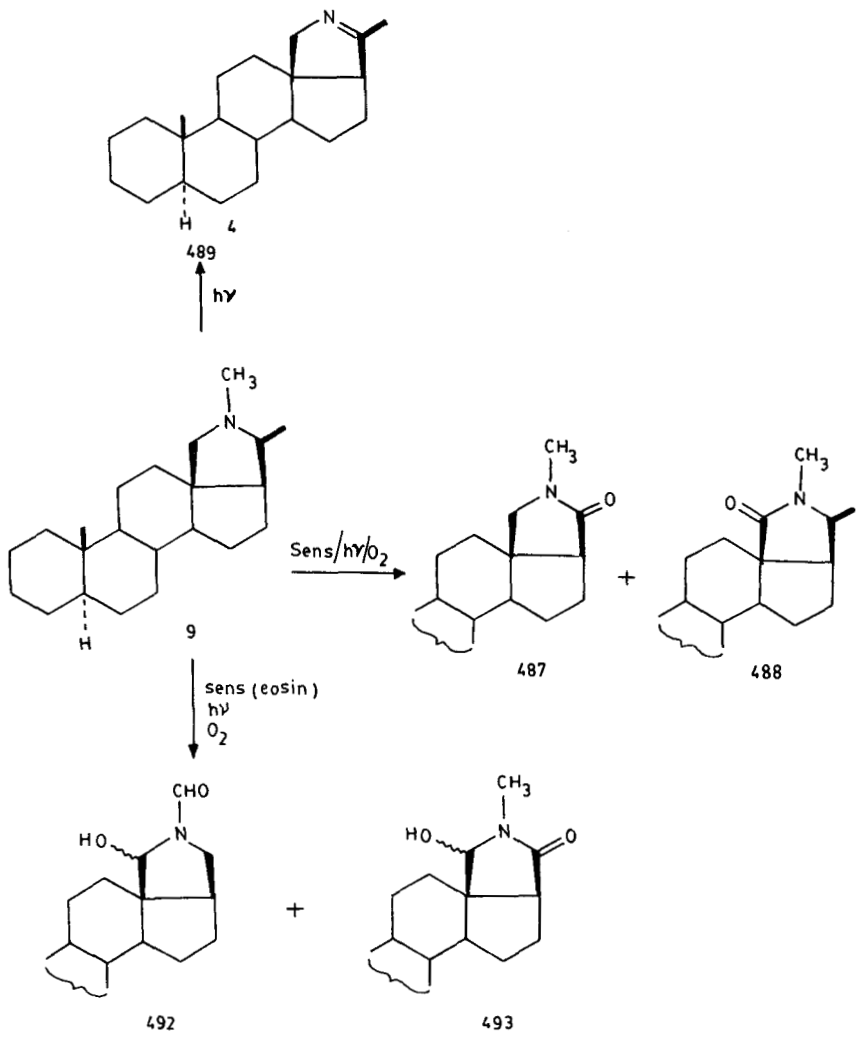
tate, paravallarine derivatives **476** were converted into the corresponding 6β-hydroxy derivatives **477.** The isomeric 4β-hydroxy compound **478** was also obtained.

C. PHOTOCHEMISTRY

Dye-sensitized photochemical transformation of tertiary amines to secondary amines and amides was achieved in 1973 (*161,162*); thus photolysis of 20α-dimethylamino-5 α-pregnane (**479**), 3α-dimethylamino-5α-pregnane (**480**), 5α-conanine (**9**), and 3β-dimethylamino cyclolaudane (**481**), in the presence of methylene blue and oxygen, led to the corresponding compounds **482–488** (Schemes 65 and 66). In the absence of oxygen, conanine gave, exclusively, the *N*-demethylated imine **489.** This was explained by a mechanism involving trans-

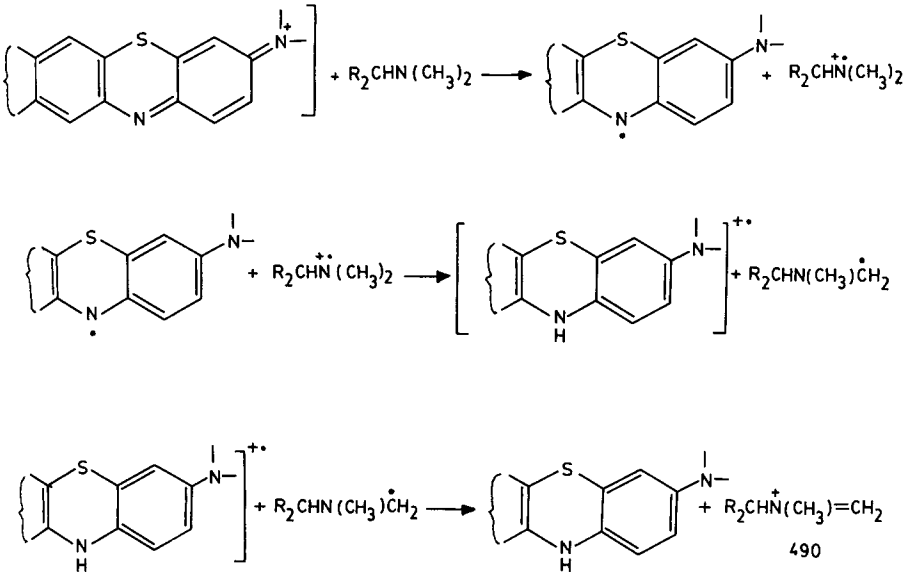


SCHEME 65



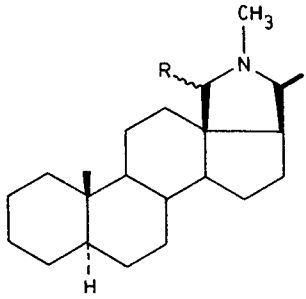
SCHEME 66

fer of charge from the excited triplet state of the dye to the amine. Hydrolysis of the immonium ion **490** yielded the resulting secondary amines (Scheme 67). This mechanism was confirmed by the isolation of the amino-nitrile **491** on irradiation of conanine in the presence of eosin and KCN. Regioselectivity of this reaction has also been studied, and it was observed that the degree of substitution of the carbon α to the nitrogen and the acidity of hydrogens affect the regioselectivity (*163*).



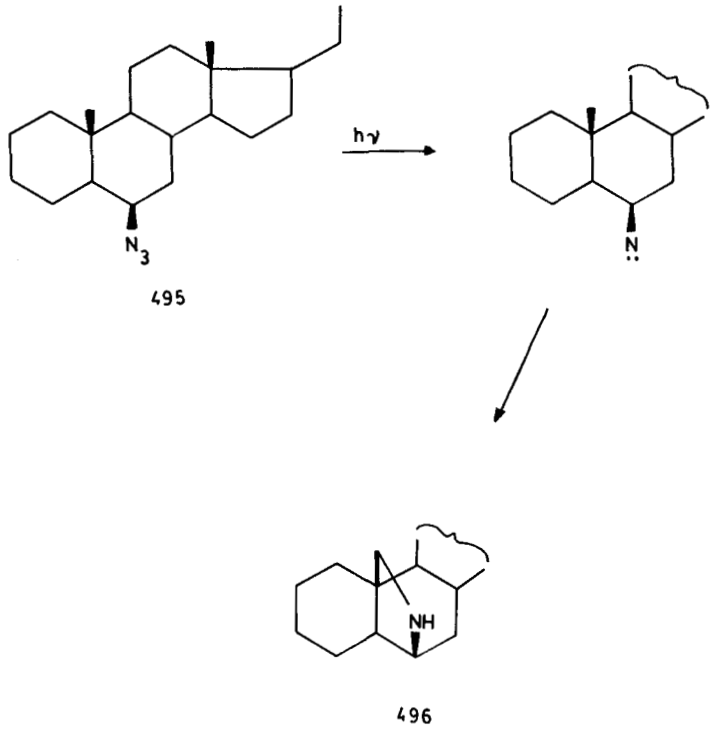
SCHEME 67

This reaction was reinvestigated and it was found that irradiation of conanine, in the presence of methylene blue and oxygen, yielded the lactams **487** and **488,** while in the presence of eosin and oxygen **492** and **493** were also obtained. When the irradiation was effected in the presence of sodium pyruvate, the epimers **494** were obtained. These results suggested that interaction of oxygen with the photosensitizer produced H_2O_2, which reacted with the tertiary amines to give the



491 R = CN

494 R = OCH_3



495

496

SCHEME 68

oxygenated compounds. Addition of sodium pyruvate prevented the formation of amides or lactams by reacting with H_2O_2 (*164*).

Formation of the pyrrolidine ring has been achieved by irradiation of a steroidal azide (*165*). 5α-Pregnan-6-one, 6-aza-B-homo-5α-pregnan-6-one-7aza- B- homo-pregn-6-ene and 6β,19-imino-5α-pregnane (**496**) were obtained through photolysis of 6β-azido-5α-pregnane (**495**) (Scheme 68).

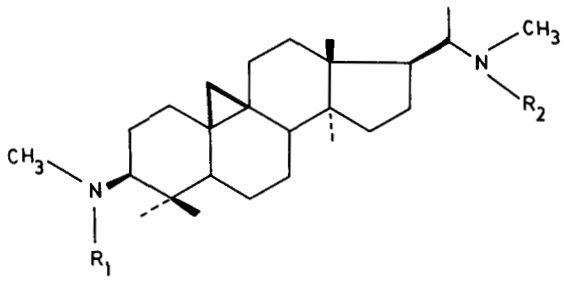
Photolysis of azido-steroids leads to alkyl migration and the products of 1,2-hydrogen transfer (*166*). Irradiation of 6β-azido-5α-pregnane (**495**) resulted in the formation of the pyrrolidine ring through an intermediate nitrene. A comparative study of the photochemistry of steroidal azides and thermolysis showed that elimination of the nitrogen molecule and migration of the alkyl group is a concerted reaction (*167*).

III. Steroidal Alkaloids of Buxaceae

A. Isolation and Structure Elucidation

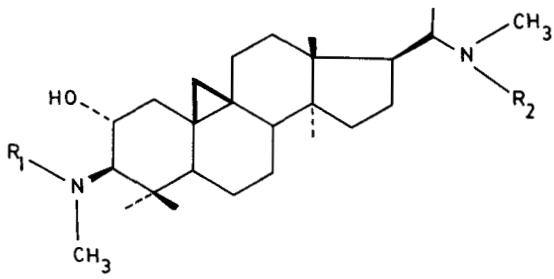
1. Derivatives of 9β-19-cyclo-5α-pregnane

a. Diamino Alkaloids. *N*-Acetylcycloprotobuxine-D (**497**), isolated from *Buxus sempervirens*, was formulated as an acetamide of cycloprotobuxine-D on



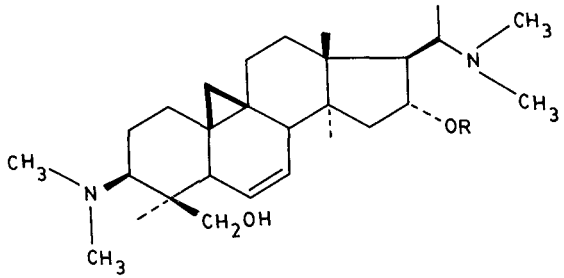
497 $R_1 = H, R_2 = Ac$
498 $R_1 = CH_3, R_2 = Ac$
499 $R_1 = Ac, R_2 = CH_3$

the basis of spectral and analytical data (*168*). Methylation of **497** gave the product **498,** which was isomeric with *N*-acetylcycloprotobuxine-C (**499**), suggesting that the new compound possessed the amide group at C-20 instead of C-3.



500 $R_1 = COC_6H_5, R_2 = H$
501 $R_1 = COC_6H_5, R_2 = H$
502 $R_1 = R_2 = H$

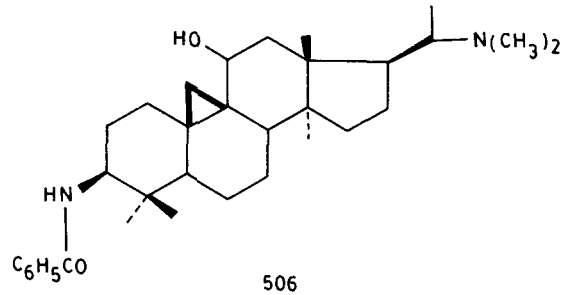
The structures of *N*-benzoylcycloprotobuxoline-C (**500**) and *N*-benzoylcycloprotobuxoline-D (**501**), isolated from *B. sempervirens,* were established on the basis of spectral data and confirmed by preparation of derivatives (*168*). A strong band at 1612 cm^{-1} in the IR spectrum of **501** was attributed to an amide function. The ^{1}H-NMR spectrum indicated the presence of two *N*-methyl groups, one proton adjacent to an OH, a cyclopropyl methylene group (two doublets at δ0.40 and δ0.55 with J = 4.0 Hz), and five aromatic protons (δ7.38). Basic hydrolysis of **501** gave cycloprotobuxoline-D (**502**). The spectral properties of *N*-benzoylcycloprotobuxoline-C (**500**) closely resembled those of **501.** A six-proton singlet at δ2.19, however, indicated the presence of an *N*-dimethyl group. Methylation of **501** to **500** with HCHO/HCOOH confirmed the assigned structures.



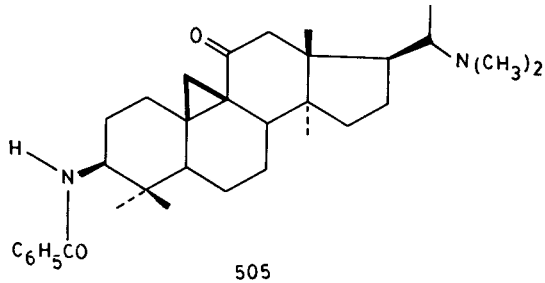
503 R = $-CO-C(CH_3)=C(CH_3)-H$

504 R = H

O-Tigloylcyclovirobuxeine-B (**503**), isolated from *B. sempervirens,* gave tiglic acid and cyclovirobuxeine-B (**504**) following saponification with methanolic potassium hydroxide (*168*). The presence of the tigloyl group was also confirmed on the basis of the characteristic signal of the vinyl proton of tiglate at δ6.82 (which appears at δ5.98 for an angelate) in the NMR spectrum.

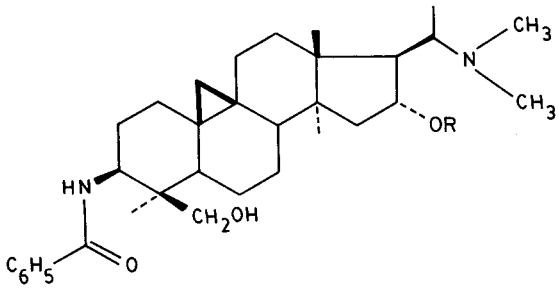


506



505

N-Benzoylcycloxobuxine-F (**505**), also described as "buxatine," has been extracted from *B. sempervirens* (*168,169*). The IR and UV spectra suggested the presence of a secondary benzamide and a carbonyl function conjugated with a cyclopropane ring. The carbonyl function was located at C-11 rather than C-1, since the methylene protons resonated as a singlet in the ^{1}H-NMR spectrum. LAH reduction of **505** yielded **506.** Confirmation of the structure was provided by correlation of **505** to cycloprotobuxine-C.



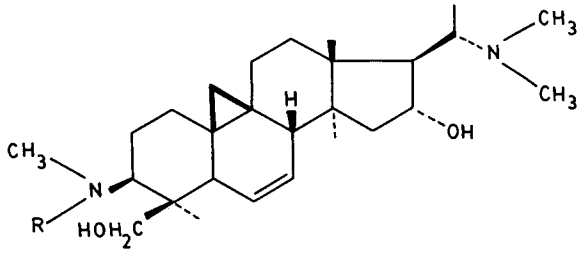
507 R = H

684 R = $COCH_3$

N-Benzoyldihydromicrophylline-F (**507**), isolated from *B. sempervirens*, showed absorption bands at 3436 cm^{-1} and 1639 cm^{-1} in the IR spectrum, indicating the presence of a secondary benzamide and an OH function. Alkaline or acid hydrolysis of **507** to the known alkaloid dihydromicrophylline-F and benzoic acid provided confirmation for the assigned structure **507** (*168*). This alkaloid has been described under the name "buxepidine" (*170*).

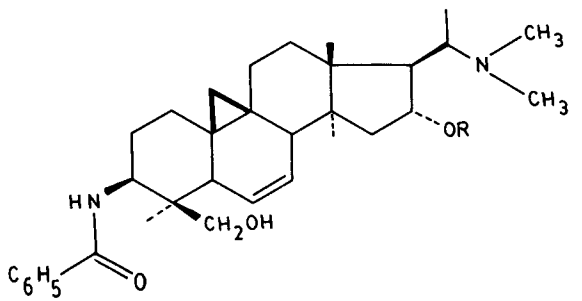
The structure of semperviramidine (**684**), isolated from *B. sempervirens* of Turkish origin, has been established on the basis of spectroscopic studies (*237*). The absorption maximum at 225 nm in the UV spectrum was characteristic of a secondary benzamide chromophore. The IR spectrum showed bands at 3350 (OH), 1721 (ester C=O), 1657 (amide C=O) and 1598 (C=C) cm^{-1}. Multi-

plets at δ7.43 and 7.68, in the ^{1}H-NMR spectrum also supported the presence of a benzamide group. A set of AB doublets resonating at δ0.38 and 0.55 ($J_{19\alpha,19\beta}$ = 3.7Hz) was assigned to the C-19 methylene protons of the cyclopropane ring. A multiplet centered at δ4.38 was attributed to the C-16 proton geminal to the acetoxy group. The molecular ion appeared at *m/z* 564.3915 ($C_{35}H_{52}N_2O_9$) in the mass spectrum. Peaks at m/z 549 and m/z 520 corresponded to the loss of methyl and 20-dimethylamino groups, respectively.



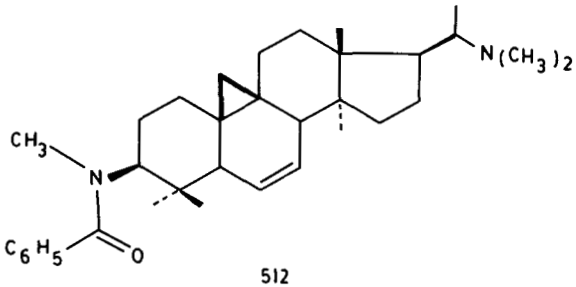
508 R= COC_6H_5
509 R= H

Cyclomicrosine (**508**), obtained from *B. microphylla* Sieb. et Zucc. *var. suffruticosa* Makino, showed the presence of *N*-benzamide (1610 cm^{-1}) and OH (3424 cm^{-1}) functions in its IR spectrum. Alkaline hydrolysis of this alkaloid yielded cyclomicrophylline-C (**509**) and benzoic acid (*171*).

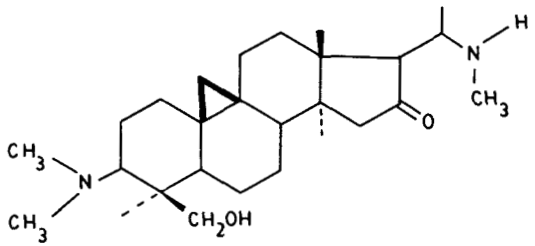


510 R = H
511 R = Ac

Buxidine (**510**) extracted from *B. sempervirens,* was deduced to be the *N*-benzoylcyclomicrophylline-F; buxandrine (**511**), from the same source was deduced to be *O*-acetyl-*N*-benzoylcyclomicrophylline-F. The deductions were made based upon their chemical behavior (*172*). Catalytic hydrogenation of (**510**) yielded buxepidine (**507**).

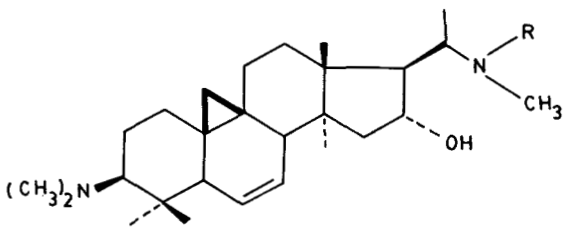


512



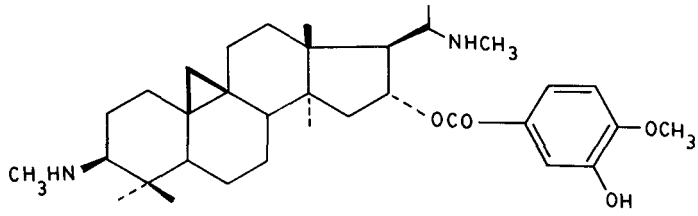
513

Buxeridine (**512**) and buxazidine-B (**513**) were isolated from *B. sempervirens* (*173*). The structures of the alkaloids were established on the basis of spectroscopic studies.



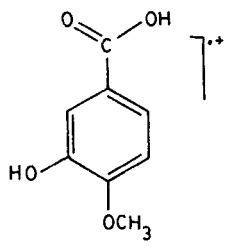
514 R = CHO
504 R = H

N-Formylcyclovirobuxeine-B (**514**), obtained from *B. malayana* Ridal. leaves, showed the presence of an amide group in its IR spectrum by absorption at 1700 cm^{-1}. Saponification of **514** with ethanolic potassium hydroxide gave cyclovirobuxeine-B (**504**) (*174*).

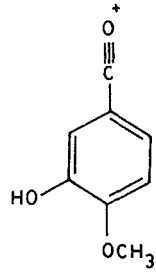


515

The IR spectrum of *O*-vanillylcyclovirobuxine-D (**515**) isolated from *B. malayana* showed absorption bands at 1710, 1600, and 1260 cm^{-1} that were characteristic of an aromatic ester. The ^1H-NMR spectrum showed signals char-

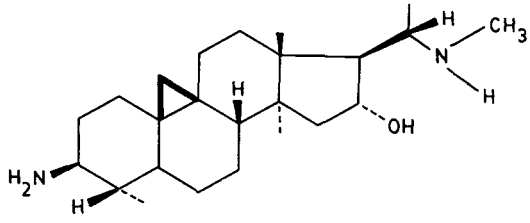


516



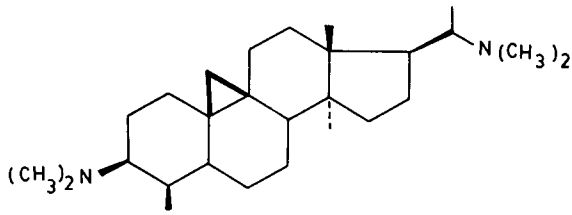
517

acteristic of cyclopropylmethylene protons, four tertiary methyl groups, a secondary methyl group, and two methyl amino groups. Signals for three protons were observed in the aromatic region. Peaks at *m/e* 168 and 151 in the mass spectrum corresponding to the fragment ions **516** and **517** also supported the presence of a vanillyl group. Cyclovirobuxine-D and vanillic acid were obtained on saponification of **515** (*174*).



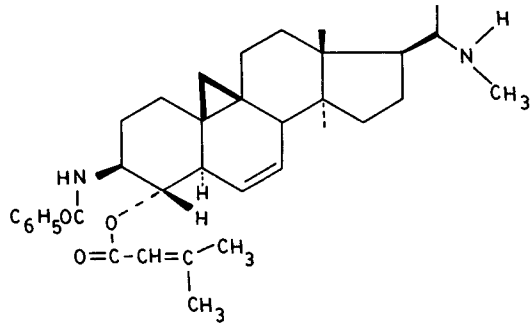
518

The structure of cyclobuxamine-H (**518**), isolated from *B. sempervirens*, was revised by Nakano, who depicted as having the 4α-methyl configuration (*171, 175–177*). The suggestion was based on the observation that replacement of the 4α-methyl by a 4β-methyl group (or introducing a 4,4-dimethyl group) caused a significant downfield shift (by δ0.20 and δ0.29) of the cyclopropylmethylene proton signals. The epimerization of the axial methyl group of the C-4 methyl derivative of cyclobuxine-D during Ruschig degradation gave indirect confirmation for this suggestion (*178*).



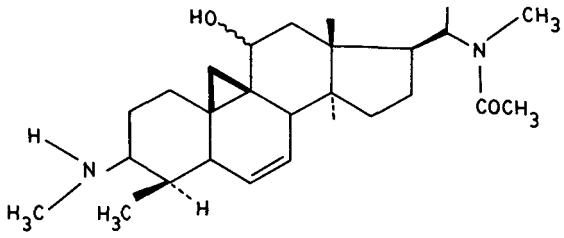
519

Buxocyclamine-A, extracted from *B. sempervirens*, has been assigned structure **519** on the basis of IR and mass spectra (*179*).



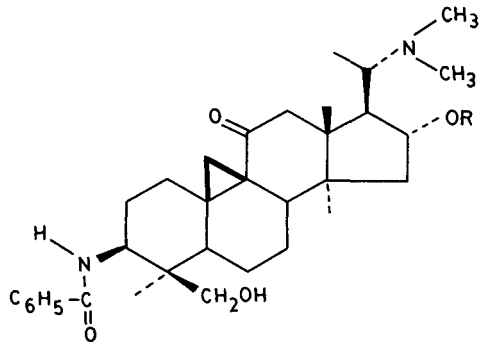
520

The structure of buxaltine (**520**), isolated from *B. sempervirens*, was determined on the basis of IR and mass spectra (*180*). The difference in molecular rotation between the alkaloid and its dihydroderivative showed the presence of a 6(7)-double bond.



521

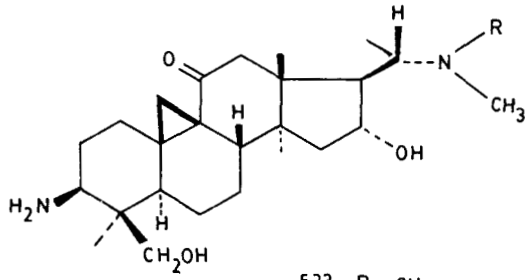
Buxiramine (**521**) was also isolated from *B. sempervirens,* together with buxaltine (**520**). The IR spectrum indicated the presence of an amide group (1640 cm^{-1}) and an olefinic linkage (1645 and 810 cm^{-1}). Peaks of *m/e* 100, 84, 70, 58, 57, and 44 were observed in the mass spectrum. The ^{1}H-NMR spectrum of **521** exhibited a one-proton multiplet at δ1.00, attributable to the hydroxyl group present on C-11 (*180*).



522 R = H
685 R = $COCH_3$

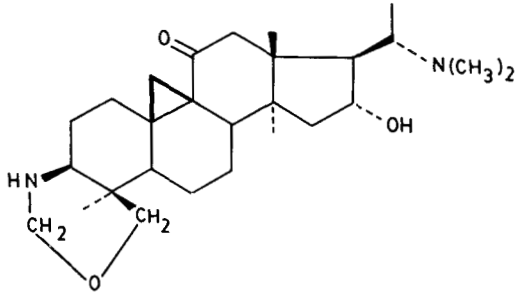
N-Benzoylcycloxobuxidine-F (**522**), also known as "*N*-benzoylbaleabuxidine-F," has been isolated from *B. balearica* (*181,182*), *B. hycrana* Pojark (*183,184*), and *B. sempervirens* (*168*). Its structure was established on the basis of spectroscopic and chemical evidence. The presence of a secondary benzamide, an alcohol, and a conjugated carbonyl group was revealed by the IR and UV spectra. Two major differences were observed in the ^{1}H-NMR spectra of **522** and **505.** The signal for one of the tertiary methyl groups (C-14) was shifted

downfield, probably due to the deshielding effect of the C-16 hydroxyl function. The upfield shift of the C-4 methyl protons (by δ0.21), however, was attributable to the shielding effect of the C-4 hydroxymethylene protons. Basic hydrolysis of **522** yielded cycloxobuxidine-F (**523**).



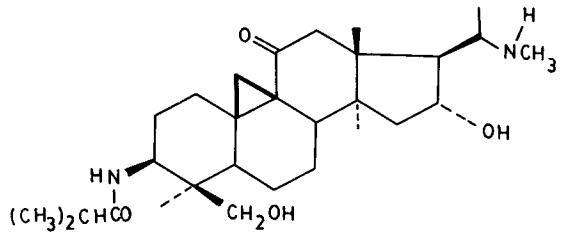
523 R = CH_3
526 R = H

N-Benzolycycloxobuxidine-F has also been isolated from *B. papilosa* together with its 16α-acetyl analogue (*238*). The structure of *N*-benzoyl-16α-acetoxy-cycloxobuxidine-F (**685**) has been established by comparing its spectral properties with *N*-benzoylcycloxobuxidine-F. The IR spectrum showed an additional band at 1730 cm^{-1} indicating the presence of an ester group. The C-16 proton resonated as a multiplet at δ4.80 in the ^{1}H-NMR spectrum. The aromatic protons appeared as two multiplets centered at δ7.45 (3H) and 7.78 (2H). The ^{13}C-NMR spectrum showed four signals at δ9.10, 10.50, 17.60, and 18.00 which are attributed to the C-21, C-30, C-18, and C-32 methyl carbons respectively. The methyl carbon of the acetyl group appeared at δ21.70 while the acetyl carbonyl carbon was observed at δ169.20. The C-31 hydroxyl-bearing methylene resonated at δ61.00.



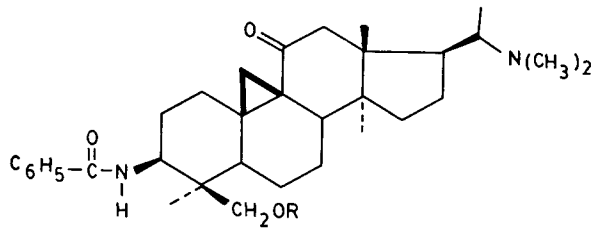
524

Cycloxobuxoxazine-C (**524**) and *N*-isobutyrylcycloxobuxidine-H (**525**) have been isolated from *B. balearica* (*182*). Cycloxobuxoxazine-C has also been described under the name "baleabuxoxazine" (*181*). Structure **524** was assigned to cycloxobuxoxazine-C and confirmed by its synthesis from cycloxobuxidine-F (**523**) by reaction with formaldehyde in dioxane. *N*-Isobutyrylcycloxobuxidine-H



525

(**525**) exhibited IR and UV spectra characteristic of 11-keto compounds possessing a cyclopropane ring. The peak at *m/e* 58 in the mass spectrum indicated the presence of a 20-methylamine group. Acid hydrolysis of **525** to cycloxobuxidine-H (**526**) confirmed the assigned structure.

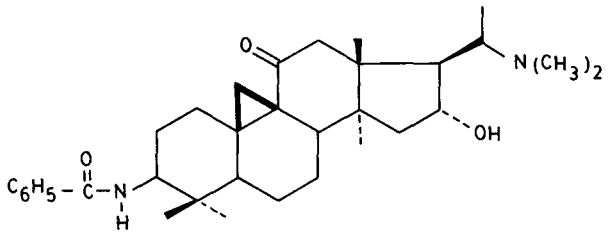


527 R=H

528 R=COCH3

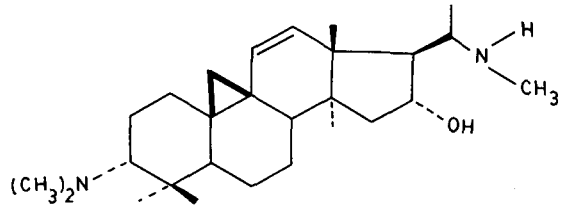
Structures **527** and **528** for *N*-benzoylcycloxobuxoline-F and *N*-benzoyl-*O*-acetylcycloxobuxoline-F, isolated from *B. sempervirens,* were proposed on the basis of spectral data and confirmed by preparation of suitable derivatives (*168*). Acetylation of **527** with acetic anhydride in pyridine afforded **528.**

Buxarine, isolated from *B. sempervirens,* has been assigned structure **529** on the basis of spectral data (*185*). Absorption band bands at 1710, 1660 (C=O), and 1460 cm^{-1} were observed in the IR spectrum. The ^{1}H-NMR spectrum



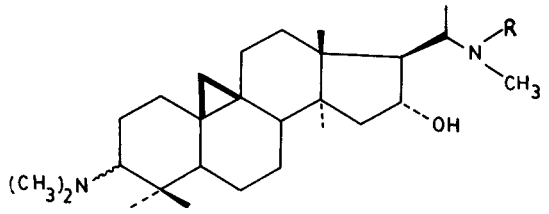
529

showed signals for five aromatic protons (δ7.42–7.75), one NH proton (δ6.07), four tertiary methyl groups, one secondary methyl group (δ0.88), a proton adjacent to a hydroxyl group (δ4.02), and two methylene protons α to the carbonyl function.

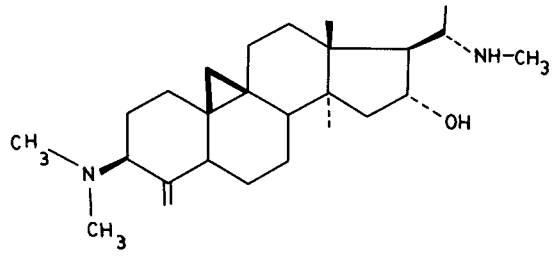


530

Cyclokoreanine-B (**530**) is the first *Buxus* alkaloid possessing α-configuration of the amino group at C-3 (*186*). The UV and NMR spectra of **530,** extracted from *B. koreana* Nakai leaves, showed the presence of a double bond adjacent to the cyclopropane ring. Catalytic hydrogenation of cyclokoreanine-B yielded dihydrocyclokoreanine-B (**531**), different from cyclovirobuxine-A (**532**), suggesting the 3 α-dimethylamine configuration for cyclokoreanine-B. This alkaloid has been reisolated from *B. microphylla* (*187*).



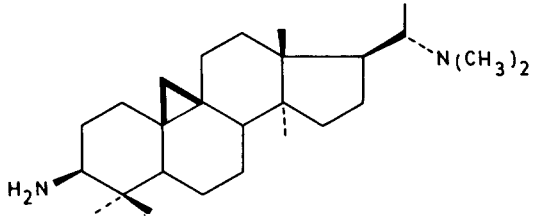
531 R = H , 3α N(CH3)2
532 R = CH3 ,3β N(CH3)2



533

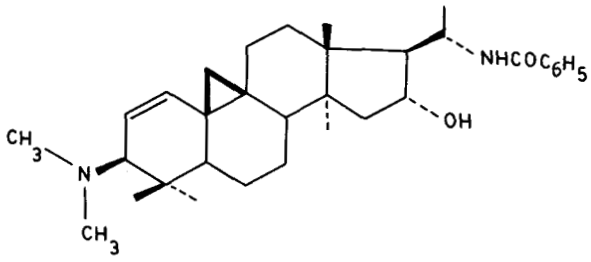
Cyclobuxine-B, isolated from the leaves of *B. sempervirens,* has been assigned structure **533** on the basis of chemical and spectral analysis (*188,189*).

The alkaloids extracted from the stem bark and roots of *B. madagascarica* Baillon subsp. *xerophilla* forma *salicicola* were identified as cycloprotobuxine-C



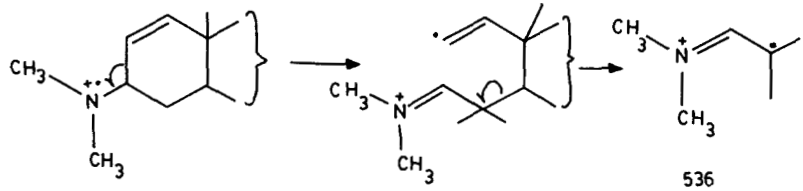
534

and cycloprotobuxine-F (**534**). The structural assignments were based on the physicochemical properties and preparation of suitable derivatives (*190*).



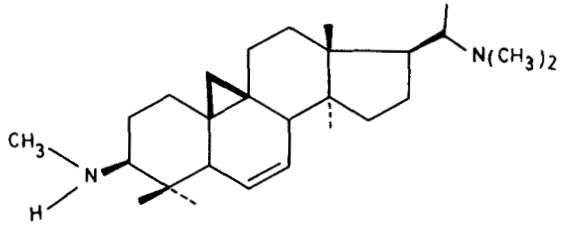
535

N-Benzoylcyclovirobuxenine-E (**535**) has been isolated from *B. sempervirens* and its structure assigned using physical and chemical methods (*191*). The IR spectrum showed bands characteristic of a secondary benzamide function (1640, 1550, and 1310 cm^{-1}), in addition to those of OH and NH functions. Signals observed in the ^{1}H-NMR spectrum were also in accord with the proposed struc-



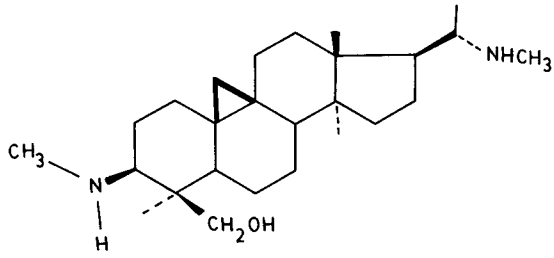
535 536

ture. *N*-Benzoylcyclovirobuxenine-E showed a peak at *m/e* 99 in its mass spectrum, corresponding to the ion **536,** indicating the presence of a 1,2-double bond in the structure.



537

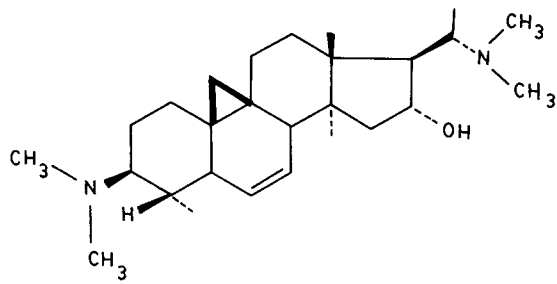
Catalytic hydrogenation of cyclobuxupaline-C (**537**), isolated from *B. papilosa* Schneider, produced dihydrocyclobuxupaline-C, which was shown to be identical with the known alkaloid cycloprotobuxine-C. This served to confirm the structure **537** assigned to cyclobuxupaline-C (*192*).



538

Cyclopapilosine-D, extracted from *B. papilosa,* was shown to have structure **538** on the basis of spectral studies (*192*). The presence of an OH group was indicated by absorption bands at 3380 cm^{-1}, the presence of an NH group by a band at 3300 cm^{-1} in the IR spectrum. The hydroxymethylene protons resonated at δ4.12 and δ4.72 (AB doublets, J = 10.0 Hz) in the ^{1}H-NMR spectrum. The ^{1}H-NMR spectrum of the acetylated derivative, however, showed a downfield shift of these doublets at δ4.58 and δ5.00. The mass spectrum of **538** showed a molecular ion at *m/e* 402 and a peak at *m/e* 58 corresponding to the CH_3—CH=$N^+H(CH_3)$ fragment.

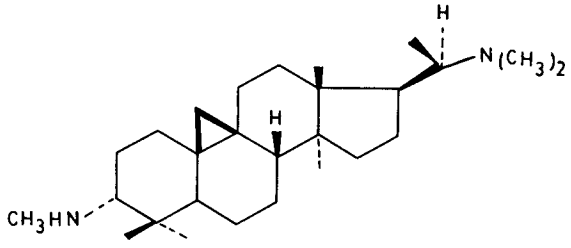
Spectral data and biogenetic considerations allowed the assignment of structure **539** to cyclobullatine-A, isolated from *B. sempervirens* var. *angustifolia*



539

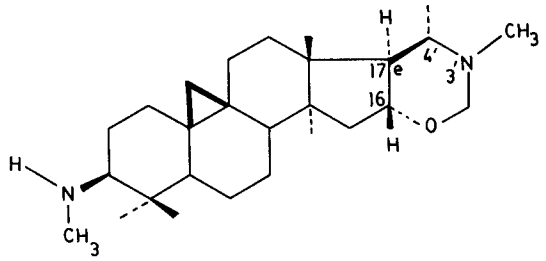
(*177*), var. *bullata* Kirchn. (*193*), var. *argentea,* and var. *rotundifolia* (*194*). The UV spectrum exhibited maximum absorption at 204 nm attributable to an isolated double bond. The IR spectrum suggested the presence of a secondary hydroxyl group (1095 cm^{-1}) and a cyclopropyl ring (3030 and 1450 cm^{-1}). Correlation of **539** with cyclobuxamine-H (**518**) confirmed the suggested structure.

Cycloprotobuxine-C, originally isolated from *B. sempervirens,* has been assigned structure **540** with the unusual (20*R*) configuration (*195,196*). The α-configuration was assigned to the 3-methylamino group on the basis of the observation that the 3-*N*-acyl derivative of *l*-cycloprotobuxine-C hydrolyzed less



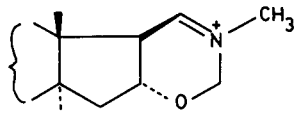
540

rapidly than compounds containing 3β-*N*-acyl substituents (*196*). Recently isolation and NMR studies of cycloprotobuxine-C have been reported from *B.* papilosa (*239*).



541

The steroidal alkaloid buxozine-C, bearing the novel structure **541,** has been isolated from *B. sempervirens* (*197,198*). Its structure was assigned mainly on



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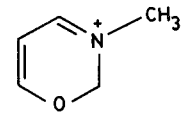
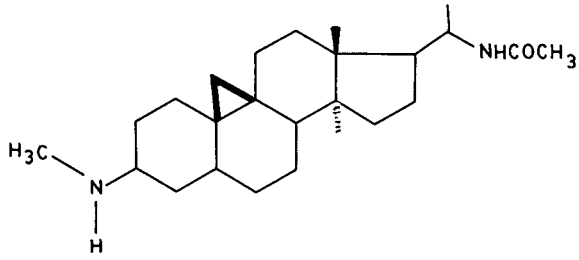


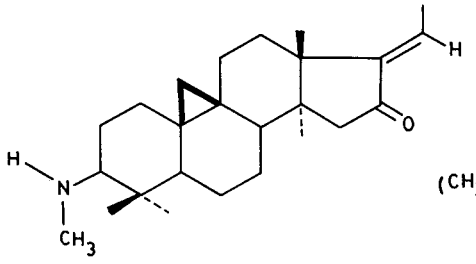
543

the basis of the fragmentation pattern in its mass spectrum, which showed the base peak at *m/e* 399, corresponding to the ion **542,** and a peak at *m/e* 98 due to the ion **543.** Confirmation of the structure came from its hydrogenolysis to the known alkaloid, cyclovirobuxine-C.



544

Cyclobuxargentine-G (**544**), extracted from *B. sempervirens* var. *argentea,* is another *Buxus* alkaloid which is not substituted at C-4 (*194*).



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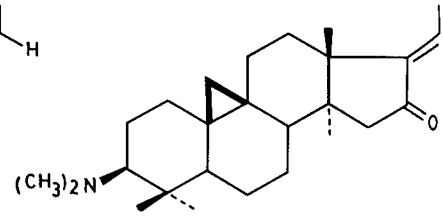
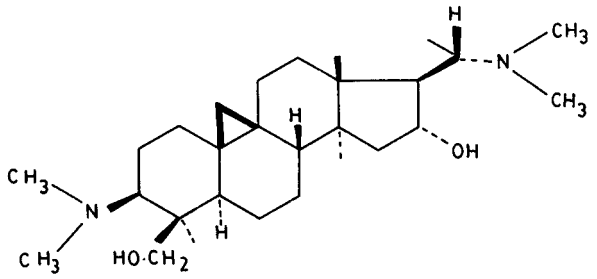


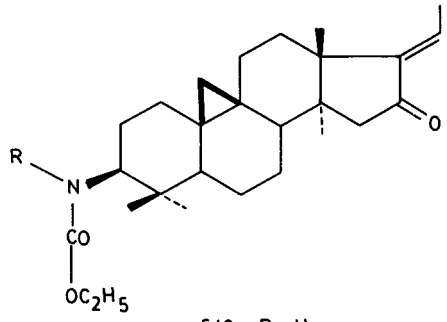
546

b. Monoamino Alkaloids C-3 Amino Derivatives. Cyclobuxophyllinine (**545**), also described as "buxenone," has been extracted from *B. microphylla* and *B. sempervirens* (*194,199*) and *B. papilosa* (*241*). The structure of cyclobuxophyllinine was proposed on the basis of spectroscopic data and confirmed by preparation of its derivatives. *N*-Methylation of **545** with methyl iodide yielded a compound identical with another alkaloid, cyclobuxophylline (**546**), isolated



547

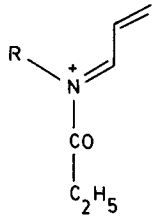
from *B. microphylla* Sieb. et Zucc. var. *suffruticosa* Makino forma major Makino (*171*). The structure assigned to cyclobuxophylline was confirmed by its synthesis from cyclomicrophylline-A (**547**).



548 R= H

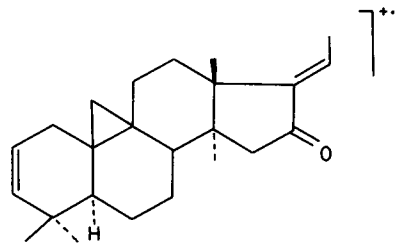
549 R= CH_3

Buxene (**548**) and *N*-methylbuxene (**549**), possessing the urethane function at C-3 have been isolated from *B. sempervirens* (*200*). Methylation of **548** with HCHO/HCOOH yielded *N*-methylbuxene (**549**). The structure of *N*-methylbuxene was confirmed by treatment of buxenone (**545**) with ethyl chloroformate which yielded **549.** Buxene showed a base peak at m/e 128, corresponding to the



550 R = H

551 R = CH_3

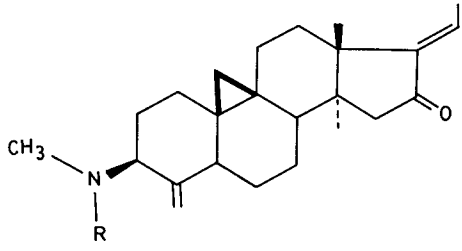


552

ion **550;** the *N*-methylbuxene peak occurred at m/e 142; corresponding to **551.** The fragment ion **552,** observed at *m/e* 338, is formed by McLafferty rearrangement.

Cyclosuffrobuxinine-M (**553**) and cyclosuffrobuxine (**554**) have been extracted from *B. microphylla* (*171*). The structures of these compounds were determined from their IR (3320, 1721, and 1650 cm^{-1}), UV (243 nm), and NMR spectra (one-proton quartet at δ6.58, three-proton doublet at δ1.85 with coupling constant J = 7.5 Hz). The syntheses of **553** and **554** were achieved by oxidation of cyclobuxine-D with chromium trioxide in glacial acetic acid to afford the 16-amino-ketone, which was deaminated with neutral alumina to give a compound identical with cyclosuffrobuxinine (**553**). Methylation of **553** with methyl iodide yielded cyclosuffrobuxine (**554**). Cyclosuffrobuxinine has recently been isolated from *B. sempervirens* and the ^{13}C-NMR assignments have been reported (*240*).

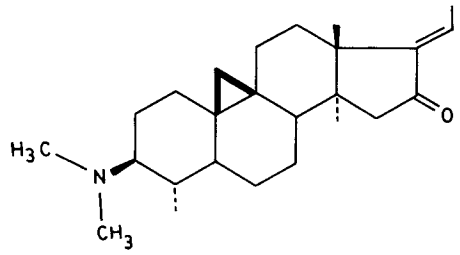
Cyclobuxosuffrine (**555**) has been isolated from *B. microphylla* and its structure established on the basis of spectral studies (*171*). The mass spectrum exhibited the molecular ion peak at *m/e* 369 with fragment peaks at *m/e* 58, 71 (base



553 R = H

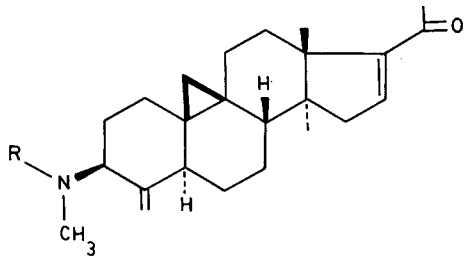
554 R = CH_3

peak), and 84 indicating the 3β-dimethylamino group. The IR and UV spectra suggested that it corresponds to a 4-methyl derivative, which could be confirmed



555

by reduction of the terminal methylene double bond of **554.** A doublet at δ0.98 (J = 6.5 Hz) also supported the presence of a 4α-methyl group. Correlation of cyclobuxine-D with cyclobuxosuffrine confirmed the equatorial (α) configuration of the 4-methyl group of cyclosufforbuxine (*201*).

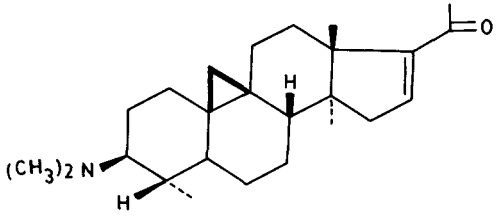


556 R = CH_3

686 R = CHO

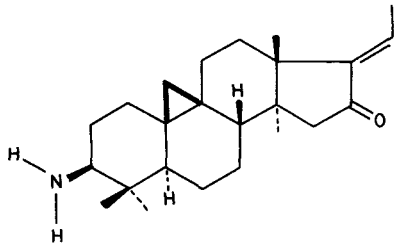
Cyclomicrobuxeine, isolated from *B. microphylla,* was shown to have structure **556** from its spectral behavior (*171*). Its structure was confirmed by direct comparison with the compound obtained on dehydration involving the 16-hydroxyl group of cyclomicrobuxine (*202*).

N-Formylcyclomicrobuxeine, isolated from *B. papilosa,* has been assigned structure **686** on the basis of spectroscopic comparison with cyclomicrobuxeine (*242*). The presence of an amide group was revealed by an absorption band at 1670 cm^{-1} in the IR spectrum. A singlet at δ8.12 in the ^{1}H-NMR spectrum also revealed the presence of the N-formyl group. NOE interactions between the C-21 methyl group and the C-16 olefinic proton suggested "E" stereochemical disposition for the enone moiety. The rest of the spectral properties resembled those of cyclomicrobuxeine.



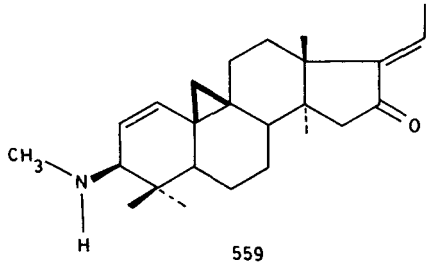
557

The spectral data of cyclobuxomicreine (**557**), extracted from *B. microphylla,* were very similar to those reported for **556.** The ^{1}H-NMR spectrum of **557,** however, showed a three-proton doublet at δ0.96 (J = 6.5 Hz), indicating the presence of a 4-methyl group. Therefore, it was suggested that this alkaloid corresponds to the compound in which the C-4 methylene group of **556** is replaced by a methyl group.



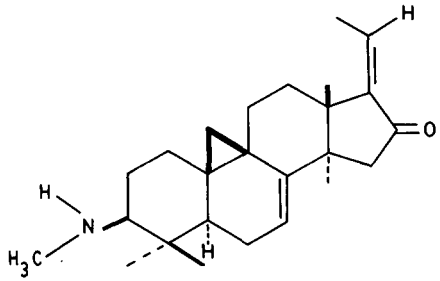
558

Cyclobuxophylline-O, isolated from *B. sempervirens* var. *rotundifolia* (*189*), was shown to have structure **558** by spectroscopic studies; the structure was confirmed by its *N*-methylation to the known alkaloid cyclobuxophylline-K (**546**).



559

Buxithienine-M (**559**) was also isolated from *B. sempervirens* and its structure was established on the basis of chemical and spectral studies (*189*). The IR spectrum showed bands at 3420 (secondary amine), 1718, and 1642 cm^{-1} (α,β-unsaturated ketone). The ^{1}H-NMR spectrum revealed the presence of a cyclopropane ring (two-proton AB quartet centered at δ0.44 with J = 5.0 Hz), two tertiary methyl groups (singlets at δ0.78 and δ1.31), and an *N*-methyl group (singlet at δ2.45). Two sets of AB doublets at δ5.74 and δ5.63 (J = 7.5 Hz) indicated the presence of a 1(2)-double bond. The presence of vinyl group was supported by the appearance of a three-proton doublet at δ1.80 (J = 8.0 Hz) coupled to the vinyl proton at δ6.53 (quartet, J = 8.0 Hz).



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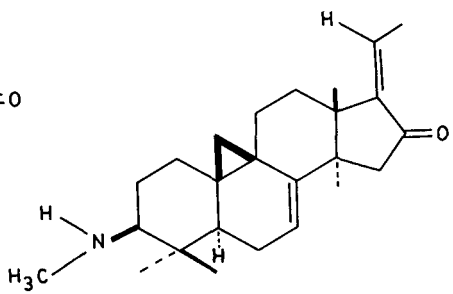
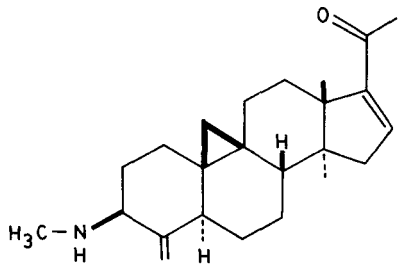


561

The structures and stereochemistry of the two alkaloids (−)-*E*-cyclobuxaphylamine (**560**) and (−)-*Z*-cyclobuxaphylamine (**561**), isolated from *B. papilosa*, have been estabilised (*203*). (−)-*E*-Cyclobuxaphylamine (**560**) showed a UV absorption at 244 nm, characteristic of an α,β-unsaturated ketone. This was supported by absorption bands at 1712 and 1636 cm^{-1} (α,β-unsaturated cyclopentanone) in the IR spectrum. The ^{1}H-NMR spectrum showed four singlets at δ1.41, δ1.17, δ1.27, and δ1.33 attributed to the four tertiary methyl groups. Half of the cyclopropyl AB quartet shifted downfield due to the deshielding effect of the neighboring olefinic functionality. The 21-methyl group appeared as a doublet at δ1.84 (J = 7.5 Hz) by coupling with the C-20 olefinic proton. A peak at *m/e* 138 in the mass spectrum gave information about the location of the allylic cleavage of ring B, indicated by the dotted lines.

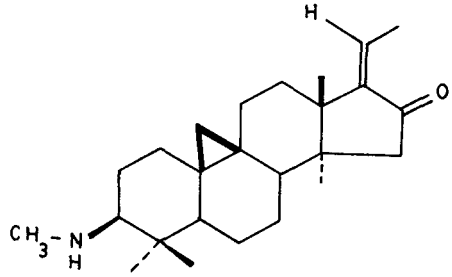
The geometrical isomer **561** of (−)-*E*-cyclobuxaphylamine exhibited an NMR spectrum similar to **560** except that the C-20 vinylic proton appeared relatively upfield (δ5.78), while the doublet due to the 21-methyl group was shifted downfield. This was explained by the deshielding effect of the neighboring C-16 carbonyl oxygen.



562

(+)-Norcyclomicrobuxeine (**562**), isolated from *B. papilosa*, was shown to possess an α,β-unsaturated ketone functionality by the UV absorption at 240 nm and IR absorption band at 1657 cm^{-1} (conjugated carbonyl). The ^{1}H-NMR spectrum of **562** showed AB doublets at δ0.10 and δ0.39 for the cyclopropyl methylene protons. Two tertiary methyl groups were observed at δ0.99 and δ1.20. A three-proton singlet at δ2.28 accounted for the methyl group next to the carbonyl functionality. The terminal methylidene protons appeared as singlets at δ4.64 and δ4.88. These spectral data resembled those reported for (+)-cyclomicrobuxeine (**556**). NOE difference measurements showed through-space interactions of H-16 and 21-methyl protons, supporting a *transoid* geometry for the enone moiety. Therefore compound **562** was assigned as the *N*-nor derivative of (+)-cyclomicrobuxeine (**556**) (*204*).

Studies on the chemical constituents of *B. sempervirens* of Turkish origin have resulted in the isolation of two new alkaloids; named (Z)-buxenone and sempervirone (*240,241*).

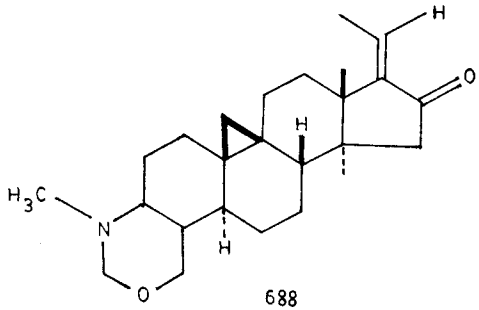


687

(Z)-Buxenone exhibited the UV and IR spectra identical to those reported for its geometrical isomer E-buxenone (**545**). "Z" -Geometry was assigned to **687** on the basis of differences observed in the ^{1}H-NMR spectrum. The C-20 vinylic proton resonated relatively downfield (at δ6.54) and the doublet for 21-methyl

protons appeared at δ1.82. The rather upfield chemical shift of the 21-methyl protons was attributed to its *anti*-disposition to the C-16 carbonyl oxygen (*241*).

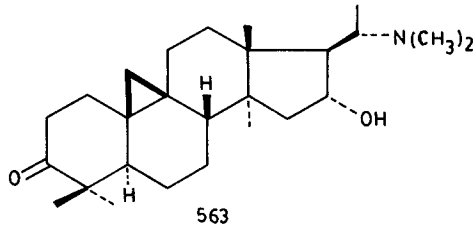
Another new alkaloids, sempervirone, showed the presence of an α,β-unsaturated cyclopentanone ring system from its UV (λ_{max} = 243 nm) and IR (1715 and 1632 cm^{-1}) spectra. The ^{1}H-NMR spectrum showed two sets of AB doublets resonating at δ3.16 and 3.77 ($J_{31\alpha,31\beta}$ = 10.6 Hz) and at δ3.63 and δ4.46 ($J_{33\alpha,33\beta}$ = 7.5 Hz) due to the C-31 and C-33 methylene protons suggesting the tetrahydrooxoazine ring system. The methylene protons of the cyclopropane ring appeared as AB doublets at δ0.43 and δ0.72 ($J_{19\alpha,19\beta}$ = 4.5 Hz). The interaction between the C-18 methyl and C-21 allylic methyl protons observed in the NOESY spectrum supported "E" geometry for the enone system. The molecular ion appeared at *m/z* 397.2982 ($C_{26}H_{39}NO_2$) in the mass spectrum with the base



688

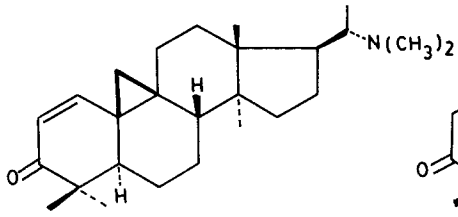
peak at *m/z* 71 corresponding to the fragment CH_2—CH=$N^+(CH_3)_2$. A major peak at *m/z* 127 was attributed to the fragment observed by cleavage of ring A along with the attached tetrahydrooxazine ring. The ^{13}C-NMR spectrum showed four signals at δ13.45, 13.79, 20.82, and 23.96 assigned to C-18, C-30, C-32, and C-21 methyl carbons, respectively. The 31-methylene carbon resonated at δ78.07 while the C-33 methylene was observed rather downfield (δ88.66) due to the deshielding influence of the neighboring nitrogen and oxygen atoms. On the basis of these studies structure **688** was assigned to sempervirone (*240*).

C-20 Aminated Alkaloids. Cyclomikuranine (**563**), isolated from *B. microphylla* showed an IR band at 3448 characteristic of a hydroxyl, and one at 1709



563

cm^{-1} corresponding to a six-membered ketone group (*171*). In the ^1H-NMR spectrum of **563,** one of the four tertiary methyl groups showed a downfield shift (δ1.14) suggesting the presence of a 16 α-hydroxyl group. The peak at *m/e* 72 in the mass spectrum indicated the presence of a 20-dimethylamino group. The alkaloid showed a negative Cotton-effect curve typical of 4,4-dimethyl-3-oxo-5αH-steroids (*205*). Cyclomikuranine may therefore be formulated as **563.**



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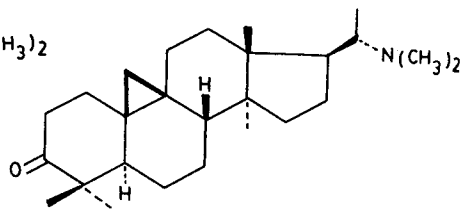
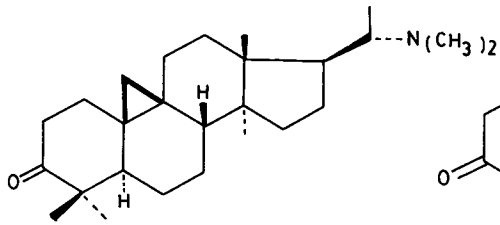


565

Cyclobuxoviridine (**564**) has been isolated from *B. microphylla* (*171*) and *B. sempervirens* var. *argentea* (*194*). The IR and UV spectra of the substance showed absorption bands corresponding to the presence of the cyclopropyl ring adjacent to the α,β-unsaturated six-membered ketone. Catalytic hydrogenation gave a ketone **565,** identical to the product obtained on Ruschig deamination at C-3 of cycloprotobuxine-C.



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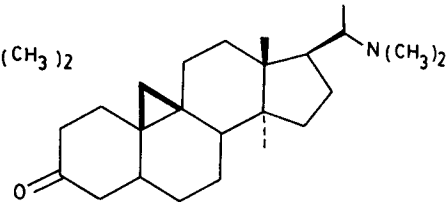
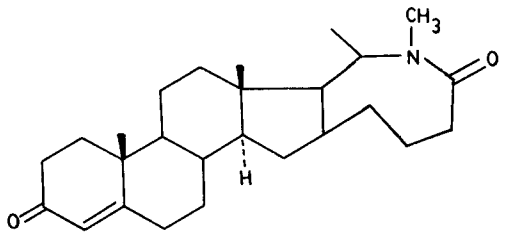


566

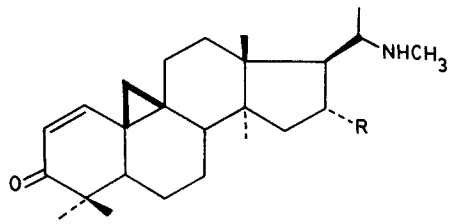
Buxandonine (**566**), isolated from *B. sempervirens* is an alkaloid having no methyl substituents on C-4 (*170*). The IR spectrum of buxandonine (**566**) exhibited an absorption band at 1670 cm^{-1} indicating the presence of a cyclic ketone. The remaining part of the structure is identical to the reduced product **567** of cyclobuxoviridine (**564**).

Buxamidine (**568**), isolated from *B. papilosa,* is the first known alkaloid bearing an eight-membered lactam ring attached to the normal steroidal skeleton. The structure was confirmed on the basis of spectroscopic studies (*206*).



568

Cyclobuxoviricine and cycloxobuxoviricine were isolated from *B. papilosa* and have been assigned structures **569** and **570** (*207,208*). The UV (268 nm) and IR (bands at 1647 and 1595 cm^{-1}) spectra of cyclobuxoviricine (**569**) suggested the presence of an α,β-unsaturated ketone. The ^{1}H-NMR spectrum showed the existence of a cyclopropane ring (AB doublets at δ0.75 and δ0.81 with coupling constant J = 5.1 Hz), a secondary methyl group (doublet, J = 6.0 Hz at δ1.18), and an *N*-methyl group (δ2.48). Two doublets at δ5.49 (J = 10.1 Hz) and δ6.75 (J = 10.1 Hz) were attributed to the olefinic protons of the α,β-unsaturated ketone function.

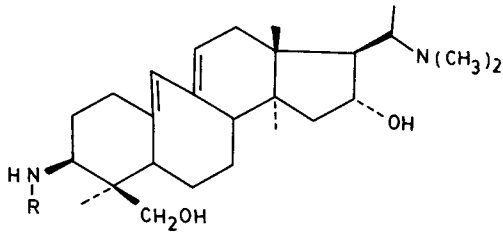


569 R = H
570 R = OH

The IR spectrum of cycloxobuxoviricine (**570**) also indicated the presence of OH function (3700 cm^{-1}) in addition to the α,β-unsaturated ketone. The ^{1}H-NMR spectrum of **570** was very similar to that of **569** except that it showed a one-proton multiplet at δ4.28 attributable to the C-16β-proton.

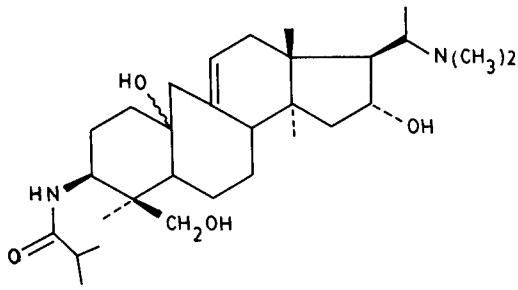
2. Derivatives of 9(10 → 19)-abeo-5α-pregnane

a. Diamino Alkaloids. *N*-Isobutyrylbaleabuxidienine-F (**571**), isolated from *B. balearica*, was found to possess the same type of conjugated heteroannular diene system which was present in buxamine-E and buxaminol-E (*181*). Hydrolysis of **571** afforded buxidienine-F (**572**).

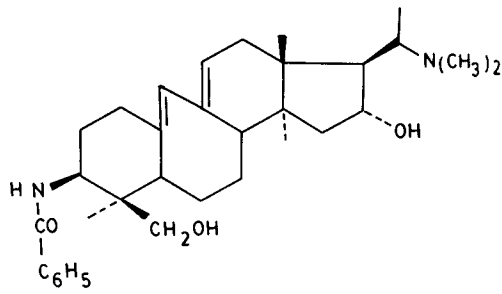


571 $R = (CH_3)_2CHCO$
572 $R = H$

N-Benzoylbuxidienine-F (**573**), isolated from *B. sempervirens* (*168*) and *B. balearica* (*182*), was shown to contain the 9(10 → 19) *abeo*-diene system which is a common feature of the group of *Buxus* alkaloids exemplified by buxamine-E and buxaminol-E. The position of the hydroxymethylene signal at δ3.29 in the ^{1}H-NMR spectrum supported the location of the amide function at C-3.



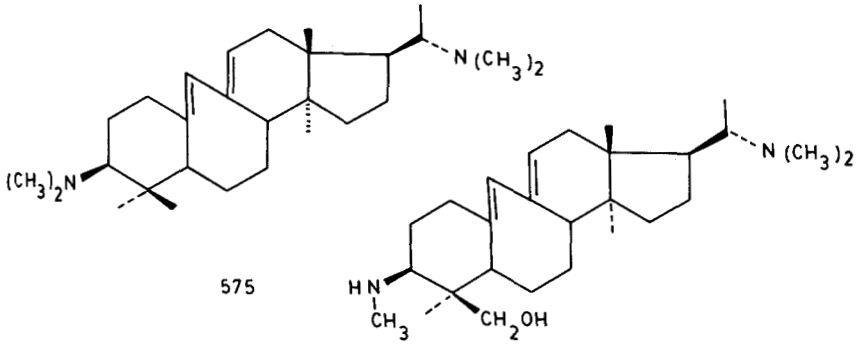
574



573

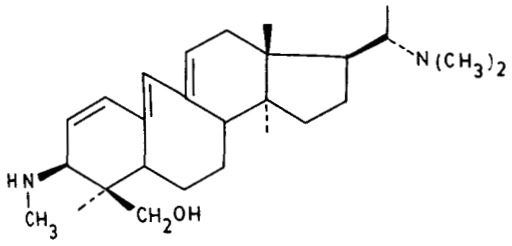
N-Isobutyrylbaleabuxaline-F (**574**) has also been isolated with *N*-benzoylbuxidienine-F (**573**) from the leaves of *B. balearica* (*181*). The ^{1}H-NMR of *N*-isobutyrylbaleabuxaline-F showed the presence of a single ethylenic proton.

The alkaloids from the stem bark and roots of *B. madagascarica* Baillon subsp. *Xerophilla* farma *salicicola* have been studied (*190*). These alkaloids have been identified as buxamine-A (**575**), 16-deoxybuxidienine-C (**576**) and bux-



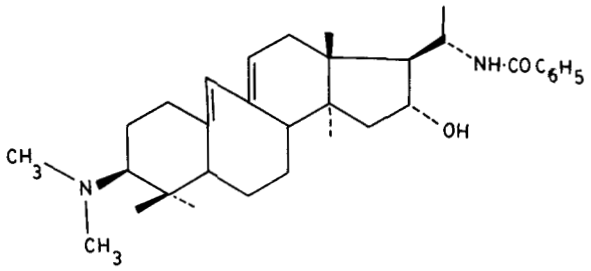
575

576



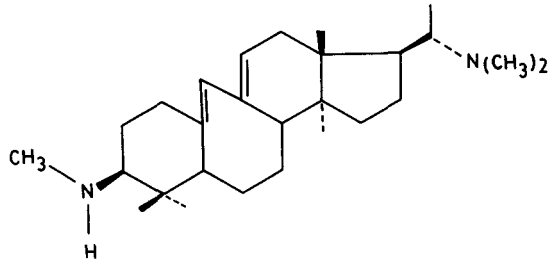
577

itrienine-C (**577**). The structural assignments were based on the physico-chemical properties and preparation of suitable derivatives. 16-Deoxybuxidienine-C has also been extracted from *B. papilosa* (*192*).



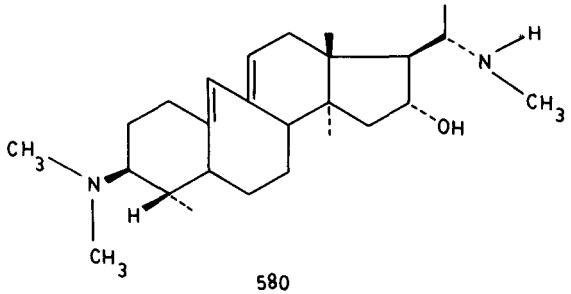
578

N-Benzoylbuxodienine-E (**578**) and its *O*-acetyl derivative have been isolated from *B. sempervirens* (*209*). The structures were assigned on the basis of spectral data.



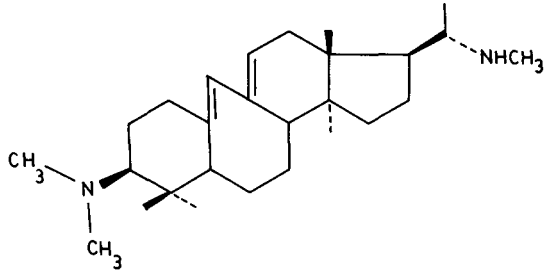
579

The UV spectrum of buxamine-C (**579**), isolated from *B. papilosa*, closely resembled that of the well-characterized alkaloid 16-deoxybuxidienine-C (**576**) (*192*). The structure was assigned on the basis of spectral data.



580

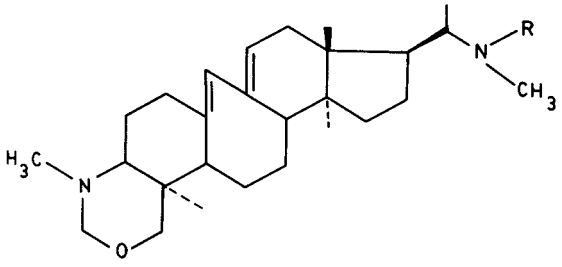
The isolation of buxaminol-B (**580**) from *B. sempervirens* var. *bullata* Kirchn. has been described (*193*). Its structure was proposed on the basis of spectral evidence and correlation with buxaminol-E. It has also been isolated from *B. papilosa* (*208*).



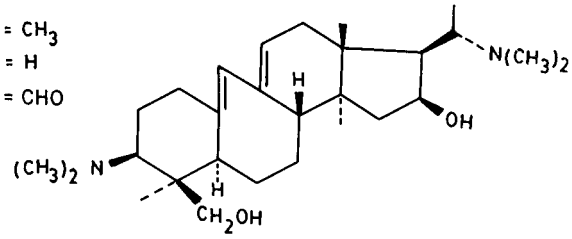
581

An investigation of the alkaloidal constituents of *B. harlandi* led to the isolation of an alkaloid, buxamine-B (**581**) (*210*). Structure **581,** proposed on the basis of spectroscopic data, was confirmed by preparation of the N-methyl deriv-

ative **575,** which was identical to buxamine-A. Buxamine-B has also been described under the name papilicine, since it was isolated from *B. papilosa* (*211*).



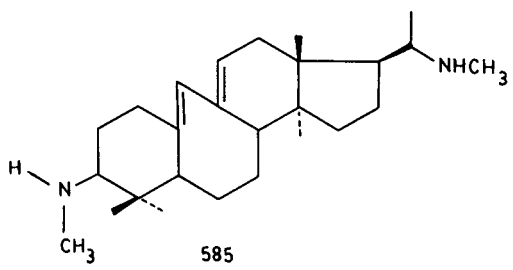
582 R = CH_3
583 R = H
689 R = CHO



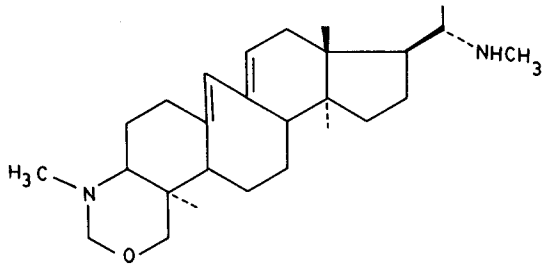
584

Thirty five new steroidal alkaloids have been isolated by the Pakistan group from the leaves of *B. papilosa* (*203,204,206–208,211–222,237–245*). Two of these, moenjodaramine (**582**) and harappamine (**583**), were found to possess a novel pentacyclic skeleton showing two sets of downfield AB double doublets for the methylene protons α- to the oxygen (*212–214*). Structure **584,** isolated by the same group from the leaves of *B. papilosa* (*215*), has been assigned to buxaminol-G based on spectroscopic studies.

Structure **689** has been assigned to papillamidine, isolated from *B. papilosa* (*243*). The spectral properties of papillamidine closely resembled those of harappamine and, in fact, it was found to be the *N*-formyl-N_b-nor derivative of (+)-harappamine (**583**) (*212,214*).

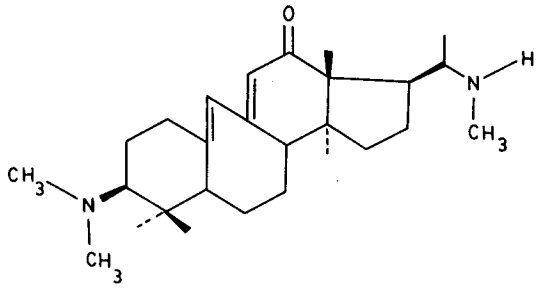


585



586

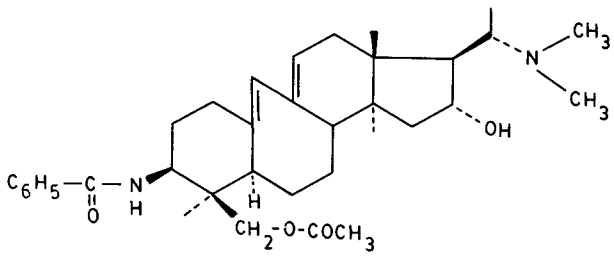
Papilamine, to which structure **585** has been assigned on the basis of spectrocopic studies, was extracted from the leaves of *B. papilosa* (*216*). The spectral data of papilinine (**586**), also isolated from *B. papilosa*, suggested that it possesses the pentacyclic skeleton (*217*).



587

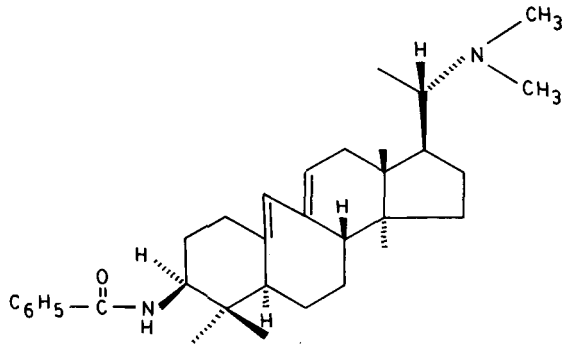
The IR spectrum of karachicine (**587**), isolated from *B. papilosa*, indicated the presence of a six-membered α,β-unsaturated ketone. The UV spectrum was indicative of the presence of a conjugated 9(10 → 19) abeo-diene system (*218*).

N-Benzoyl-30-acetoxybuxidienine (**588**) has also been isolated from *B. papilosa* (*219*). The IR spectrum of **588** showed intense absorptions at 3400 (OH),



588

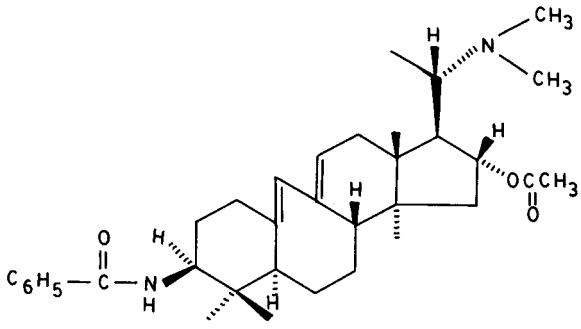
3350 (NH), 1716 (ester C=O), 1662 (amide C=O), and 1610 cm^{-1} (C=C). Absorption maxima at 238, 245, 253, 268, and 290 nm were characteristic of a 9(10 → 19) *abeo*-diene system and an aromatic ring. The mass spectrum showed the molecular ion at *m/e* 562 ($C_{35}H_{50}N_2O_4$) and a fragment peak at *m/e* 503, corresponding to the loss of an acetate group. Three singlets at δ0.76, δ0.77, and δ0.94 in the ^{1}H-NMR spectrum were assigned to three tertiary methyl groups. Another three-proton singlet at δ2.12 was assigned to the acetate methyl protons. NOE studies established the α-orientation of the C-29 methyl group.



589

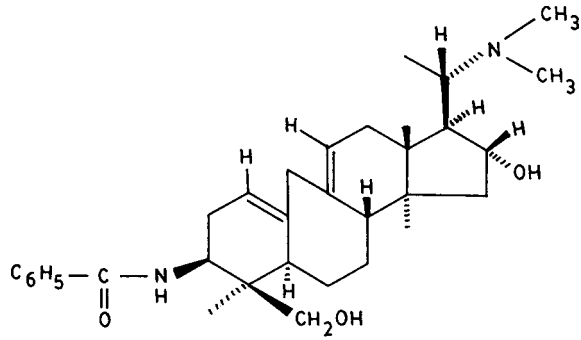
(+)-Buxabenzamidienine (**589**), isolated from the weakly basic fraction of *B. papilosa*, exhibited a UV spectrum characteristic of a tetracyclic 9(10 → 19) *abeo* diene skeleton (*220*). The mass spectrum showed the molecular ion at *m/e* 488 with the base peak at *m/e* 72 representing the trimethyliminium side chain. A peak at *m/e* 383 corresponded to the loss of the benzoyl substituent. Intense bands at 3680 (–NH) and 1652 cm^{-1} (aromatic amide) were observed in the IR spectrum. The 21-methyl protons appeared as a doublet at δ0.93 (J = 6.3 Hz). A singlet at δ6.00 was assigned to the vinylic H-19 proton, while a double doublet centered at δ5.56 (J_1 = 2.5 Hz, J_2 = 1.8 Hz) was attributed to the vinylic H-11 proton which is coupled to the C-12 methylene protons. Four tertiary methyl groups appeared as singlets. These spectral studies led to the assignment of structure **589** to buxabenzamidienine.

16α-Acetoxybuxabenzamidiene (**590**) was also isolated from *B. papilosa*, together with **589** (*220*). It has also been isolated from *B. sempervirens* (*237*). The spectral data of **590** were nearly identical with that of **589.** The IR spectrum showed an additional band at 1732 cm^{-1} because of the ester carbonyl function.



590

The relatively downfield shift (δ1.26) of the 21-methyl protons was attributed to the presence of an acetoxyl function on C-16. A singlet at δ1.80 was assigned to the acetyl methyl group which was absent in the case of buxabenzamidienine (**589**), which lacks the 16-acetoxyl function.

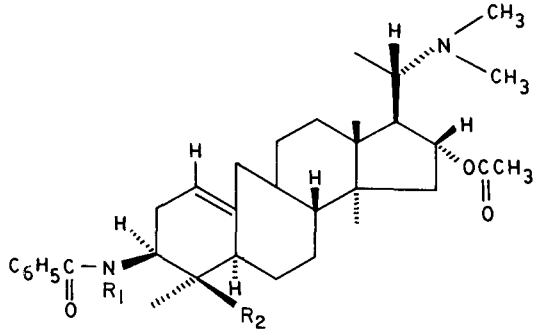


591

Another alkaloid, (+)-buxanoldine (**591**), extracted from *B. papilosa* showed a benzamide chromophore by the absorption at 228 nm in the UV spectrum. The presence of benzamide function was also revealed by the absorption band at 3360 cm^{-1}. The ^1H-NMR spectrum showed three singlets at δ0.69, δ0.72, and δ0.95 due to the three tertiary methyl groups. The C-30 methylene protons appeared as two doublets centered at δ3.23 and δ3.49. A multiplet at δ4.11 was assigned to H-16, its downfield shift being due to the presence of the hydroxyl group at C-16. A detailed NOE study of **591** led to the establishment of the stereochemical disposition of the hydroxyl group at C-16. Irradiation of the H-16 multiplet

(δ4.11) resulted in no enhancement of the H-17 signal, thus establishing the α-configuration of the hydroxyl function that is usual for all other *Buxus* alkaloids hydroxylated at C-16. The C-19 methylene protons were shown to be in close proximity with the vinylic H-1 and H-11 protons (δ5.43 and δ5.26), since these absorptions showed reciprocal enhancements. The chemical shifts of H-16 and C-30 methylene protons were confirmed by preparation of the diacetate, which showed downfield shifts for these protons (*220*).

Structure **592** was assigned to (+)-buxanaldinine isolated from *B. papilosa* (*220*). It is the first *Buxus* alkaloid in which the C-30 hydroxyl or ester function is replaced by an aldehyde group. The UV spectrum displayed absorption at 228 nm due to the benzamide function. The IR spectrum showed bands at 3660 (NH), 1735 (ester C=O), 1722 (aldehyde C=O), and 1656 cm^{-1} (aromatic amide). Three singlets at δ0.80, δ0.88, and δ0.89 in the ^{1}H-NMR spectrum were assigned to the tertiary methyl groups. Only one proton (H-1) resonated in the olefinic region (δ5.69) as a doublet of doublets. The aldehydic proton absorbed as a singlet at δ9.5. Reduction of **592** with $NaBH_4$ resulted in the disappearance of the signal at δ9.5 in the NMR spectrum, confirming the presence of aldehyde proton.

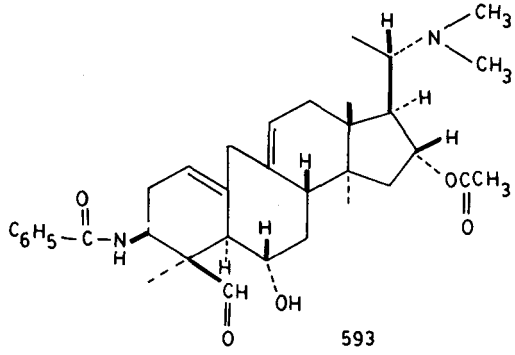


592 R_1 = H, R_2 = CHO

690 R_1 = HC_3, R_2 = CH_2OH

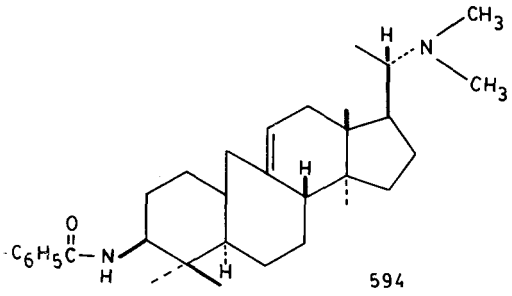
(+)-Buxabenzacine (**690**). $C_{36}H_{54}N_2O_4$, has recently been isolated from *B. papilosa* (*244*). The UV spectrum showed absorption at 224 nm, indicating a tertiary benzamide chromophore. The IR spectrum displayed intense absorption at 3400 (OH), 1722 (ester C=O), 1626 (α,β-unsaturated C=O), and 1617 (C=C) cm^{-1}. The presence of a benzamide group was also indicated by three multiplets centered at δ7.38, 7.45, and 7.74 in the ^{1}H-NMR spectrum. The C-19 methylene protons appeared as a multiplet at δ2.74. A broad singlet at δ5.62

corresponding to the C-1 olefinic proton showed coupling with the C-2 methylene protons. A set of AB doublets resonating at δ3.82 and 3.99 ($J_{31\alpha,31\beta}$ = 9.3 Hz) was assigned to the C-31α and C-31β protons, respectively. These values were shifted downfield (δ4.16 and 4.20) in the acetylated product. The C-16 methine proton geminal to the acetoxy group appeared as a multiplet at δ4.73. A relatively intense peak at *m/z* 171 ($C_9H_{17}NO_2$) in the mass spectrum was attributed to the cleavage of ring D along with the nitrogen containing side-chain. The composition of this ion indicated that the acetoxy group is located on ring D. The probable position of the acetoxy group on C-16 was also indicated by the ion at *m/z* 157 ($C_8H_{15}NO_2$) resulting from the cleavage of ring D along with the substituents.



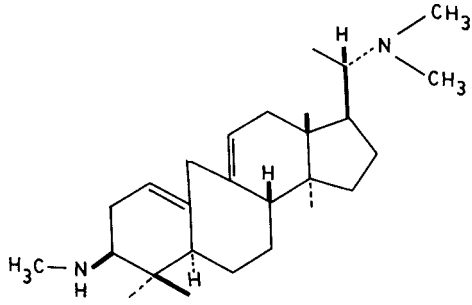
593

(−)-Buxapapinolamine, extracted from *B. papilosa*, was formulated as **593** on the basis of spectroscopic studies (*203*). The UV spectrum indicated the presence of a benzamidic chromophore. The IR spectrum showed absorption bands at 3670 (N-H), 3310 (OH), 1732 (COOR), 1722 (CHO), and 1658 (aromatic amide) cm^{-1}. Three singlets at δ0.73, δ0.88, and δ1.01 in the ^{1}H-NMR spectrum of **593** were assigned to the three tertiary methyl groups present in the molecule. A three-proton doublet at δ0.92 was observed due to the 21-methyl group.



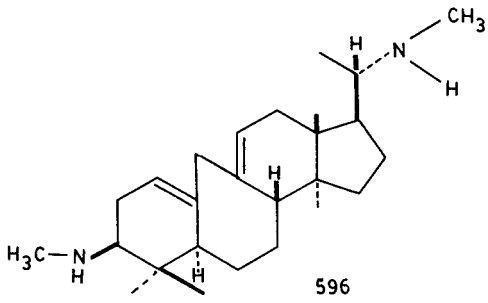
594

The weakly basic alkaloidal fraction of *B. papilosa* leaves yielded three new alkaloids—(+)-buxabenzamidine (**594**), (+)-buxupapine (**595**), and (+)-N_b-norbuxupapine (**596**) (*204*). Spectral studies of these alkaloids resulted in the establishment of their structures. The IR (1650 cm^{-1}) and UV spectra (λ_{max} 226 nm) of (+)-buxabenzamidine (**594**) were diagnostic of a benzamidic chromophore. The molecular ion appeared at *m/e* 490 in the mass spectrum, with the base peak at *m/e* 72 corresponding to the trimethyliminium cation, $H_3C—CH=N^+(CH_3)_2$. The 360 MHz ^{1}H-NMR spectrum revealed only one proton in the olefinic region (a double doublet at δ5.34, J = 2.1, 1.6 Hz) and four tertiary methyl groups at δ0.69, δ0.77, δ1.00, and δ1.01. The 21-methyl group appeared as a doublet centered at δ0.91 (J = 6.3 Hz). The downfield shift of H-3 (δ4.12) was attributed to the presence of benzamide group on C-3.



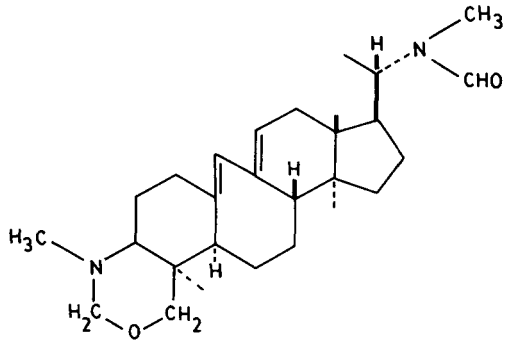
595

(+)-Buxupapine (**595**) showed the presence of three *N*-methyl groups, two nonconjugated olefinic protons, and four tertiary methyl groups. Fragment ions at *m/e* 355 and 85 in the mass spectrum corresponding to loss of $(CH_3)_2C=CH—{}^+NHCH_3$ from ring A, and retro-Diels–Alder cleavage of ring A, suggested the presence of secondary amine at C-3. The base peak at *m/e* 72 indicated the trimethyliminium side chain. These spectral evidences led to the assignment of structure **595** to (+)-buxupapine (*204*).



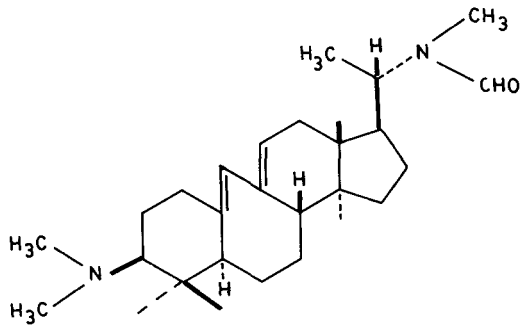
596

(+)-Buxupapine was accompanied in the plant by its *N*-demethyl analog, (+)-N_b-norbuxupapine (**596**) (*204*). The ^{1}H-NMR spectrum of **596** was strikingly similar to that of **595,** the main difference being the lack of an *N*-methyl signal. The base peak appeared at *m/e* 58 instead of *m/e* 72, suggesting 20-*N*-methylamino functionality.



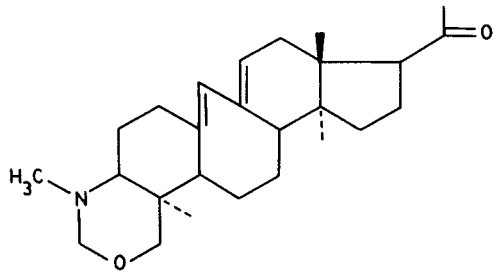
597

Both (+)-*N*-formylharappamine (**597**) and *N*-formylpapilicine (**598**), isolated from *B. papilosa,* have been found to possess the 9(10 → 19)-*abeo*-pregnane skeleton (*221*). (+)-*N*-Formylharappamine (**597**) displayed a ^{1}H-NMR spectrum similar to that reported for harappamine (**583**) (*213,214*). An additional singlet at δ8.11 represented the N_b-formyl proton. Similarly, a singlet at δ2.74 was diagnostic of the N_b-methyl group. The mass spectrum of **597** exhibited the molecular ion peak at *m/e* 440, with the base peak at *m/e* 86 representing the *N*-formyldimethyliminium cation.



598

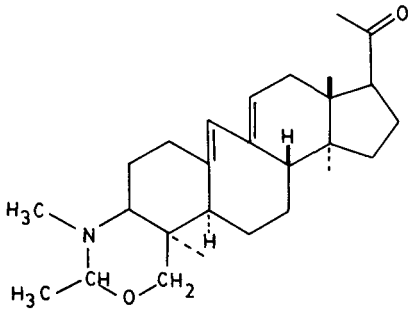
(+)-*N*-Formylpapilicine was assigned structure **598** by comparison of its spectral data with those of the known alkaloid papilicine (**581**). The formyl proton resonated as a singlet at δ8.11 in the NMR spectrum while a singlet at δ2.74 was diagnostic of N_b-methyl group. The mass spectrum showed the molecular ion at *m/e* 426, and a relatively intense peak at *m/e* 426 corresponding to the *N*-formyldimethyliminium cation. On the basis of these data, **598** was identified as the *N*-formyl derivative of papilicine (*221*).



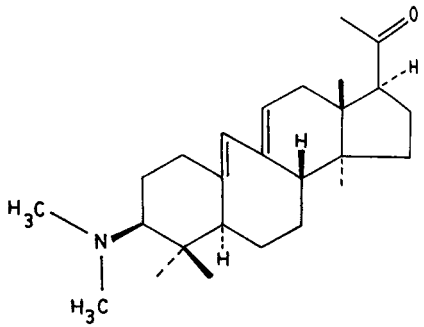
599

b. Monoamino Alkaloids C-3 Amino Derivatives. Buxaquamarine (**599**) isolated from *B. papilosa* showed a UV spectrum characteristic of the 9(10→19) *abeo*-diene system. An absorption band at 1645 cm^{-1} in its IR spectrum indicated the presence of a ketone function. The ^{1}H-NMR spectrum revealed the presence of tetrahydrooxazine ring. A set of AB doublets at δ3.61 and δ4.45 (J = 7.5 Hz) was assigned to the C-31 methylene protons adjacent to the C-3 nitrogen, while another set of AB doublets at δ3.27 and δ3.84 (J = 10.6 Hz) was attributed to the C-29 methylene protons. The isolated olefinic proton at C-19 appeared as a singlet at δ6.00. Another singlet at δ2.12 was assigned to the *N*-methyl group. The mass spectrum showed an important fragment at *m/e* 127, which may arise by cleavage of ring A accompanied by an intramolecular proton transfer. On the basis of these spectral evidences structure **599** was assigned to buxaquamarine; it was suggested that buxaquamarine may arise in nature from **582** or **583** by oxidation of the *N*-bearing side chain to the corresponding ketimine, followed by its hydrolytic removal (*222*).

The structure of (+)-homobuxaquamarine (**600**), isolated from *B. papilosa*, was established on the basis of spectral behavior (*204*). (+)-Homobuxaquamarine showed a UV spectrum similar to moenjodaramine (**582**), harappamine (**583**), and buxaquamarine (**599**). A strong absorption band at 1695 cm^{-1} indicated the presence of carbonyl group. The ^{1}H-NMR spectrum of **600** was similar to that of **599.** The C-31 proton, however, resonated as a quartet at



600

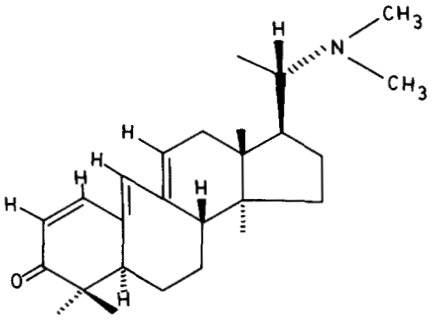


691

δ3.63 (J = 5.4 Hz) and an additional three-proton doublet at δ1.33 (J = 5.4 Hz) suggested that this methyl group is substituted in C-31 of the tetrahydrooxazine ring. The fragment at m/e 141 in the mass spectrum could arise by the cleavage of ring A.

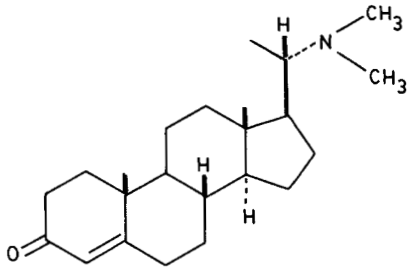
Buxaminone, isolated from *B. papilosa,* was found to possess the 9(10 → 19) *abeo* diene system, characterized by absorption maxima at 238 and 248 nm in the UV spectrum. The IR spectrum showed bands at 1690 (ketonic C=O) and 1569 (C=C) cm^{-1}. The compound showed the base peak at m/z 71.7072 attributed to the loss of CH_2=CH—$N^+(CH_3)_2$ ion, common in *Buxus* alkaloids containing a dimethylamino substituent at C-3. The ^{1}H-NMR spectrum of buxaminone was strikingly similar to that reported for (+)-papilicine (**581**). It showed four singlets at δ0.66, 0.76, 0.77, and 1.07 for the four tertiary methyl groups. A downfield singlet at δ2.10, integrating for three protons, was assigned to the 21-methyl group. These spectroscopic studies led to the assignment of structure **691** to buxaminone.

C-20 Amino Derivatives. (+)-Cyclobuxotriene (**601**), isolated from *B. papilosa,* exhibited an absorption at 324 nm in its UV spectrum, indicative of a highly conjugated system. Absorption at 1652 cm^{-1} in the IR spectrum showed the presence of a conjugated ketone; absorption at 1600 cm^{-1} indicated an olefinic linkage. The ^{1}H-NMR spectrum showed four signals in the olefinic region; at δ6.00 (doublet, J = 10.0 Hz), δ6.24 (double doublet, J_1 = 2.5, J_2 = 1.8 Hz), δ6.71 (doublet, J = 10.0 Hz), and δ7.42 (doublet, J = 2.2 Hz),



601

attributable to C-2, C-11, C-1, and C-19 protons, respectively. This was in accord with the high degree of conjugation revealed by the UV spectrum. The mass spectrum showed the molecular ion at *m/e* 381 and an interesting peak at *m/e* 167 resulting from retro-Diels–Alder cleavage of ring C. The base peak at *m/e* 72 was diagnostic of dimethyliminium side chain. On the basis of these studies, structure **601** was assigned to (+)-cyclobuxotriene (*220*).



602

Another alkaloid, (+)-buxaprogestine (**602**), was extracted from the weakly basic fraction of an extract of *B. papilosa* leaves (*203*). This yielded the second example of occurrence of a simple pregnane derivative in Buxaceae. The presence of α,β-unsaturated ketone functionality was concluded from the UV and IR spectra of **602.** The ^{1}H-NMR spectrum displayed singlets at δ0.72 and δ1.18 due to the 18- and 19-methyl groups. A doublet at δ5.73, showing long-range coupling (J = 1.1 Hz) with H-6, was assigned to the C-4 olefinic proton. The mass spectrum showed peaks at *m/e* 343 (M^+), 328, 84, 72 (base peak), and 58. These studies led to the establishment of structure **602** for (+)-buxaprogestine.

4. Mass Spectroscopy

Mass fragmentation patterns of *Buxus* alkaloids provides useful information about individual structural types, positions of functional groups, and the position and extent of unsaturation in different rings. The lower mass region of the spectrum has considerable importance in this connection.

Compounds bearing oxygenated function on ring D, such as **532,** exhibit characteristic peaks in their mass spectra. The considerably large fragment ion of *m/e* 129 ($C_7H_{15}NO$) is formed in **532** by the cleavage of –OH-containing ring D along with the $N(CH_3)_2$-bearing side chain.

Compounds bearing a monomethyl amino substituent at C-20 show the base peak at *m/e* 58 due to the fragment CH_3—CH=N^+HCH_3, while compounds containing a dimethylamino side chain exhibit a fragment at *m/e* 72 as the base peak. Interestingly, fragment ions arising by cleavage of a ring D side chain are usually more abundant as compared to fragments arising by cleavage of ring A. Compounds which contain a dimethylamino group at C-3 give rise to the base peak at *m/e* 71, while compounds containing monomethylamino ($HNCH_3$) group at C-3 give rise to the base peak at *m/e* 57.

The mode of unsaturation in different rings of *Buxus* alkaloids has a pronounced effect on the fragmentation pattern. Compounds having isolated double bonds show fragment ions at *m/e* 58 resulting from the retro-Diels–Alder cleavage of ring A; fragment ions at *m/e* 167 result from cleavage of ring D.

Buxus alkaloids having unsubstituted tetrahydrooxazine ring (exemplified by **599**) show considerably large *m/e* 127 ions in their mass spectra, while alkaloids containing a methyl substituent on the tetrahydrooxazine ring exhibit large fragment ions at *m/e* 141 formed by the cleavage of ring A. Some diagnostic ions of *Buxus* alkaloids are shown in Table II; Table III lists the physical data of the new steroidal alkaloids.

TABLE II
SOME COMMON FRAGMENTS IN ALKALOIDS OF BUXACEAE

m/e	Formula	Structure
44	C_2H_6N	CH_2=$\overset{+}{N}H(CH_3)$
57	C_3H_7N	CH_3—$\dot{C}$=$\overset{+}{N}H(CH_3)$
58	C_3H_8N	CH_3—CH=$\overset{+}{N}HCH_3$
71	C_4H_9N	CH_3—$\dot{C}$=$\overset{+}{N}(CH_3)_2$
72	$C_4H_{10}N$	CH_3—CH=$\overset{+}{N}(CH_3)_2$

(*continued*)

TABLE II
(*Continued*)

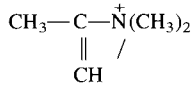
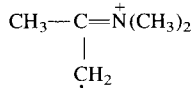
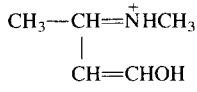
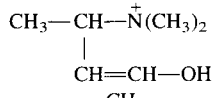
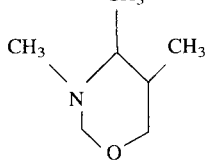
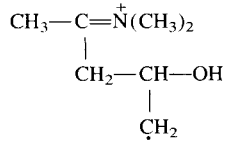
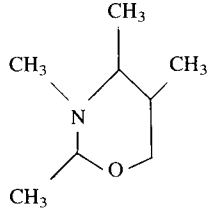
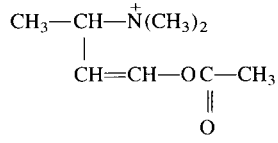
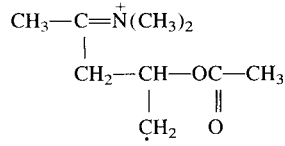
m/e	Formula	Structure
84	$C_5H_{10}N$	
85	$C_5H_{11}N$	
101	$C_5H_{11}NO$	
115	$C_6H_{13}NO$	
127	$C_7H_{15}NO$	
129	$C_7H_{15}NO$	
141	$C_8H_{14}NO$	
157	$C_8H_{15}NO_2$	
171	$C_9H_{17}NO_2$	

TABLE III
NEW STEROIDAL ALKALOIDS

Alkaloid	Source (refs.)	Molecular weight	Molecular formula	Structure number	Physical data						
					mp(°C)	$[\alpha]_D$	UV	IR	Mass	^{1}H-NMR	^{13}C-NMR
Alkaloids of family Apocynacea											
Conanine type											
Holarrheline	*H. floribunda* [4,5]	358	$C_{23}H_{38}N_2O$	**1**	190	+18° (EtOH)	—	[5]	[5]	[5]	—
Holadienine	*H. floribunda* [4,5]	325	$C_{22}H_{31}NO$	**2**	110 (sub.)	+80° ($CHCl_3$)	[5]	[6]	[6]	[6]	—
Holaromine	*H. floribunda* [4,5]	309	$C_{22}H_{31}N$	**3**	198	+90 ($CHCl_3$)	[4,5]	[4,5]	[5]	[5]	—
Holaline	*H. floribunda* [4,5]	374	$C_{24}H_{42}N_2O$	**4**	267	+36° ($CHCl_3$: MeOH/1:1)	—	[5,6]	[5,6]	[5,6]	—
Maingayine	*P. maingayi* [7]	309	$C_{21}H_{27}NO$	**10**	150–152	+2.1° ($CHCl_3$)	[7]	[7]	—	[7]	—
N-Formylconkurchine	*H. crassifolia* [8]	340	$C_{22}H_{32}N_2O$	**15**	247	−50°	—	[8]	[8]	[8]	—
Malouetamide	*M. hondelotii* [9]	341	$C_{22}H_{31}NO$	**17**	184	+144° ($CHCl_3$)	[9]	[9]	[9]	[9]	—
Malouetafrine	*M. hondelotii* [9]	311	$C_{21}H_{29}NO$	**21**	205	+89° ($CHCl_3$)	[9]	[10]	[9]	[9]	—
Holarrhesine	*H. floribunda* [10]	454	$C_{29}H_{23}N_2O_2$	**22**	oil	−14.8°	—	[11,12]	[10]	[10]	—
Holacine	*H. antidysenterica* [11,12]	416	$C_{26}H_{44}N_2O_2$	**23**	270–271	−49° (EtOH)	—		[11,12]	[11,12]	—
Pregnane type											
Methylholaphylline	*H. floribunda* [13]	343	$C_{23}H_{37}NO$	**24**	121	+29° ($CHCl_3$)	—	[13]	—	[13]	—
Holaphyllinol	*H. floribunda* [13]	331	$C_{22}H_{37}NO$	**26**	—	—	—	[13]	—	[13]	—
Holaphyllidine	*H. floribunda* [13]	331	$C_{22}H_{37}NO$	**27**	226–227	−71° ($CHCl_3$)	—	—	—	[13]	—
Dihydroholaphyllamine	*H. floribunda* [13]	317	$C_{21}H_{35}NO$	**29**	300	+79° ($CHCl_3$)	—	[13]	—	[13]	—
Bokitamine (12β-hydroxyfuntumine)	*H. wulfabergii* [16]	333	$C_{21}H_{35}NO_2$	**30**	191	—	—	[16]	[16]	[16]	—
Kisantamine	*H. congolensis* [20]	315	$C_{21}H_{33}NO$	**31**							—
Holarricine	*H. antidysenterica* [11,12]	360	$C_{21}H_{32}N_2O_3$	**32**	350–351	−31° (ETOH)	[11,12]	[11,12]	[11,12]	[11,12]	—
Holacetine	*H. antidysenterica* [21]	361	$C_{23}H_{39}NO_2$	**36**	258	+6.9°	—	[21]	[21]	[21]	—
20-Epiirehdiamine-I	*F. elastica* [20]	372	$C_{25}H_{44}N_2$	**42**	151–153	−36° ($CHCl_3$)	—	—	[20]	[20]	
N-Desmethylholacurtine	*H. curtisii* [24]	477	$C_{28}H_{47}NO_5$	**43**							
Paravallarine type											
7α-Hydroxyparvallarine	*P. microphylla* [25,26]	359	$C_{22}H_{33}NO_3$	**44**	213	−113°	—	[25,26]		[25,26]	—
7β-Hydroxyparavallarine	*P. microphylla* [25,26]	359	$C_{22}H_{33}NO_3$	**47**	210	−113°	—	[25,26]		[26]	—
11α-Hydroxyparavallarine	*P. microphylla* [25,26]	359	$C_{22}H_{33}NO_3$	**52**	235	−55°	—	[25,26]	[25,26]	—	—

2α-Hydroxy*N*-methyl-20-epiparavallarine	*K. ofitingensis* [35,36]	373	$C_{23}H_{35}NO_3$	**61**	200	−19°	—	[35]	[38]	[38]	—
Gitingensine	*K. ofitingensis* [35,36]	329	$C_{21}H_{31}NO_2$	**62**			—	—	[35,37]	—	—
Lanitine (2α-hydroxy-*N*-methyl-paravallarine)	*K. gitingensis* [41]	375	$C_{23}H_{37}NO_3$	**63**							
2β-Hydroxy-*N*-methyl-paravallarine	*K. gitingensis* [41]	375	$C_{23}H_{37}NO_3$	**64**							
20-Epikibataline	*P. microphylla* [42]	357	$C_{23}H_{35}NO_2$	**65**	220–223 (CH_3OH)	−30° ($CHCl_3$) −11.8° (EtOH)	—	[42]	[42]	[42]	—
Amino-glyco-steriods											
Holacurtine	*H. curtisii* [43]	491	$C_{29}H_{49}NO_5$	**66**	162	+42°	—	[43]	—	[43]	—
Mitiphylline	*H. mitis* [45]	531	$C_{31}H_{49}NO_6$	**70**	—	+20°	[45]	[45]	[45]	[45]	—
Holantosine-A	*H. antidysenterica* [46]	493	$C_{28}H_{47}NO_6$	**72**	260–261*	−28°*	—	[46]*	[46]*	[46]*	—
Holantosine-B	*H. antidysenterica* [46]	461	$C_{28}H_{45}NO_5$	**73**	290*	−29°	—	[46]*	[46]*	[46]*	—
Holantosine-C	*H. antidysenterica* [47]	493	$C_{28}H_{47}NO_6$	**77**	245*	−73°	—	[47]*	[47]*	[47]*	—
Holarosine-A	*H. antidysenterica* [47]	517	$C_{30}H_{47}NO_6$	**79**	268–270*	−39°*	—	[47]*	[46]*	[47]*	—
N-Demethylmitiphylline	*H. mitis* [48]	517	$C_{30}H_{47}NO_6$	**81**	amorphous	+9°	—	—	[48]	[48]	—
Holantosine-D	*H. antidysenterica* [47]	475	$C_{28}H_{45}NO_5$	**82**	262–263*	−62°	—	[47]	[47]	[47]	—
Holarosine-B	*H. antidysenterica* [49]	517	$C_{30}H_{47}NO_6$	**84**	—	+4°	—	[49]	[49]	[49]	—
Holantosine-E	*H. antidysenterica* [49]	493	$C_{28}H_{47}NO_6$	**85**	152*	−47°	—	[49]*	[49]*	[49]*	—
Holantosine-F	*H. antidysenterica* [49]	475	$C_{28}H_{45}NO_5$	**86**	134*	−43° ($CHCl_3$)	—	[49]*	[49]*	[49]*	—
Alkaloids of family Buxaceae											
9β,19-*cyclo*-5α-*pregnane type*											
Cycloprotobuxine-F	*B. madagascarica* [190]	386	$C_{26}H_{46}N_2$	**534**	163	+42°	—	[190]	—	[190]	—
N-Benzoylcycloviro-buxenine-E	*B. sempervirens*	504	$C_{33}H_{48}N_2O_2$	**535**	120/190–193	+32° ($CHCl_3$)	[191]	[191]	[191]	[191]	—
Cyclobuxupaline-C	*B. papilosa*	398	$C_{27}H_{46}N_2$	**537**	111–113	−37° ($CHCl_3$)	—	[192]	[192]	[192]	—
Cyclopapilosine-D	*B. papilosa*	402	$C_{26}H_{46}N_2O$	**538**	233–235	+54° ($CHCl_3$)	—	[192]	[192]	[192]	—
Cyclobullatine-A	*B. sempervirens* var. *angustifolia* [177] var. *bullata* [193] var. *argentea* [194] var. *rotundifolia* [194]	414	$C_{27}H_{46}N_2O$	**539**	275	−99° (EtOH)	[177]	[177]	[177]	—	—
l-Cycloprotobuxine-C	*B. sempervirens* [195,196]	400	$C_{27}H_{48}N_2$	**540**	195–197	−62°	—	[195,196]	[195,196]	[195,196]	
Buxozine-C	*B. sempervirens* [197,198]	416	$C_{27}H_{48}N_2O$	**541**	137	+65° ($CHCl_3$)	—	[197]	—	[198]	—
Cyclobuxargentine-G	*B. sempervirens* [194]	386	$C_{25}H_{42}N_2$	**544**	283	−51°	[194]	[194]	[194]	—	—
Cyclobuxophylline-O	*B. sempervirens* [189]	357	$C_{24}H_{39}NO$	**558**	219–222 (acetone)	−61.5° ($CHCl_3$)	[189]	[189]	—	[189]	—

(*continued*)

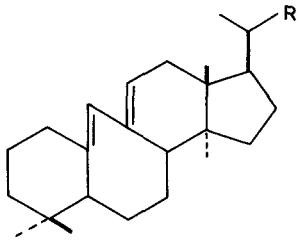
TABLE III (*Continued*)

Alkaloid	Source (refs.)	Molecular weight	Molecular formula	Structure number	Physical data						
					mp(°C)	$\lvert\alpha\rvert_D$	UV	IR	Mass	^{1}H-NMR	^{13}C-NMR
Buxithienine-M	*B. sempervirens* [*189*]	367	$C_{25}H_{37}NO$	**559**	166–169 (acetone + 5% CH_3OH)	−75.6° ($CHCl_3$)	[*189*]	[*189*]	[*189*]	[*189*]	—
(−)-*E*-Cyclobuxaphylamine	*B. papilosa* [*203*]	367	$C_{25}H_{37}NO$	**560**	—	−30° ($CHCl_3$)	[*203*]	[*203*]	[*203*]	[*203*]	—
(−)-*Z*-Cyclobuxaphylamine	*B. papilosa* [*203*]	367	$C_{25}H_{37}NO$	**561**	—	−76° ($CHCl_3$)	[*203*]	[*203*]	[*203*]	[*203*]	—
(+)-Norcyclomicrobuxeine	*B. papilosa* [*204*]	353	$C_{24}H_{35}NO$	**562**	—	+34° ($CHCl_3$)	[*204*]	[*204*]	[*204*]	[*204*]	—
Buxamidine	*B. papilosa* [*206*]	397	$C_{26}H_{39}NO_2$	**568**	—	−11°	[*206*]	[*206*]	[*206*]	[*206*]	[*206*]
Cyclobuxoviricine	*B. papilosa* [*207*]	369	$C_{25}H_{39}NO$	**569**	—	−54° ($CHCl_3$)	[*207*]	[*207*]	[*207*]	[*207*]	[*207*]
Cycloxobuxoviricine	*B. papilosa* [*208*]	385	$C_{25}H_{39}NO_2$	**570**	—	−41.2° ($CHCl_3$)	[*208*]	[*208*]	[*208*]	[*208*]	—
Sempervіramidine	*B. sempervirens* [*237*]	564	$C_{35}H_{52}N_2O_4$	**684**	—	—	[*237*]	[*237*]	[*237*]	[*237*]	—
(+)-*N*-Benzoyl-16α-acetoxycycloxobuxidine-F	*B. papilosa* [*238*]	578	$C_{35}H_{50}N_2O_5$	**685**	—	—	[*238*]	[*238*]	[*238*]	[*238*]	[*238*]
N-Formylcyclomicrobuxeine	*B. papilosa* [*242*]	381	$C_{25}H_{35}NO_2$	**686**	—	+16° ($CHCl_3$)	[*242*]	[*242*]	[*242*]	[*242*]	—
(−)-Z-Buxenone	*B. sempervirens* [*241*]	369	$C_{25}H_{39}NO$	**687**	—	−67° ($CHCl_3$)	[*241*]	[*241*]	[*241*]	[*241*]	—
Sempervirone	*B. sempervirens* [*240*]	397	$C_{26}H_{39}NO_2$	**688**	—	−65° ($CHCl_3$)	[*240*]	[*240*]	[*240*]	[*240*]	—
9(10 → 19)-abeo-5α-pregnane type											
Buxamine-A	*B. madagascarica* [*190*]	412	$C_{28}H_{48}N_2$	**575**	134	+40°	[*190*]	—	[*190*]	[*190*]	—
16-Deoxybuxidienine-C	*B. madagascarica* [*190*] *B. papilosa* [*192*]	414	$C_{27}H_{46}N_2O$	**576**	200–201	+55°	[*190*]	—	[*190*]	[*190*]	—
Buxitrienine-C	*B. madagascarica* [*190*]	412	$C_{27}H_{44}N_2O$	**577**	192	+57°	[*190*]	[*190*]	[*190*]	[*190*]	—
N-Benzoylbuxodienine-E	*B. sempervirens* [*209*]	504	$C_{33}H_{49}N_2O$	**578**	235–237	+34.6 ($CHCl_3$)	[*209*]	[*209*]	[*209*]	[*209*]	—
Buxamine-C	*B. papilosa* [*192*]	398	$C_{27}H_{46}N_2$	**579**	153–155	+24° ($CHCl_3$)	[*192*]	[*192*]	[*192*]	[*192*]	—
Buxaminol-B	*B. sempervirens* [*193*]	414	$C_{26}H_{44}N_2O$	**580**	225	+20° (CH_3OH)	[*193*]	[*193*]	[*193*]	[*193*]	[*193*]
Buxamine-B (papilicine)	*B. harlandi* [*210*] *B. papilosa* [*211*]	398	$C_{27}H_{46}N_2$	**581**	248	+61.4°	[*210*]	[*210*]	[*210*]	[*211*]	[*210,211*]
Moenjodaramine	*B. papilosa* [*212,213*]	426	$C_{28}H_{46}N_2O$	**582**	177	+33.3 ($CHCl_3$)	[*212,213*]	[*212,213*]	[*212,213*]	[*212,213*]	[*213*]
Harappamine	*B. papilosa* [*212,214*]	412	$C_{27}H_{44}N_2O$	**583**	—	—	[*212,214*]	[*212,214*]	[*212,214*]	[*212,214*]	[*214*]
Buxaminol-G	*B. papilosa* [*215*]	444	$C_{28}H_{48}N_2O_2$	**584**	—	+83.3° ($CHCl_3$)	[*215*]	[*215*]	[*215*]	[*215*]	—

Papilamine	*B. papilosa* [216]	384	$C_{26}H_{44}N_2$	**585**	—	+23.3° ($CHCl_3$)	[216]	—	[216]	[216]	—
Papilinine	*B. papilosa* [217]	398	$C_{26}H_{42}N_2O$	**586**	—	+29.4° ($CHCl_3$)	[217]	[217]	[217]	[217]	—
Karachicine	*B. papilosa* [218]	412	$C_{27}H_{44}N_2O$	**587**	—	—	[218]	[218]	[218]	[218]	—
N-Benzoyl-3(0)-acetoxybuxidienine	*B. papilosa* [219]	562	$C_{35}H_{50}N_2O_4$	**588**	—	−4° ($CHCl_3$)	[219]	[219]	[219]	[219]	[219]
(+)-Buxabenzamidienine	*B. papilosa* [220]	488	$C_{33}H_{48}N_2O$	**589**	—	+6° ($CHCl_3$)	[220]	[220]	[220]	[220]	—
16α-Acetoxybuxabenzamidiene	*B. papilosa* [220]	546	$C_{35}H_{50}N_2O_3$	**590**	—	+6° ($CHCl_3$)	[220]	[220]	[220]	[220]	—
(+)-Buxanoldine	*B. papilosa* [220]	520	$C_{33}H_{48}N_2O_3$	**591**	—	−27.4 ($CHCl_3$)	[220]	[220]	[220]	[220]	—
(+)-Buxapapinolamine	*B. papilosa* [220]	562	$C_{35}H_{50}N_2O_4$	**592**	—	+12° ($CHCl_3$)	[220]	[220]	[220]	[220]	—
(−)-Buxapapinolamine	*B. papilosa* [203]	576	$C_{33}H_{48}N_2O_5$	**593**	—	−16° ($CHCl_3$)	[203]	[203]	[203]	[203]	—
(+)-Buxabenzamidine	*B. papilosa* [204]	490	$C_{33}H_{50}N_2O$	**594**	—	+47° ($CHCl_3$)	[204]	[204]	[204]	[204]	—
(+)-Buxupapine	*B. papilosa* [204]	398	$C_{27}H_{46}N_2$	**595**	—	+11° ($CHCl_3$)	[204]	[204]	[204]	[204]	—
(+)-N_b-Norbuxupapine	*B. papilosa* [204]	384	$C_{26}H_{44}N_2$	**596**	—	+20° ($CHCl_3$)	[204]	[204]	[204]	[204]	—
(+)-*N*-Formylharappamine	*B. papilosa* [221]	440	$C_{28}H_{44}N_2O_2$	**597**	—	+40° ($CHCl_3$)	[221]	[221]	[221]	[221]	—
N-Formylpapilicine	*B. papilosa* [221]	426	$C_{28}H_{46}N_2O$	**598**	—	+36° ($CHCl_3$)	[221]	[221]	[221]	[221]	—
Buxaquamarine	*B. papilosa* [222]	397	$C_{26}H_{39}NO_2$	**599**	—	+24° ($CHCl_3$)	[222]	[222]	[222]	[222]	—
(+)-Homobuxaquamarine	*B. papilosa* [204]	411	$C_{27}H_{41}NO_2$	**600**	—	+21.9°	[204]	[204]	[204]	[204]	—
(+)-Cyclobuxotriene	*B. papilosa* [220]	381	$C_{26}H_{39}NO$	**601**	—	+13.5	[220]	[220]	[220]	[220]	—
(+)-Buxaprogestine	*B. papilosa* [203]	343	$C_{23}H_{37}NO$	**602**	—	+26° ($CHCl_3$)	[203]	[203]	[203]	[203]	—
Papillamidine	*B. papilosa* [243]	426	$C_{27}H_{42}N_2O_2$	**689**	—	0° ($CHCl_3$)	[243]	[243]	[243]	[243]	—
Buxabenzacine	*B. papilosa* [244]	578	$C_{36}H_{54}N_2O_4$	**690**	216 (dec.)	+48° ($CHCl_3$)	[244]	[244]	[244]	[244]	[244]
Buxaminone	*B. papilosa* [245]	381	$C_{26}H_{41}NO$	**691**		−22° ($CHCl_3$)	[245]	[245]	[245]	[245]	—

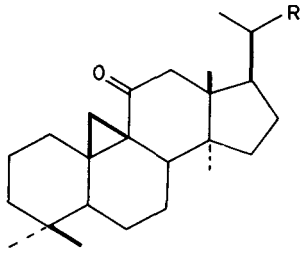
5. *General Rules Relating Specific Rotation to Molecular Structure*

A comparison of the specific rotations (recorded in chloroform) of different *Buxus* alkaloids has led to the establishment of the following general rules for the relationships between structure and specific rotation (*220*).



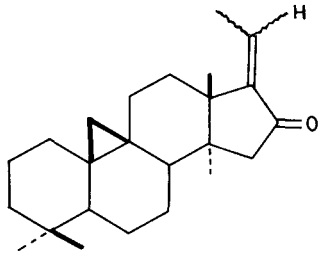
603

1. 9(10 → 19) *abeo* dienes **603** are dextrorotatory. Specific examples are buxamine-E (442°), papilamine (+23.3°), buxamine-C (+24°), desoxy-16-buxidienine (+33°), buxaquamarine (+24°), papilicine (+47°), buxamine-A (+40°), papilanine (+29.4°), buxaminol-B (+20°), buxaminol-E (+40°), and moenjodaramine (+33.3°). An exception to this is *N*-benzoylbuxidienine (−36°), whose optical activity needs to be redetermined.



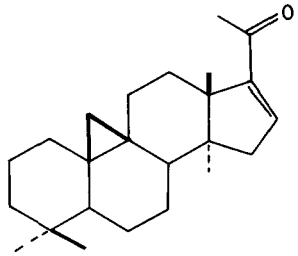
604

2. 9β,19-Cyclo-11-oxo compounds **604** are also dextrorotatory, but with larger magnitude. Examples include baleabuxoxazine-C (+116°), *N*-benzoylbaleabuxidine-F (+52°), *N*-isobutyroylcycloxobuxidine-H (+76°), baleabuxidine (+127°), *N*-isobutyroylcycloxobuxidine-F (+71°), *N*-isobutyroylbaleabuxidine-F (+76°), *N*-benzoylcycloxobuxine-F (+90°), *N*-benzoylcycloxobuxoline-F (+76°), *N*-isobutyroylcycloxobuxine (+115°), buxarine (+98°), *N*-benzoylcycloxobuxidine-F (52°), *N*-benzoyl-*O*-acetylcycloxobuxoline-F (114°), and buxatine (+108°).



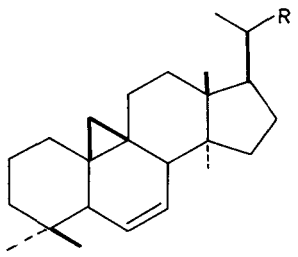
605

3. 9β,19-Cyclo-16-oxo-Δ (17 → 20) alkaloids **605** are levorotatory, regardless of the geometrical isomerism about the C-17(20) double bond. Some examples are cyclobuxophylline-O (−61.5°), buxenone (−48°), cyclobuxophylline-K (−67°), cyclobuxophylline (−72°), methylbuxene (−104°), and cyclobuxosuffrine (−62°).



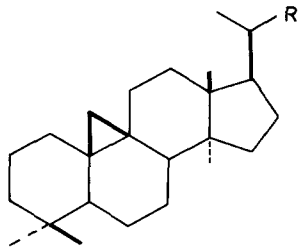
606

4. 9β,19-Cyclo-20-oxo-Δ (16 → 17) derivatives **606** are dextrorotatory. Only two examples are available, cyclomicrobuxeine (+126°) and cyclobuxomicreine (+37°). The former of these also incorporates an exocyclic methylene at C-4 instead of the usual *gem* dimethyl group.



607

5. 9β,19-Cyclo-Δ (6 → 7) compounds **607** are levorotatory, as exemplified by cyclobuxupaline-C (−37°), cyclobullatine-A (−99°), cyclovirobuxeine-B (−80°), cyclovirobuxeine-A (−87°), *N*-benzoyldihydrocyclomicrophylline-F (−20°), cyclomicrosine (−33°), and cyclomalayanine-B (−60°).



608

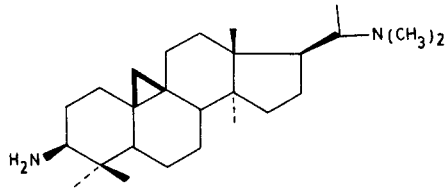
6. Simple saturated *Buxus* alkaloids with a 9β,19-cyclo system **608** are dextrorotatory. Relevant examples are cycloprotobuxine-F (+42°), cycloprotobuxine-C (+68°), buxocyclamine-A (+87°), cyclovirobuxine-D (+63°), cyclopapilosine-D (54°), cyclovirobuxine-C (+62°), cyclorofloxazine (+106°), buxozine (+65°), *N*-acetylcycloprotobuxine-D (+53°), 16-deoxycyclobuxoxazine (+56°), cyclorolfeibuxine-C (+52°), dihydrocyclomicrophylline-A (+46°), and *N*-benzoylcycloprotobuxoline (+42°).

It was concluded from these features that substitution at C-30 does not have any pronounced effect on specific rotation. Similarly, direction of rotation remains unchanged by replacement of substituents on C-16 of ring D.

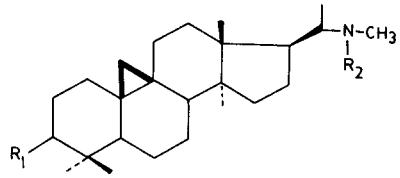
B. Syntheses and Chemical Transformations of Steroidal Alkaloids of Buxaceae

The transformation of cycloprotobuxine-F (**534**) into the lactone **618** and the tertiary alcohol **612,** which are important intermediates for the synthesis of holothurinogenin, has been described (*223*).

Cycloprotobuxine-F (**534**) was oxidized with ruthenium tetroxide to the 3-oxo-20-formylmethylamino derivative, which was then reduced to the corresponding alcohol **609.** This was deformylated to the methylamino derivative **610** on hydrogenolysis. Deamination to the 20-oxo compound **611** followed by treatment with the appropriate Grignard reagent, gave the tertiary alcohol **612.** Acetylation of the deamination product **611** and reduction of the acetylated substance **613** yielded the (20*R*) alcohol **614.** Conversion to (20*R*) nitrite **615** followed by its irradiation gave the 18-oximino (20*R*) hydroxy compound **616.** Subsequent oxidation to the lactone **617** followed by hydrolysis yielded **618.**

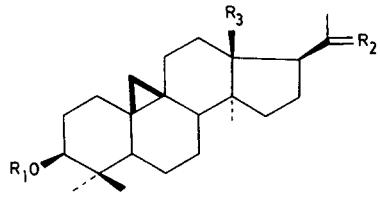


534



609 $R_1 = \langle^{H}_{OH}$, R_2 = CHO

610 $R_1 = \langle^{H}_{OH}$, R_2 = H



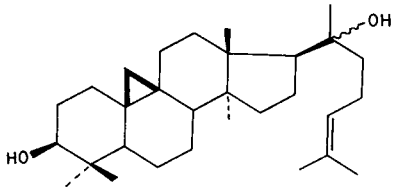
611 R_1 = H, R_2 = O, R_3 = CH_3

613 R_1 = Ac, R_2 = O, R_3 = CH_3

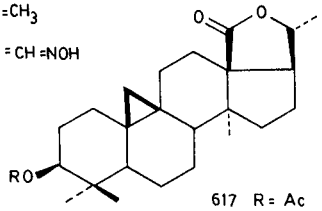
614 R_1 = Ac, $R_2 = \langle^{H}_{OH}$, R_3 = CH_3

615 R_1 = Ac, $R_2 = \langle^{H}_{ONO}$, R_3 = CH_3

616 R_1 = Ac, $R_2 = \langle^{H}_{OH}$, R_3 = CH=NOH



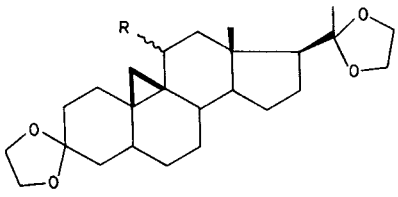
612



617 R = Ac

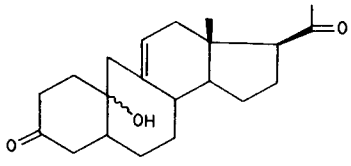
618 R = H

The 9β,19-cyclo-11-keto steroid system showed a marked difference in reactivity from the corresponding triterpenoid derivatives, caused by conformational differences between the two systems (*224*). The triterpenoid steroids 11α- and 11β-hydroxy-9,19-cyclo-5α,9β-pregnan-3,20-dione bis (ethylene ketal) (**619**)

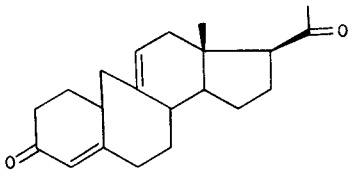


619 R = αOH

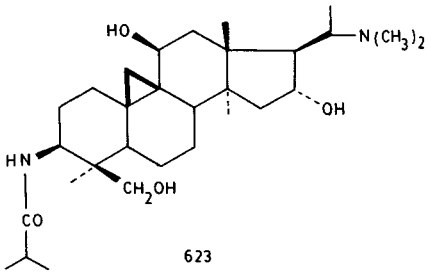
620 R = βOH



621

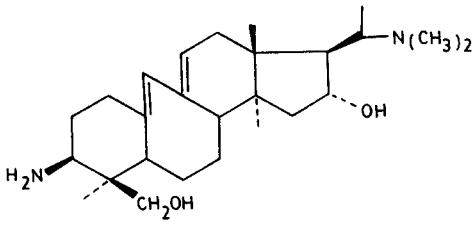


622

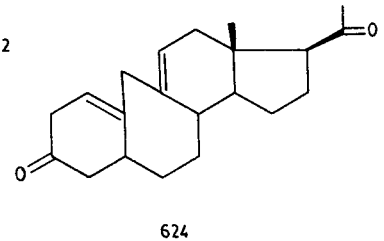


623

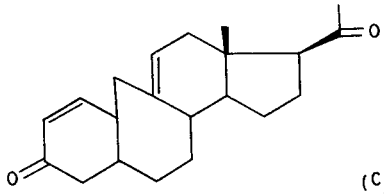
and (**620**), derived from the corresponding 11-keto compound by metal hydride reduction, have been found to undergo ring opening in acidic medium to give the alcohol **621** and the diene **622** respectively, in contrast to the *Buxus* alkaloid **623** which gave the 9(10 → 19)-*abeo*-pregnan-9(11),10(19)-diene (**572**). Treatment of **619** and **620** with acid under more vigorous conditions resulted in the formation of dienes **624** and **625.** Reduction of 11-oxosteroidal amines such as **626** by



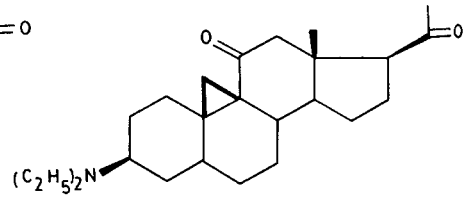
572



624



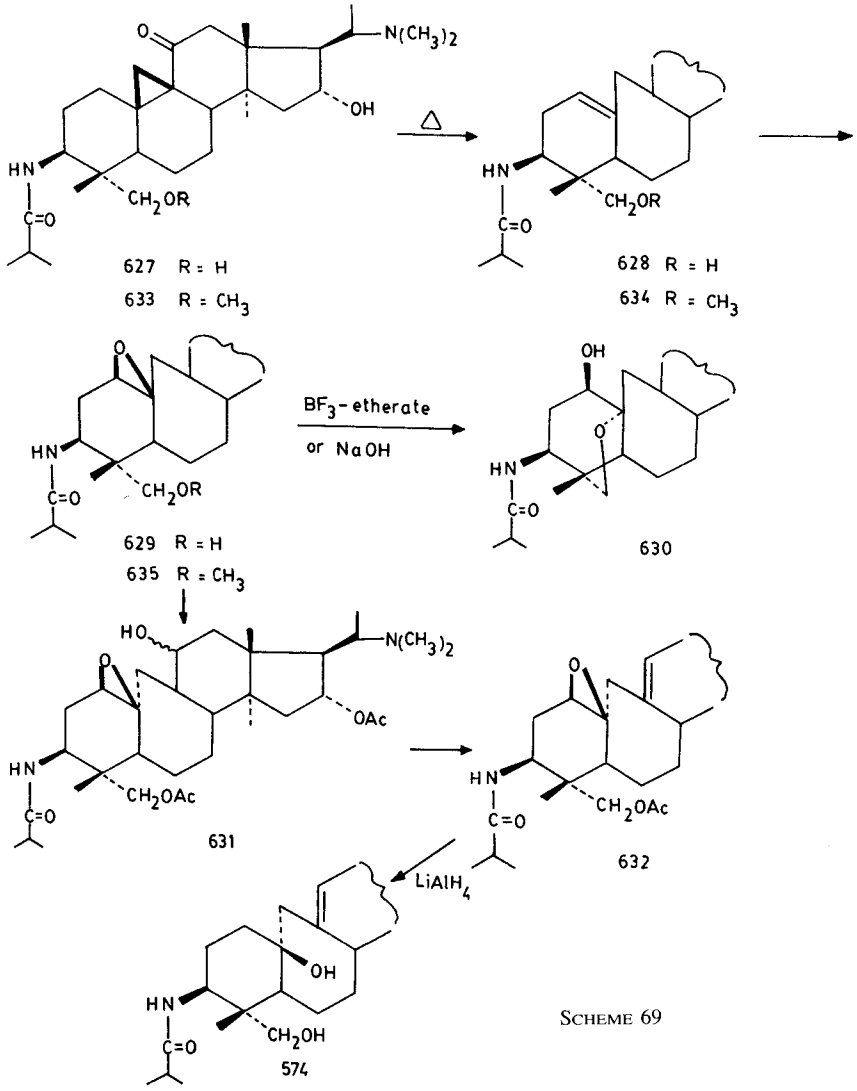
625



626

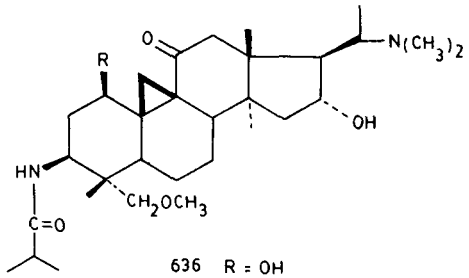
hydride ion also proceeded in a different manner from that of the triterpenoid alkaloids, since reduction of the 11-keto group to a methylene group was not observed.

Investigations of the epoxidation of 1(10)-olefins in the 9(10 → 19)-*abeo*-4α-hydroxy methylene-4β,14α-dimethyl-5α-pregnane series have been used to establish the structure and stereochemistry of *N*-isobutyrylbuxaline-F (**574**) (*225*). The olefinic compound **628,** obtained on thermolysis of *N*-isobutyrylcyclox-



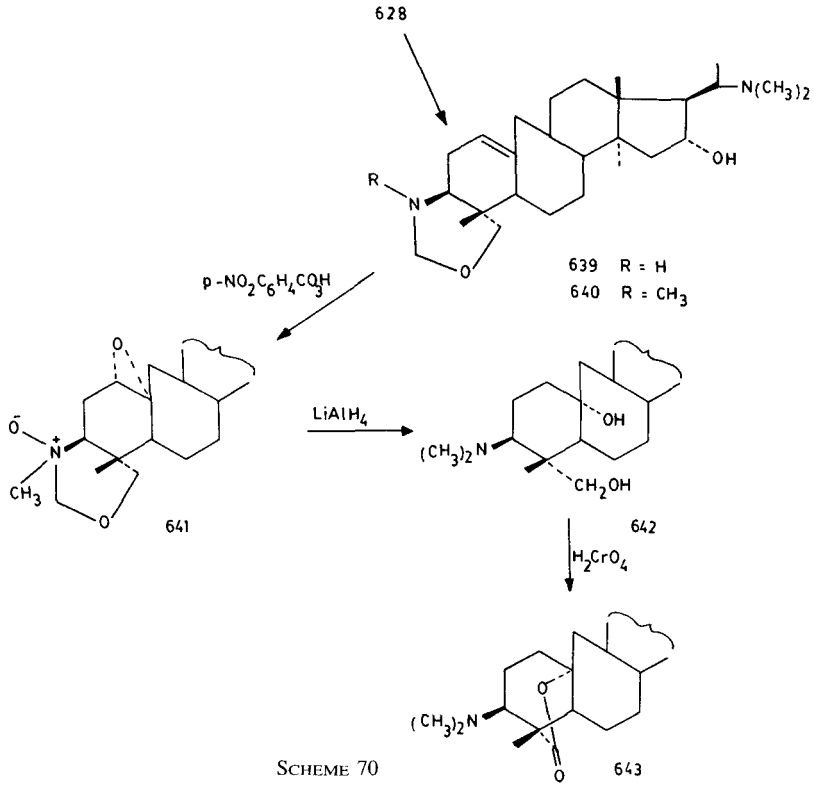
SCHEME 69

obuxidine-F (**627**) was converted into the 1β,10β-epoxide **629** on treatment with NBA in the presence of perchloric acid. In BF_3-etherate or sodium hydroxide, the epoxide **629** isomerized to the ether **630.** Acetylation followed by reduction of **629** with $NaBH_4$ yielded the alcohol **631,** which was then dehydrated to the olefin **632.** The epoxide ring of **632** was reduced with LAH to give the alcohol, which is identical to the known *Buxus* alkaloid *N*-isobutyrylbuxaline-F (**574,** Scheme 69).



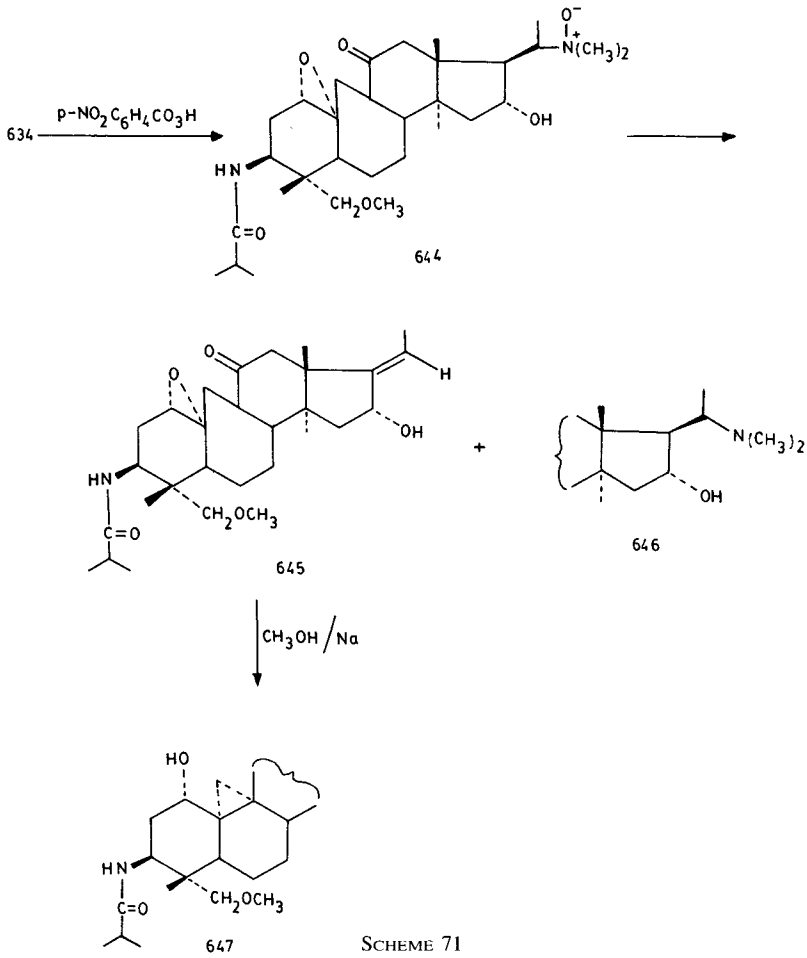
636 R = OH
637 R = Cl
638 R = H

The epoxide **635** (obtained from the olefin **634** of the alkaloid **633**) underwent an intramolecular S_N2 type of reaction with sodium methoxide to give **636.** The chloro derivative **637,** obtained by reaction of **636** with thionyl chloride, was transformed into **634** and the 9β,19-cyclo steroid **638** by catalytic hydrogenolysis. Wolff–Kishner reduction of **628** and reaction with formaldehyde gave the tetrahydrooxazine **639,** which was then *N*-methylated by reaction with for-



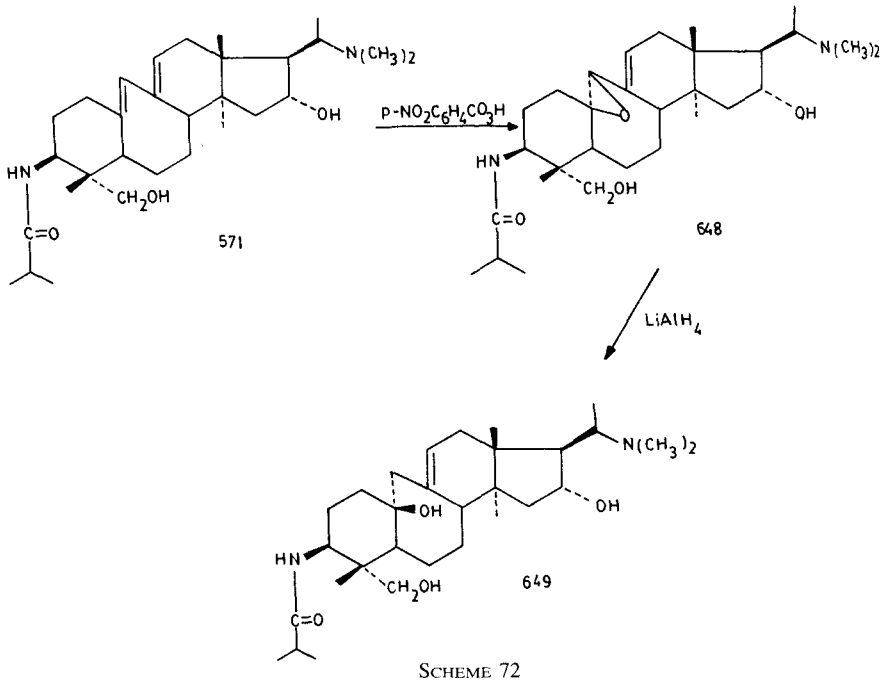
SCHEME 70

maldehyde and $NaBH_4$ to **640.** Treatment of **640** with *p*-nitroperbenzoic acid yielded the *N*-oxy epoxide **641,** which was reduced with LAH to give the tertiary alcohol **642.** Chromic acid oxidation of **642** to the lactone **643** established the α-configuration of the epoxide ring in **641** (Scheme 70).



SCHEME 71

Conversion of the olefin **634** into the N_b-oxy epoxide **644** with *p*-nitroperbenzoic acid followed by its reaction with triphenylphosphine in benzene produced the olefinic compound **645** with the amino derivative **646** in small yields. The S_N2 reaction of the olefin **645** with sodium methoxide resulted in the formation of the hydroxy compound **647** having the unusual 9α,10α-stereochemistry of the cyclopropane ring (Scheme 71).

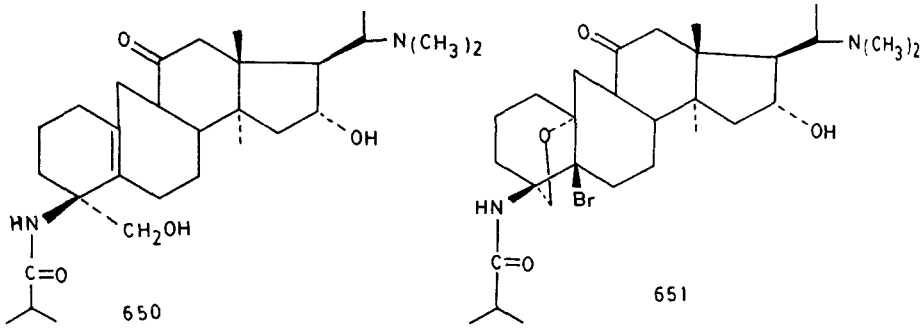


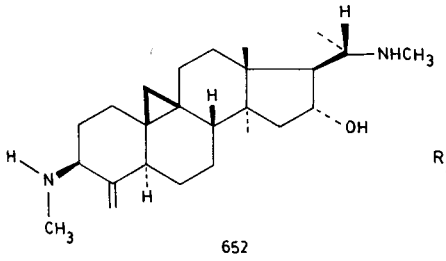
SCHEME 72

Reaction of *N*-isobutyrylbuxidienine-F (**571**) with *p*-nitroperbenzoic acid and subsequent reduction with $NaBH_4$ led to the epoxide **648.** Regioselective and stereoselective reduction of **648** with LAH yielded the alcohol **649** (Scheme 72).

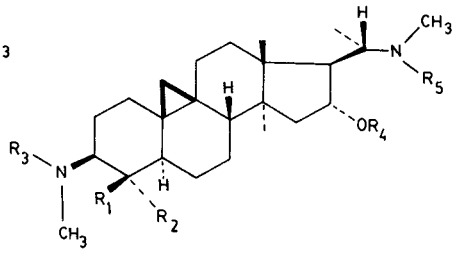
The 5(10)-dehydro compound **650,** a rearranged product of cyclobuxidine-A, was transformed into the bromoether **651** on treatment with NBA (225).

Several transformations of cyclobuxine-D have been reported and it has been found that the variation in the sequence of acetylation and hydrogenation results in differences in the stereochemistry at C-4 of the resulting compounds (226).





652

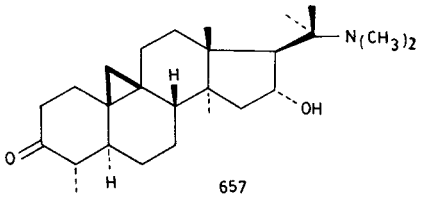


653 $R_1 = H, R_2 = CH_3, R_3 = R_4 = R_5 = Ac$

654 $R_1 = CH_3, R_2 = H, R_3 = R_4 = R_5 = Ac$

655 $R_1 = H, R_2 = CH_3, R_3 = Cl, R_4 = H, R_5 = CH_3$

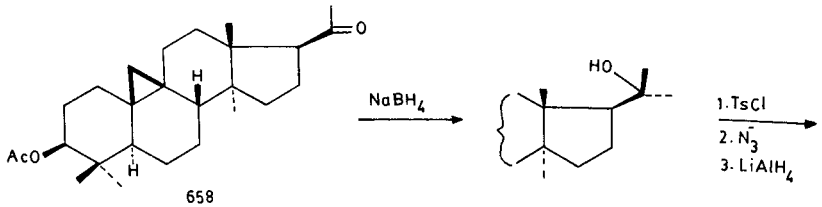
656 $R_1 = CH_3, R_2 = H, R_3 = Cl, R_4 = H, R_5 = CH_3$



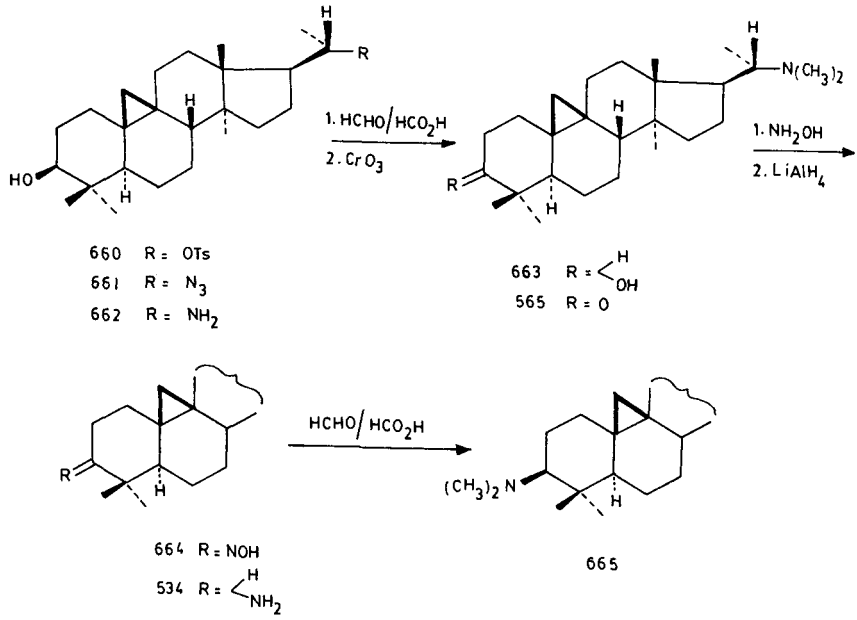
657

When cyclobuxine-D (**652**) was acetylated prior to hydrogenation, it yielded the 4α-methyl derivative **653,** whereas catalytic hydrogenation followed by acetylation gave the 4β-methyl steroid **654.** The corresponding *N*-chloroderivatives **655** and **656** underwent Ruschig degradation to give the 4α-methyl ketone **657.** These results supported the suggestion that epimerization of the 4β-methyl substituent into the 4α-methyl compound is possible during Ruschig degradation and indirectly confirmed the 4α-methyl configuration of cyclobuxamine.

Stereospecific transformation of 9,19-cyclo-4,4,14α-trimethyl-3β-acetoxy-5α,9β-pregnan-20-one (**658**) into the *Buxus* alkaloids cycloprotobuxine-F (**534**), buxandonine (**566**), and cycloprotobuxine-A (**644**) has been achieved (*227*). Reduction of the steroidal ketone **658,** a degradation product of cycloartenol (*228*), with $NaBH_4$ yielded the 20*R* alcohol **659** as the predominant product. Tosylation of the alcohol **659** to **660,** followed by S_N2 reaction with sodium

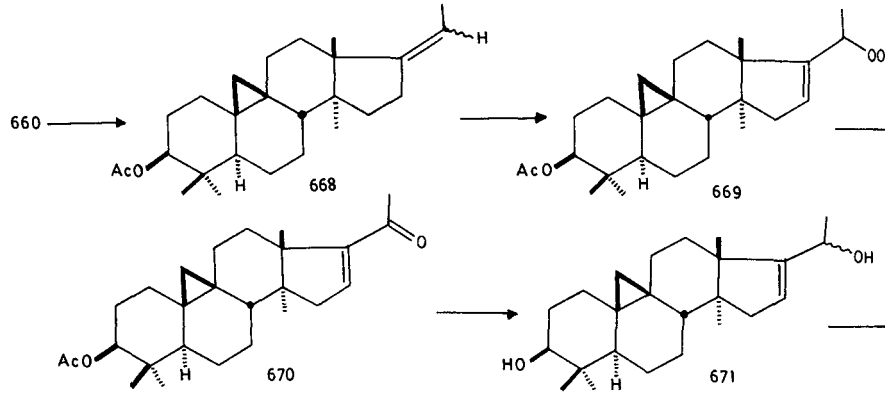


658 659

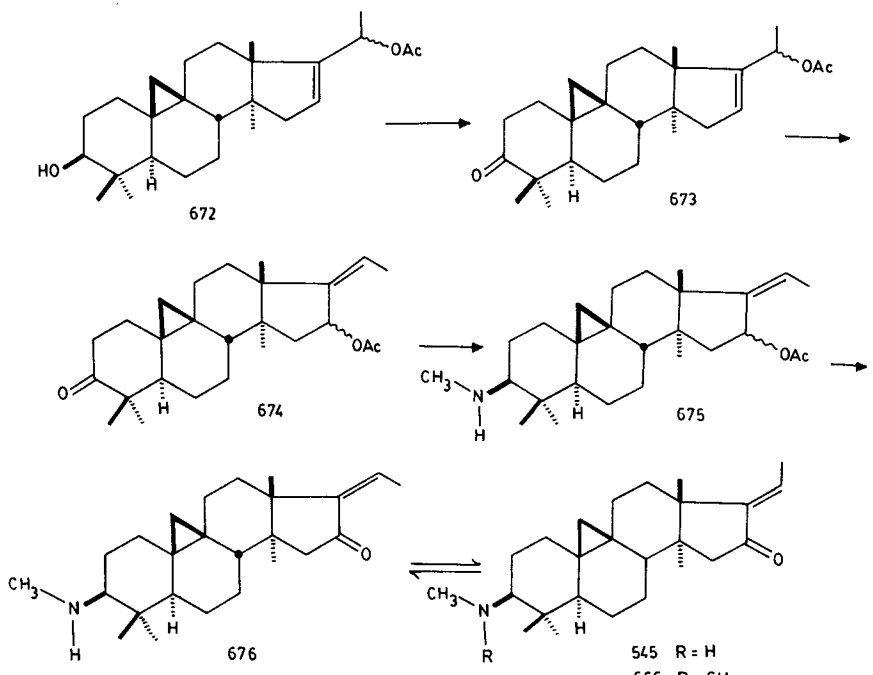


SCHEME 73

azide in HMPTA, gave the azide **661.** Reduction of the azide **661** with LAH led to the corresponding amine **662,** *N*-methylation and subsequent alkaline hydrolysis of which yielded the compound **663.** Oxidation of **663** with CrO_3-H_2SO_4 yielded a compound identical to buxandonine (**566**). The oxime **664,** obtained from buxandonine (**566**), was reduced with LAH to cycloprotobuxine-F (**534**), which was transformed into cycloprotobuxine-A (**665**) by *N*-methylation with HCHO/HCOOH (Scheme 73).



SCHEME 74 *(continued)*

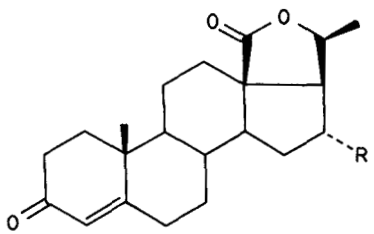


SCHEME 74

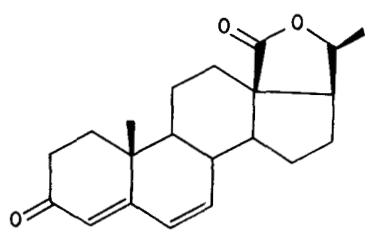
Partial synthesis of cyclobuxophyllinine-M (**545**), which is known to be converted to cyclobuxophylline-K (**666**) and buxanine-M (**667**), has also been achieved and is outlined in Scheme 74 (*229*). The tosylated product **660** was converted to the corresponding olefin **668** by reaction with *sym*-collidine. The α,β-unsaturated ketone **670** was obtained by photochemical oxidation of **668** to the hydroperoxide **669.** Reduction of **670** with LAH to the epimeric diols **671** and acetylation gave the monoacetates **672,** which were then oxidized with pyridinium-chromate to the 3-oxo-compound **673.** In the presence of acid, the acetates **673** underwent allylic rearrangement to give the 17(20)-olefin **674** having Z configuration. The corresponding 3β-methyl amino derivative **675** was obtained by reductive amination of the acetate **674.** Saponification and oxidation of the 16-acetoxy-3-methylamino derivative **675** produced the corresponding 16-keto derivative **676,** which yielded the E-isomer cyclobuxophyllinine-M (**545**) on equilibration with methanolic KOH.

IV. Biosynthesis

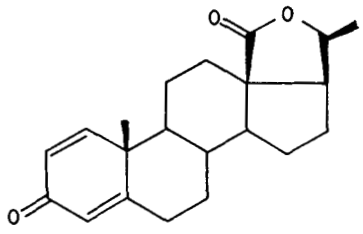
The early suggestions concerned with the biosynthesis of Apocynaceae and Buxaceae alkaloids involved the intermediacy of 3- or 20-ketones bearing the



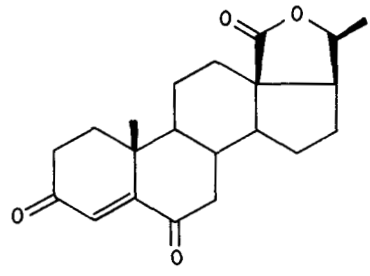
677 R = H
678 R = OH



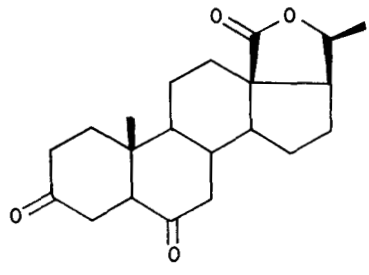
679



680



681

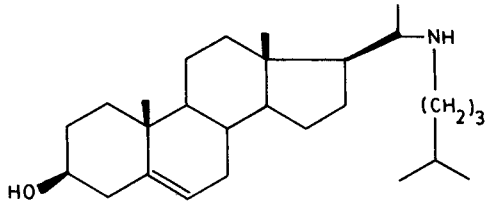


682

sterol or triterpene skeleton. Some labeling experiments in this connection were discussed in Volumes 9 and 14 of this series. The occurrence of C-3 steroidal ketones **677–682** in *P. microphylla* having the characteristic 18 → 20 lactone function of paravallarine and paravallaridine further supported the earlier hypothesis (*230*).

V. Pharmacology

Irehdiamine-A possesses potentializing activity on hepatocarcinogenesis in rats (*231*). Binding of irehdiamine-A resulted in the uncoiling of closed circular



683

DNA (*232*). Mitiphylline (**70**) was shown to have cardiotonic activity (*233*). The amoebicidal and trichomonocidal properties of paravallarine derivatives were also studied (*234*). Azanorcholesterol (**683**), prepared from holafebrine, is known for its antihyperadrenocortical activity (*235*). Cyclosuffrobuxinine (**553**) exhibited a marked inhibitory action on choline esterase of horse and human serum (*236*).

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CHAPTER 3

DISTRIBUTION OF ALKALOIDS IN TRADITIONAL CHINESE MEDICINAL PLANTS

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I. Introduction

Because alkaloids as a class of compounds have shown a wide range of biological activity and toxicity, they have attracted much interest among scientists.

The distribution of alkaloids in the plant kingdom is wide. It has been shown that there are at least 50 alkaloid-containing plant families and 120 alkaloid-containing plant genuses. Alkaloids occur abundantly in dicotyledons but are rarely found in cryptogamia and monocotyledons.

As to the plants used in traditional Chinese medicine, a still finer distinction can be made between the regular prescriptions (totally, about 300 to 500 plant species) and the folk-lore practice (involving about 5000 species), the latter being, however, restricted to usage by the local people. As a result, the alkaloids reviewed here come mostly from the second category.

The alkaloids described in this chapter will be limited to publications which have appeared since 1980, except for a few background papers.

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II. Diterpene Alkaloids

A. Occurrence and Distribution

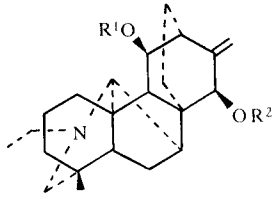
Diterpene alkaloids occur predominently in the genera *Aconitum* and *Delphinium* of the family Ranunculaceae. Interest in this group of compounds arises from the use of *Aconitum* roots. They are widely used as antirheumatics, analgesics, anesthetics, and to improve blood circulation. The chemistry and importance of diterpene alkaloids in medicine was previously reviewed (*1*). The present review covers work published during the years 1980–1987 by Chinese researchers.

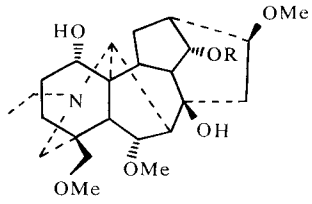
Wang and Liang (*2*) reported the MS-spectral characteristics of diterpene alkaloids. The ^{13}C-NMR spectra data of 186 diterpenoids also were reported (*3*).

Wang *et al.* (*4*) have reported on the investigation of Big aconites and Vine aconites, used as substitutes for aconite in the Southwest region of China. High-pressure liquid chromatography (HPLC) was used to evaluate the alkaloidal content of these drugs. Twelve aconites from different species were analyzed and the alkaloids identified and quantified. It was shown that all aconite samples contained yunaconitine-type alkaloids, suggesting that these plants are phytochemically related. The results are summarized as in Table I.

B. Alkaloids from Different Aconitum Species

Chen and Sung (*5*) investigated the roots of A. *jinyangense* W. T. Wang, a new species growing in Sichuan province of Southwestern China. In addition to denudatine (**1**) and 14-acetylneoline (**2**), a new base named jynosine (**3**, $C_{24}H_{35}NO_3$) was isolated. From the ^{1}H-NMR, IR, and MS spectral data, jynosine was identified as 15-acetyldenudatine. Denudatine showed prophylactic inhibition of cardiac arrhythmia caused by aconitine in rats.





1 Denudatine $R^1 = R^2 = H$ **2** 14-Acetylneoline $R = Ac$

3 Jynosine $R^1 = H$ $R^2 = Ac$ **5** Neoline $R = H$

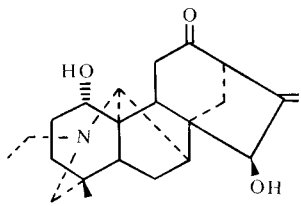
Further studies of the roots of *A. nagarum* Stapf Var. Lasiandrum W. T. Wang, which grows in the Yunnan province and is used for the treatment of

TABLE I
SOURCE AND MEDICINAL USE OF BIG ACONITES AND VINE ACONITES[a]

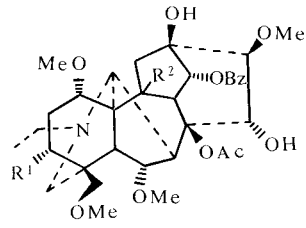
Species	Alkaloids[b] I%	II%	III%	Medicinal use
Aconitum forrestii Diels	0.16	0.11	—	antirheumatic, tonic, poison antidote
A. contortum Finet et Gagnep.	—	—	—	antirheumatic, fracture, analgesic
A. taipaicum Hand-Mazz.	0.35	—	—	antirheumatic
A. kongboense Lauener.	—	—	0.45	antipyretic, insecticide, analgesic
A. austroyunnanense W.T.Wang	0.04	0.12	—	antirheumatic, paralysis, fracture
A. crassicaule W.T.Wang	0.92	0.67	—	analgesic
A. stapfianum Hand-Mazz.	0.85	0.39	—	antirheumatic analgesic
A. sungpanense Hand-Mazz.	0.60	0.04	—	fracture, arthralgia, antirheumatic
A. hemsleyanum Pritz.	0.19	0.67	—	fracture, pain tubercular condition
A. episcopale Levl.	0.40	0.35	—	analgesic
A. transsectum Diels	0.08	0.44	—	antirheumatic
A. vilmorinianum Kom.	0.31	—	—	

[a] From Wang *et al.* [*4*].
[b] I: yunaconitine (**25**) II: crassicauline A (**21**) III: vilmorrianine A (**41**).

cancer were made (*6*). In addition to 14-acetylneoline (**2**), songorine (**4**), neoline (**5**), aconitine (**6**), and deoxyaconitine (**7**), a "new base" $C_{34}H_{47}NO_{12}$, melting point (mp) 198–200°C was obtained. Based on spectral data the structure of 10β-hydroxy aconitine was proposed for this new base. However Pelletier and Page (*7*) noted this "new base" to have identical mp and the same structure as the known alkaloid aconifine (**8**).



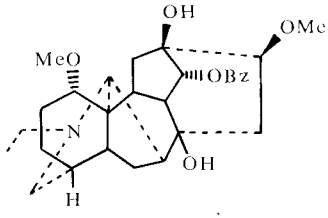
4 Songorine



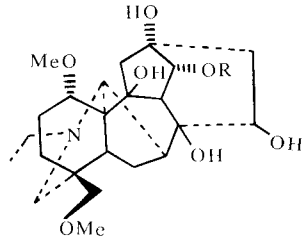
6 Aconitine $R^1 = OH$ $R^2 = H$
7 Deoxyaconitine $R^1 = H$ $R^2 = H$
8 Aconifine $R^1 = OH$ $R^2 = OH$

Zhu and Zhu (*8*) isolated a new base, named delavaconitine (**9**, $C_{29}H_{39}NO_6$) from the roots of *A. delavyi*. Based on spectral data and derivatives, the structure was confirmed as (**9**).

A new base named guayewuanine A was isolated from *A. hemsleyanum* (*9*). Its structure was proposed as (**10**).



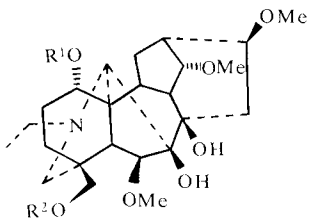
9 Delavaconitine



10 Guayewuanine R = Anisoyl

A. finetianum Hand-Mazz is a plant indigenous to China. The roots of this plant are used in folk medicine for the treatment of rheumatoid, inflammatory, and dysenteric diseases. Cheng *et al.* (*10*) have isolated delsoline (**11**), avadharidine, (**12**) and lycoctonine (**13**). Delsoline was proved to be the active principal for the treatment of dysentery and enteritis.

Jiang *et al.* (*11, 12*) have reported on further study of *A. finetianum* Hand-Mazz. In addition to avadharidine (**12**), lycoctonine (**13**), *N*-deacetyllappaconitine (**14**), and ranaconitine (**15**), three new bases were isolated. From ^{1}H-NMR, IR, and MS data, they were determined to be *N*-deacetyl ranaconitine (**16**, $C_{30}H_{42}N_2O_3$), *N*-deacetylfinaconitine (**17**, $C_{30}H_{42}N_2O_9$), and finaconitine (**18**,

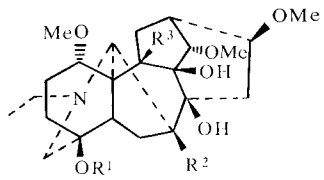


11 Delsoline R^1 = H R^2 = Me

12 Avadharidine R^1 = Me

R^2 = CO-C_6H_4-*o*-NHCO$(CH_2)_2$CONH

13 lycoctonine R^1 = Me R^2 = H



14 *N*-Deacetyllappaconitine

R^1 = CO-C_6H_4-*o*-NH_2 R^2 = R^3 = H

15 Ranaconitine

R^1 = CO-C_6C_4-*o*-NHAc R^2 = OH R^3 = H

16 *N*-Deacetylranaconitine

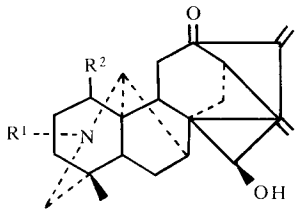
R^1 = CO-C_6H_4-*o*-NH_2 R^2 = OH R^3 = H

17 *N*-Deacetylfinaconitine

R^1 = CO-C_6H_4-*o*-NH_2 R^2 = R^3 = OH

18 Finaconitine

R^1 = CO-C_6H_4-*o*-NHAc R^2 = R^3 = OH

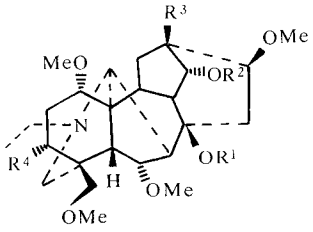


19 Finetianine R^1 = Me R^2 = OH
20 1-Dehydrosongorine R^1 = Et R^2 = O

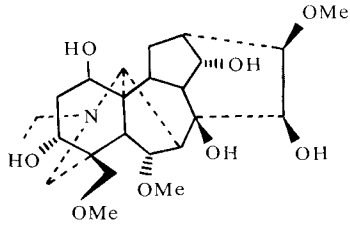
$C_{32}H_{44}N_2O_{10}$). Pharmacological studies showed that all three alkaloids exhibited analgetic activity.

Tian *et al.* (*13*) also isolated two new bases, finetianine (**19,** $C_{22}H_{29}NO_3$) and 1-dehydrosongorine (**20,** $C_{22}H_{29}NO_2$), from the same plant species.

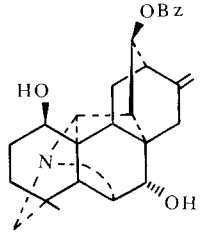
Wang *et al.* (*14, 15*) reported on the study of roots of *A. crassicaule* W. T. Wang, which were prescribed in folk medicine in Yunnan province of China for bruises and injuries. Three new alkaloids—crassicauline-A (**21,** $C_{35}H_{49}NO_{10}$), crassicauline-B (**22,** $C_{27}H_{31}NO_4$), and crassicaulidine (**23,** $C_{24}H_{39}NO_2$), along with two known diterpene alkaloids chasmanine (**24**) and yunaconitine (**25**)—were obtained.



21 Crassicauline-A
R^1 = Ac R^2 = Anisoyl
R^3 = OH R^4 = H
24 Chasmanine
$R^1 = R^2 = R^3 = R^4$ = H
25 Yunaconitine
R^1 = Ac R^2 = Anisoyl
$R^3 = R^4$ = OH



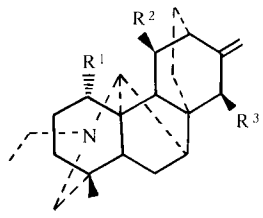
23 Crassicaulidine



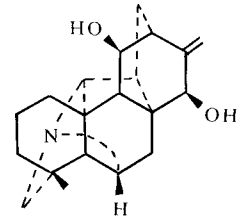
22 Crassicauline-B

Sung *et al.* (*16*) have reported on the isolation of lepenine (**26**), lepedine (**27**), and lepetine (**28**), together with the known alkaloids denudatine (**1**), neoline (**5**),

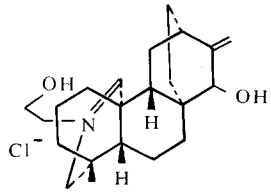
yunaconitine (**25**), kobusine (**29**), atisinium chloride (**30**), and isoatisine (**31**), from *A. pseudohuiliense* Chang.



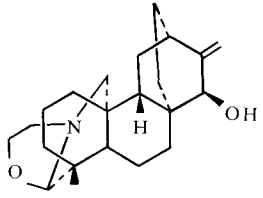
26 Lepenine $R^1 = R^2 = R^3 = OH$
27 Lepedine $R^1 = OMe$ $R^2 = R^3 = OH$
28 Lepetine $R^1 = R^3 = OH$ $R^2 = OAc$



29 Kobusine

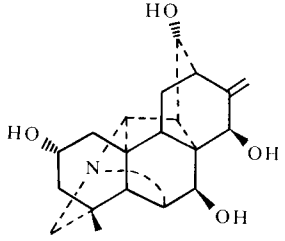


30 Atisinium chloride

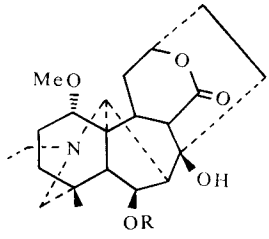


31 Isoatisine

A new base named tanwusine-A (**32**), together with heteratisine (**33**) and benzoylheteratisine (**34**), were isolated from the roots of *A. tanguticum* (Maxim) Stapf. (*16*).

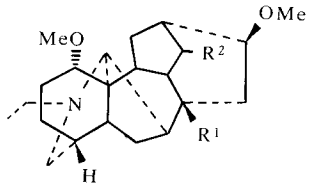


32 Tanwusine-A



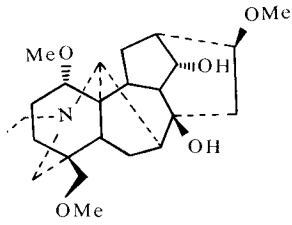
33 Heteratisine R = H
34 Benzoylheteratisine
R = C_6H_5-CO-

A new base, 14-dehydro-8-deoxyaconosine (**35**), along with the known alkaloids aconosine (**36**), talatisamine (**37**), crassicauline-A (**21**), and yunaconitine (**25**) were isolated from the roots of *A. stapfianum* Hand-Mazz (*16*).



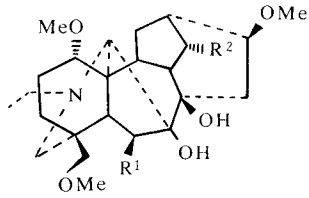
35 14-Dehydro-8-deoxyaconosine
$R^1 = H$ $R^2 = O$

36 Aconosine $R^1 = OH$ $R^2 = \alpha OH$



37 Talatisamine

Three new lycoctonine-type alkaloids, vaginatine (**38**), vaginaline (**39**), and vaginadine (**40**) were isolated from the roots of *A. scaposum* var. *vaginatum* (*16*).



38 Vaginatine $R^1 = OH$ $R^2 = OH$
39 Vaginaline $R^1 = OH$ $R^2 = O$
40 Vaginadine $R^1 = O$ $R^2 = O$

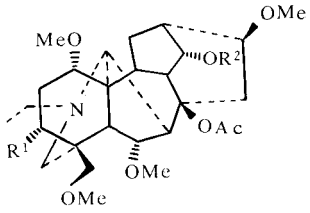
Yang *et al.* (*17, 18*) reported on the alkaloids of *A. vilmorrianum* Kem, a popular Chinese folkloric medicinal herb. Three new alkaloids, vilmorrianine-A (**41,** $C_{35}H_{49}NO_{10}$,mp 182–184°C), vilmorrianine-C (**42,** $C_{35}H_{49}NO_9$, mp 156–157°C), and vilmorrianine-D (**43**) were isolated.

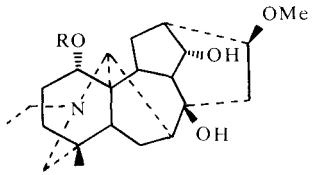
It was proved by ^{13}C-NMR that vilmorrianine-B (**44**) has the same structure as karakoline, isolated from *A. karakalium* by Yunosov (*18*).

Four known alkaloids—aconitine (**6**), deoxyaconitine (**7**), neoline (**5**), and songorine (**4**)—were isolated from the roots of *A. karakolium* Rap. It was rich in its aconitine content (yield 0.49%) (*19*).

Xiao *et al.* (*20*) have reported four known alkaloids (talatisamine (**37**), 14-acetyltalatisamine (**45**), condelphine (**46**), and isotalatisidine (**47**)) to be present in roots of *A. gymnandrum* Maxim.

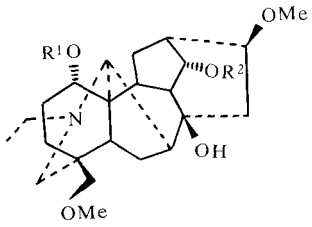
Chen and Sung (*21*) have isolated franchetine ($C_{31}H_{41}NO_6$) as a minor new alkaloid. Franchetine was isolated from a weak base fraction of *A. franchetii* Fin. et Gagn. and assigned structure (**48**) on the basis of IR, ^{1}H-NMR, ^{13}C-NMR, and MS data, which possesses a dihydropyrane ring moiety. In addition, five





41 *Vilmorrianine-A*
R^1 = OH R^2 = Anisoyl
42 Vilmorrianine-C
R^1 = H R^2 = Anisoyl

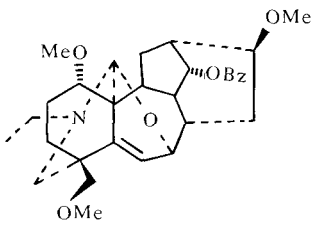
43 *Vilmorrianine-D* R = Me
44 Vilmorrianine-B R = H
(karakoline)

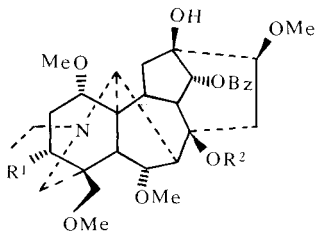


45 14-Acetylalatisamine R^1 = Me R^2 = Ac
46 Condelphine R^1 = H R^2 = Ac
47 Isotalatisidine $R^1 = R^2$ = H

known alkaloids (indaconitine (**49**), benzoylpseudaconine (**50**), chasmaconitine (**51**), chasmanine (**24**), and talatisamine (**37**)) were isolated from the roots.

The roots of *A. flavum* Hand Mazz. are used for the treatment of rheumatic arithritis, rheumatic pain, and bleeding in the Northwestern region of China. Chang *et al.* (*22*) and Liu and Chang (*23*) have reported on studies of roots of *A. flavum* Hand-Mazz. Two new alkaloids, 3-acetylaconitine (**52,** $C_{36}H_{47}NO_{12}$, mp



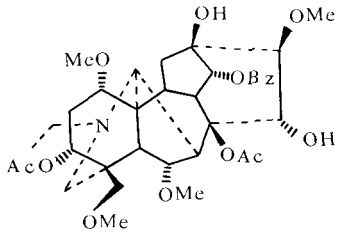


48 Franchetine

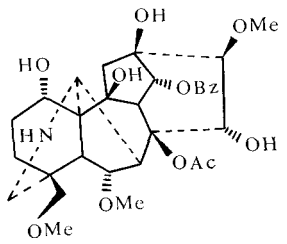
49 Indaconitine R^1 = OH R^2 = Ac
50 Benzoylpseudaconine R^1 = OH R^2 = H
51 Chasmaconitine R^1 = H R^2 = Ac

196–197°C) and flavaconitine (**53,** $C_{31}H_{41}NO_{11}$) were obtained. From UV, IR, NMR, and MS data, structures (**52**) and (**53**) were assigned.

Tang *et al.* (*24*) found that 3-acetylaconitine (**52**) inhibited at 0.18–0.3 mg/kg the increased vascular permeability induced by ip injection of 0.7% acetic acid in mice, and at 0.3–0.5 mg/kg, both the swelling of the hind paw induced by injection of 0.1 ml of 2.5% formaldehyde, and edema produced by injection of 1% carrageenin.



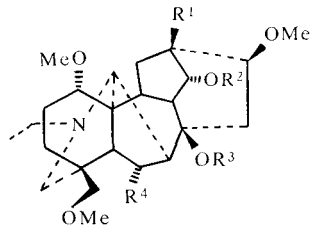
52 3-Acetylaconitine



53 Flavaconitine

Wang *et al.* (*25*) have reported on Studies of *A. forestii* Stapf. A new alkaloid, liwaconitine (**54,** $C_{41}H_{53}NO_{11}$, mp 201–201.5°C) along with yunaconitine (**25**), crassicauline-A (**21**), aconosine (**36**), chasmaconitine (**51**), cammaconine (**55**) and foresaconitine (**56**) were isolated. Liwaconitine is the first example of a diaroyl ester of a C_{19}-diterpene alkaloid.

Chen and Sung (*26*) have reported six known alkaloids—denudatine (**1**), crassicauline-A (**21**), chasmanine (**23**), yunaconitine (**24**), talatisamine (**37**), and vilmorianine-C (**42**)—to be present in roots of *A. pseudogeniculatum* W. T. Wang.

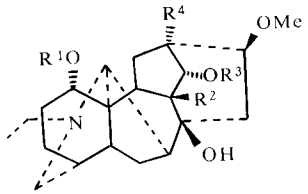


54 Liwaconitine R^1= OH R^2 = R^3 = Anisoyl R^4 = OMe
55 Cammaconine R^1= R^2 = R^3 = R^4 = H
56 Foresaconitine R^1 = H R^2 = Anisoyl
R^3 = AC R^4 = OMe

A. episcopale Levl. is a plant indigenous to China. The tubers of the plant were used as an antidote for poisoning by aconitine. Wang and Fang (*27*) have

carried out an extensive series of investigations with *A. episcopale*. Through ion-exchange chromatography and centrifugal preparative thin-layer chromatography, four new alkaloids, episcopalisine (**57**), episcopalisinine (**58**), episcopalitine (**59**), and episcopalidine (**60**) were isolated from the roots. The structure and relative stereochemistry of (**60**) was deduced from X-ray analysis.

A new alkaloid, episcopaline (**61**), was previously isolated from the same plant (*18*).

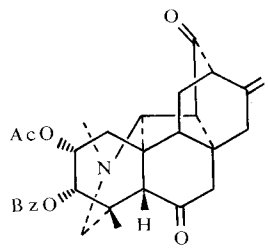


57 Episcopalisine R^1 = Me R^2 = OH R^3 = Bz R^4 = H

58 Episcopalisinine R^1 = Me R^2 = OH R^3 = H R^4 = H

59 Episcopaitine R^1 = Me R^2 = H R^3 = Ac R^4 = OH

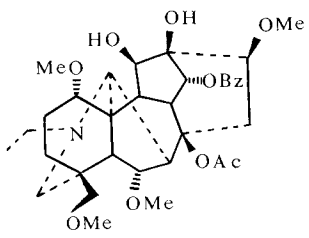
61 Episcopaline R^1 = R^2 = R^3 = R^4 = H



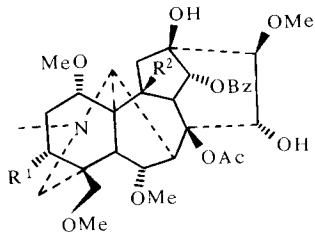
60 Episcopalidine

Wang *et al.* (*28*) reported on studies of *A. duclouxii* levl., used as analgesics in Chinese folk medicine. Aconitine (**6**) and new alkaloid named duclouxine (**62**, $C_{34}H_{47}NO_{10}$, mp 168–169°C) were obtained.

A new alkaloid named beiwutine (**63**), along with four known alkaloids—aconitine (**6**), deoxyaconitine (**7**), hypaconitine (**64**), and mesaconitine (**65**)—were isolated from *A. kusnezoffi* (*29*). Beiwutine showed analgesic activity.



62 Duclouxine



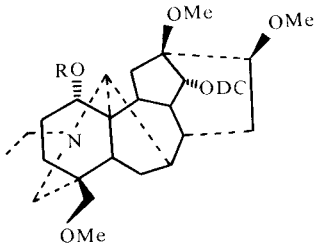
63 Beiwutine R^1 = OH R^2 = OH

64 Hypaconitine R^1 = R^2 = H

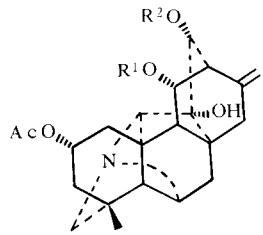
65 Mesaconitine R^1 = OH R^2 = H

Jiang *et al.* (*30*) reported on the isolation work on *A. gymnandrum* Maxim. Two new alkaloids, gymnaconitine (**66**, $C_{34}H_{47}NO_8$) and methyl gymnaconitine (**67**, $C_{35}H_{49}NO_8$) were isolated.

Three new alkaloids, guan-fu base A (**68**), guan-fu base G (**69**) and guan-fu rensu (**70**) were isolated from *A. coreanum* (Lovl) Raipaies (*31, 32*). The structures were confirmed based on spectral data and chemical reactions.



66 Gymnaconitine R = H
DC = COCH = $CHC_6H_3(OMe)_2$(3.4)
67 Methyl Gymnaconitine R = Me

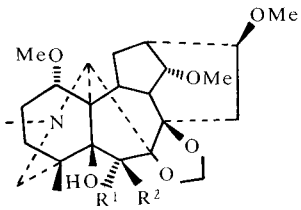


68 Guan-fu base A R^1 = Ac R^2 = H
69 Guan-fu base G R^1 = Ac R^2 = Ac
70 Guan-fu rensu R^1 = H R^2 = H

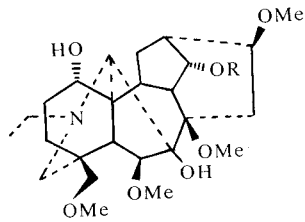
C. Alkaloids from Delphinium and Spiraea Species

Jiang and Sung (*33, 34*) reported on the isolation of alkaloids from *D. brovalotii* Franch. Seven new diterpene alkaloids—bonvalotine (**71**), bonvalol (**72**), bonvalone (**73**), delbotine (**74**), delbonine (**75**), delbine (**76**), and delboxine (**77**)—were isolated. Structures of delbotine and delboxine were confirmed through X-ray analyses. Delboxine and delbine are the first examples of C-6 oxygenated C_{18}-alkaloids.

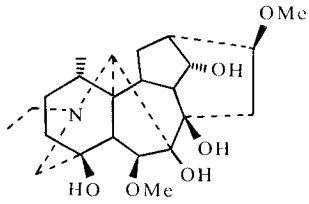
Jin (*35*) isolated a new base, anhweidelphinine (**78**) from *D. anhweiense* W. T. Wang. The structure was confirmed using spectral data.



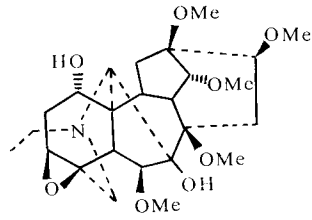
71 Bovalotine R^1 = H R^2 = OAc
72 Bonvalol R^1 = H R^2 = OH
73 Bonvalone R^1 = H R^2 =O



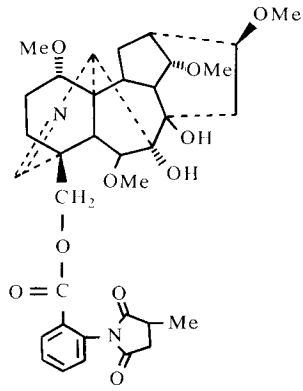
74 Delbotine R = Me
75 Delbonine R = Ac



76 Delbine



77 Delboxine



78 Anhweidelphinine

Sun *et al.* (*36, 37*) isolated from *Spiraea japonica* L.f. var. *fortunei* (planch.) Rehd. 15 new alkaloids, designated as spirasine I to spirasine XV, along with spiredine (**79**) and spiradine-A (**80**), which have known structures. They are assembled in Table II.

The oxazolidine ring-containing spirasines exist as C_{19}-epimeric pairs in solution in about equal proportions. Only one epimer with C_{19}-S was found in the solid state, presumably as a result of some transannular interaction between the amino nitrogen and the 6-keto group.

Epimeric pairs with 16-OH groups were distinguished by CD differences and by x-ray analysis of Spirasine VI.

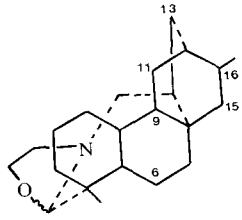
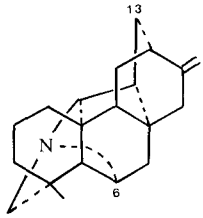
III. *Stephania* Alkaloids

A. Occurrence and Distribution

The root tubers of some species of the genus *Stephania* (Menispermaceae Family) were known in Chinese folk medicine as "Shan-Wu-Gui." There are 18 species distributed in the south and southwestern regions of China. Many al-

TABLE II

ALKALOIDS ISOLATED FROM SPIRAEA *JAPONICA* L. F.VAR. *FORTUNEI* (PLANCH.) REHD.[a]

Alkaloid		Structure
		
79	Spiredine	6, 11 - diketo, Δ^{16}
81	*Spirasine I*	9β - OH, 6 keto, Δ^{15}
82	Spirasine II	9β - OH, 11- diketo, Δ^{16}
83	Spirasine III	9β - OH, 6 - keto, Δ^{16}
84	Spirasine V	16α - OH, 6 - keto
85	Spirasine VI	16β - OH, 6 - keto
86	Spirasine VII	9β, 16α - diOH, 6 - keto
87	Spirasine VIII	9β, 16 - diOH, 6 - keto
		
80	Spiradine - A	6 - OH, 11 - keto
88	Spirasine IV	13 - keto
89	Spirasine IX	11 - keto
90	Spirasine X	13α - OH, 11 - keto
91	Spirasine XI	13 - αOH
92	Spirasine XII	6, 13α - diOH, 11- keto
93	Spirasine XIII	6, 13β - diOH, 11- keto
94	Spirasine XIV	6, 13α - diOH
95	Spirasine XV	6, 13β - diOH

[a] From Sun *et al.* (*36,37*).

kaloids which exhibited physiological activities have been isolated from these plants, such as (−)-tetrahydropalmatine, (−)-dicentrine, and (+)-cepharanthine.

New alkaloids and alkaloids of known structure that have been obtained recently from the *Stephania* species named "Shan-Wu-Gui" are listed in Table III.

TABLE III
ALKALOIDS FROM *STEPHANIA* SPECIES (SHAN-WU-GUI)

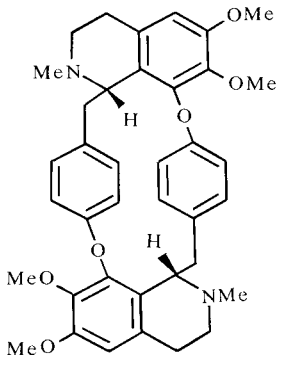
Plant source	Alkaloid	Reference
Stephania epigeae H.S.Lo.	**96** (−)-cycleanine	*38*
	97 (−)-curine	
	98 (+)-isocorydine	
	99 (+)-cepharanthine	
S. viridiflavens H.S.Lo et. M.Yang, sp. nov. mss.	**100** (−)-tetrahydropalmatine	*39*
	101 (−)-xylopinine	
	102 palmatine	
	103 jatrorrhizine	
S. brachyandra Diels	**98** (+)-isocorydine	*40*
	100 (−)-tetrahydropalmatine	
	104 (−)-dicentrine	
	105 (−)-sinomenine	
	106 (+)-corytuberine	
	107 (−)-sinoacutine	
	108 dehydrodicentrine	
	109 (+)-isoboldine	
	110 (−)-8,14-dihydrosalutaridine	
	111 (+)-*N*-methyllaurotetanine	
S. intermedia H.S.Lo	**100** (−)-tetrahydropalmatine	*41*
	112 (−)-corydalmine	
	113 (−)-stepholidine	
	114 (−)-discretamine	
	115 (+)-stepharine	
	116 stepharanine	
	103 jatrorrhizine	
	102 palmatine	
	117 dehydrocorydalmine	
	118 dehydrodiscretamine	
S. dicentrinifera H.S.Lo et M.Yang, sp.nov. mss.	**104** (−)-dicentrine	*42*
	100 (−)-tetrahydropalmatine	
S. dielsiana Y.C.Wu	**107** (−)-sinoacutine	*43,44*
	119 (−)-stephanine	
	120 (−)-crebanine	
	100 (−)-tetrahydropalmatine	
	101 (−)-xylopinine	
	121 dehydrostephanine	
S. micrantha H.S.Lo et. M.Yang, sp. nov. mss.	**121** dehydrostephanine	*45*
	100 (−)-tetrahydropalmatine	
	122 (−)-capaurine	
	123 (−)-tetrahydrocolumbamine	
	124 (−)-corypalmine	
	101 (−)-xylopinine	

(*continued*)

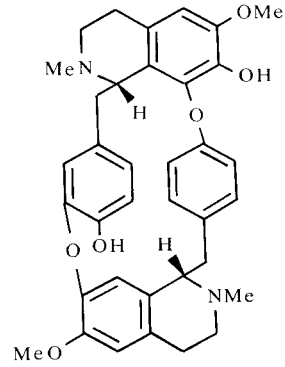
TABLE III (*Continued*)

Plant source	Alkaloid	Reference
	125 dehydroroemerine	
	119 (−)-stephanine	
	98 (+)-isocorydine	
	107 (−)-sinoacutine	
	105 (−)-sinomenine	
S. mashanica H.S.Lo et B.N.Chang, sp.nov. mss.	**107** (−)-sinoacutine	*46*
	123 (−)-tetrahydrocolumbamine	
	100 (−)-tetrahydropalmatine	
	126 (−)-tetrahydrojatrorrhizine	
	127 dicentrinone	
	104 (−)-dicentrine	
S. kwangsiensis H.S.Lo, sp. nov. mss.	**100** (−)-tetrahydropalmatine	*47,48*
	122 (−)-capaurine	
	98 (+)-isocorydine	
	128 (−)-roemerine	
	125 dehydroroemerine	
	121 dehydrostephanine	
	129 dihydropalmatine	
	119 stephanine	
	102 palmatine	
S. sinica Diels	**99** (+)-cepharanthine	*49,50*
	96 (−)-cycleanine	
S. cepharantha Hayata	**99** (+)-cepharanthine	
	96 (−)-cycleanine	
S. delavayi Diels	**99** (+)-cepharanthine	
	96 (−)-cycleanine	
S. yunnanensis. H.S.Lo, sp.nov.mss.	**107** (−)-sinoacutine	
S. kuinanensis H.S.Lo et M.Yang, sp.nov.mss.	**104** (−)-dicentrine	
	100 (−)-tetrahydropalmatine	
S. dolichopoda Diels	**100** (−)-tetrahydropalmatine	
S. succifera H.S.Lo et Y.Tsoong	**100** (−)-tetrahydropalmatine	
S. hainanensis H.S. Lo et. Y. Tsoong	**100** (−)-tetrahydropalmatine	
S. excentrica H.S.Lo	**99** (+)-cepharanthine	

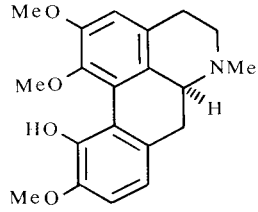
(−)-Tetrahydropalmatine (**100**) exhibits sedative and analgesic effects and has been examined for its mode of action (*51*). (+)-Cepharanthine (**99**) was used in China for the prevention of leukocytopenia caused by radiation therapy and chemotherapy (*52, 53*). Wang and Cao (*54*) reported the use of dimethylcycleanine bromide in producing controlled hypotension during general anesthesia, and it was shown that its use was efficacious and safe. (−)-Dicentrine (**104**) and (−)-xylopinine (**101**) exhibit analgesic and sedative effects (*40*).



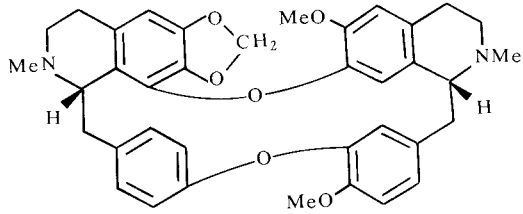
96 (-) - Cycleanine



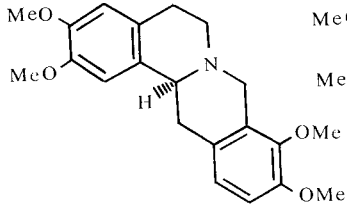
97 (-) - Curine



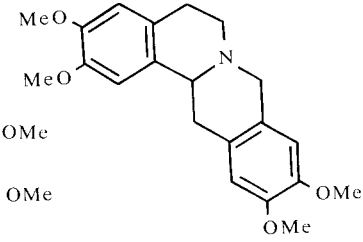
98 (+) - Isocorydine



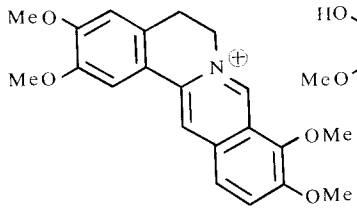
99 (+) - Cepharanthine



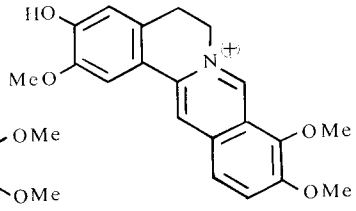
100 (-) - Tetrahydropalmatine



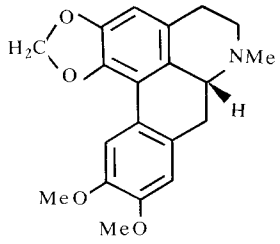
101 (-) - Xylopinine



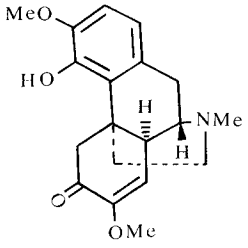
102 Palmatine



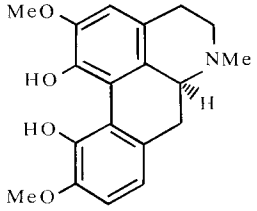
103 Jatrorrhizine



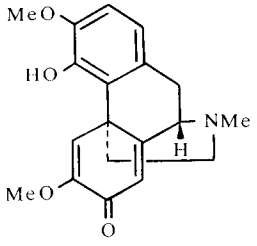
104 (-) - Dicentrine



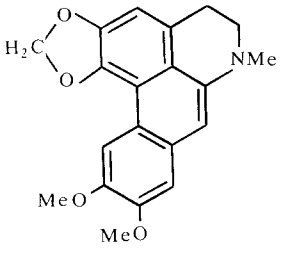
105 (-) - Sinomenine



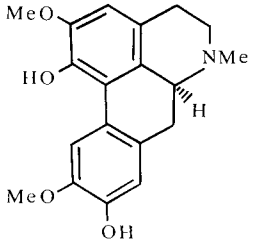
106 (+) - Corytuberine



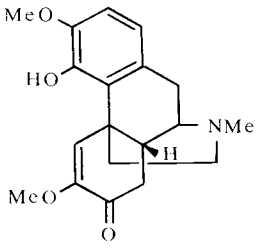
107 (-) - Sinoacutine



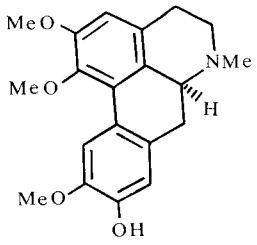
108 Dehydrodicentrine



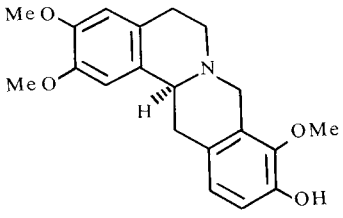
109 (+) - Isoboldine



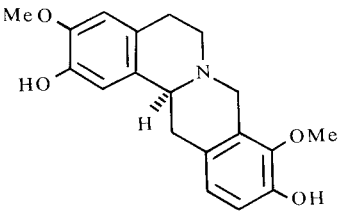
110 (-) -8, 14 - Dihydrosalutaridine



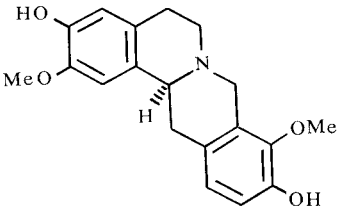
111 (+) - *N* - Methyllaurotetanine



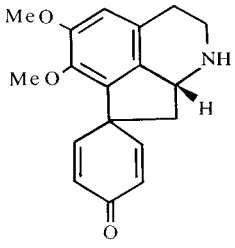
112 (-) - Corydalmine



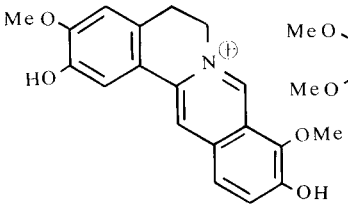
113 (-) - Stepholidine



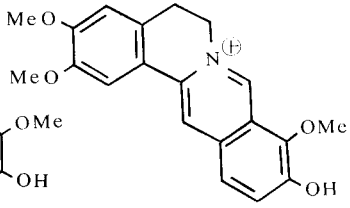
114 (-) - Discretamine



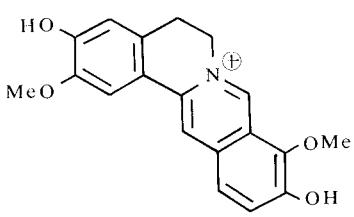
115 (+) - Stepharine



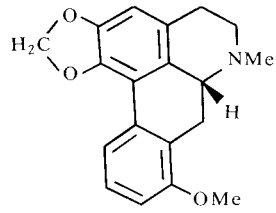
116 Stepharanine



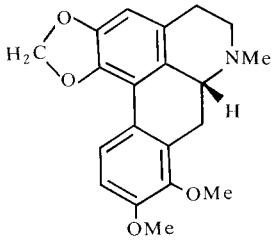
117 Dehydrocorydalmine



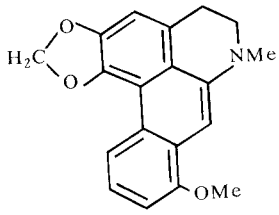
118 Dehydrodiscretamine



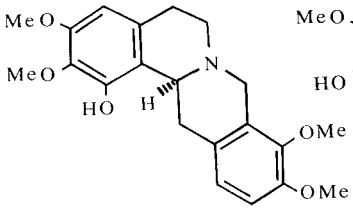
119 (-) - Stephanine



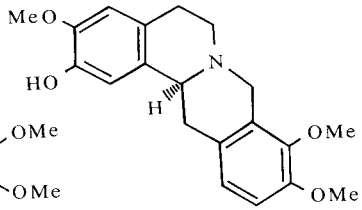
120 (-) - Crebanine



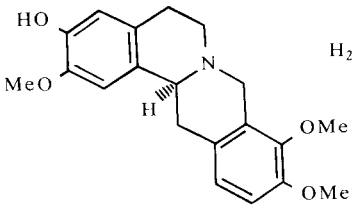
121 Dehydrostephanine



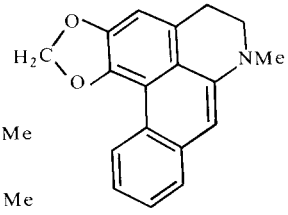
122 (-) - Capaurine



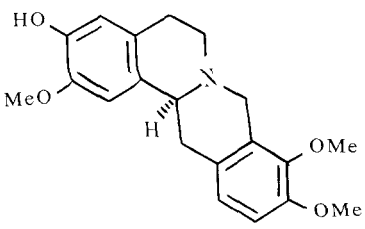
123 (-) - Tetrahydrocolumbamine



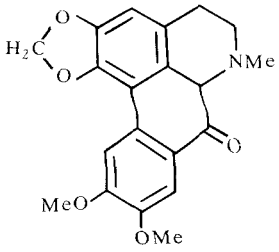
124 (-) - Corypalmine



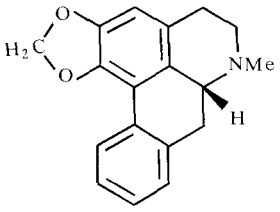
125 Dehydroroemerine



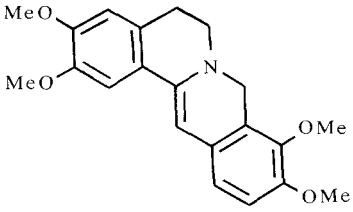
126 (-) - Tetrahydrojatrorrhizine



127 Dicentrinone



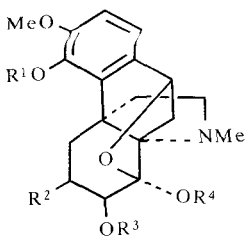
128 (-) - Roemerine



129 Dihydropalmatine

B. Alkaloids of *Stephania longa* L.

Lao *et al.* (*55*) reported on studies of *S. longa* L., which grows wild in the Hainan Island of China and is used as an antirheumatic in Chinese folk medicine. In addition to stephaboline (**130**), two new hasubanan alkaloids, named longanine (**131,** $C_{19}H_{25}NO_6$, mp 197–199°C) and longanone (**132,** $C_{20}H_{25}NO_6$, mp 161–163°C), were isolated from the roots and stems.



130	Stephaboline	$R^1 = R^2 = R^3 = R^4 = H$
131	Longanine	$R^1 = R^4 = H$, $R^2 = OH$, $R^3 = Me$
132	Longanone	$R^1 = H$, $R^2 = O$, $R^3 = R^4 = Me$

Based on spectral data and chemical derivation, the structures of longanine and longanone were assigned as (**131**) and (**132**), respectively. Pharmacological studies of longanine and longanone are in progress.

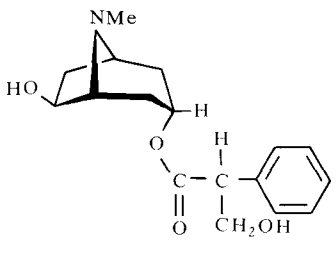
It is known the *Stephania* alkaloids exhibit antibacterial, anti-inflammatory, sedative, analgesic, and diuretic activities, and some are clinically used in China.

IV. Tropane Alkaloids

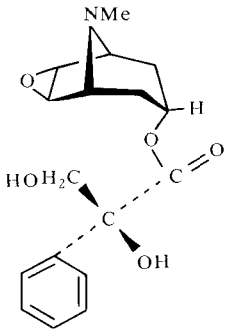
A. Anisodamine and Anisodine

Anisodamine (**133**) and anisodine (**134**) were obtained in 1975 and 1976 from *Anisodus tanguticus* (Maxim) Pascher, which grows in the northwest region of China (*56*).

Both alkaloids were isolated by ion-exchange and preparative thin-layer chromatography (*57, 58, 59*).



133 Anisodamine



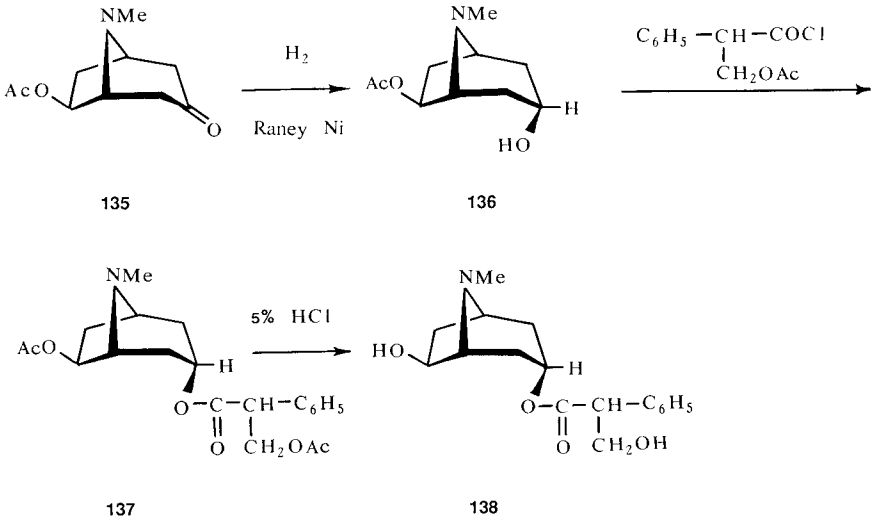
134 Anisodine

On the basis of pharmacological and clinical studies, anisodamine is used as an anticholinergic drug for the treatment of acute microcirculatory disturbance.

B. Synthesis of Anisodamine and Anisodine

Xie *et al.* (*60*) have reported the synthesis of anisodamine as shown in Scheme 1.

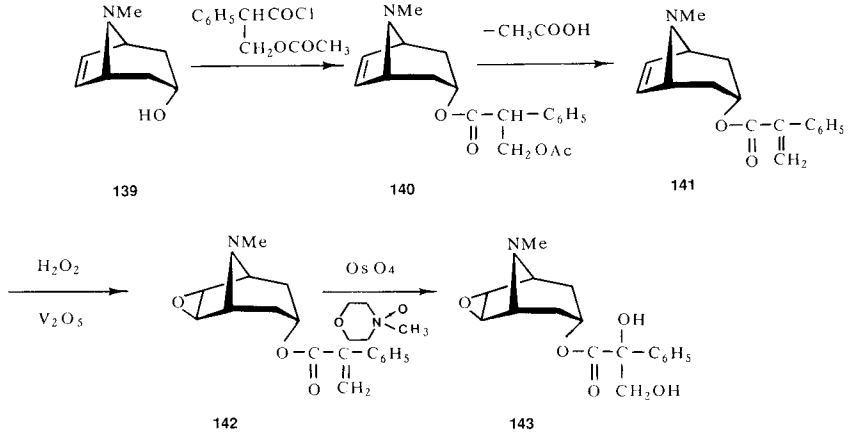
Reduction of (**135**) gave 3α-hydroxy (**136**), which was esterified with *O*-acetyltropoyl chloride to give (**137**). Removal of the acetyl groups gave anisodamine (**138**) as a diastereomeric mixture.



SCHEME 1

Natural and synthetic specimens (**133**) and (**138**) have different solid-phase spectra. Interestingly enough, the IR spectra of samples containing one molecule of benzene became accidentally superposed. The trade name of the synthetic product is 654-2.

Xie *et al.* (*59, 61*) have reported the improvement on the total synthesis of anisodine as shown in Scheme 2.



SCHEME 2

As 3α-hydroxytropene-6 (**139**) reacted with *O*-acetyltropoyl chloride in chloroform, using pyridine as catalyst, an ester (**140**) was formed which, after elimination of one molecule of acetic acid from the side chain, gave 3α-atropoyltropene-6 (**141**) as the chief product following crystallization. The latter was then oxidized with H_2O_2, using V_2O_5 as catalyst, to form the 6,7-epoxy derivative (**142**). Racemic anisodine (**143**) was obtained after dihydroxylation of the remaining double bond with *N*-methylmorpholine-*N*-oxide and osmium tetroxide.

Clinically, anisodamine is very effective in the treatment of shocks, especially septic shock.

V. Miscellaneous Alkaloids

A. Crotalaria Alkaloids

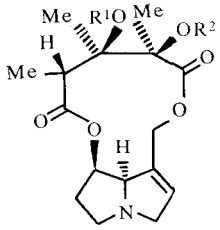
The genus *Crotalaria* (Family Leguminose) is essentially restricted to the southern part of China. It is rich in pyrrolizidine alkaloids. The alkaloids of the *Crotalaria* species are more varied, although macrocyclic diesters are more numerous. The chemistry and toxicities of pyrrolizidine alkaloids have been reviewed (*62, 63*).

The plant *Crotalaria sessiliflora* L. was first used for the treatment of skin cancer in Chinese folk medicine. A macrocyclic diester of retronecine, monocrotaline (**144**) was isolated from the aerial part of the plant. It was active against a number of tumor systems in mice, such as Walker 256 and Sarcoma 180. Monocrotaline was found to be efficacious for the external treatment of skin cancer and cervical carcinoma (*64, 65*).

Bull and Culvenor (*62*) have carried out extensive investigation of pyrrolizidine alkaloids. The structural requirement for antitumor activity was the allylic ester group of the molecule. It was also found to be responsible for hepatotoxicity. Mattocks (*66*) showed that the hepatotoxic effect of pyrrolizidine alkaloids was produced from metabolic products, for example, pyrrole derivatives.

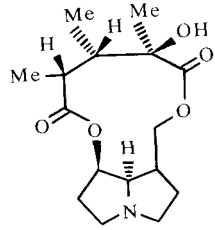
Wang *et al.* (*67*) also studied structure–activity relationships of pyrrolizidine alkaloids. Several plants of *Crotalaria* species growing in China were studied (*68, 69*). In addition to the main alkaloid monocrotaline, two minor alkaloids, retusine (**145,** $C_{16}H_{25}NO_7$) and spectabline (**146,** $C_{18}H_{25}NO_7$), were isolated from the seeds of *C. retusa* L. Another alkaloid, usaramine (**147**), was obtained from the seeds of *C. mucronata* Desv. It was found that retusine was more active against Hela cells than monocrotaline. In order to further study the antitumor activity of retusine, dihydromonocrotaline (**148**) was prepared by homophase catalytic hydrogenation (*70*).

The effects of dihydromonocrotaline on lines of human liver-cancer cells (BEL-7402) and on Ehrlich's Aseitic cancer cells of mice *in vitro* were reported by Sun *et al.* (*71*). It was found that growth, the survival of rats, and the mitotic index of the line of human liver cancer cells (BEL-7402) may be significantly reduced by dihydromonocrotaline. Dihydromonocrotaline is capable of killing the human liver cells directly, as well as inhibiting the growth of Ehrlich's Ascitic cells of mice. The allylic ester group of monocrotaline is not the primary structural requirement for antitumor activity. Dihydromonocrotaline exhibits antitumor effects, and its toxicity on the livers of mice was lower than that of the monocrotaline. The acute LD_{50} of dihydromonocrotaline in mice was found to be 1042 mg/kg (ip).

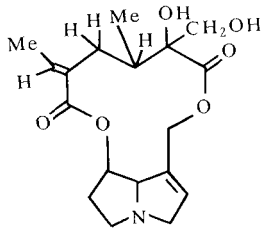


144 Monocrotaline $R^1 = R^2 = H$

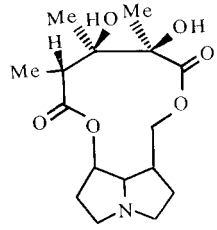
146 Spectabiline $R^1 = Ac$ $R^2 = H$



145 Retusine



147 Usaramine

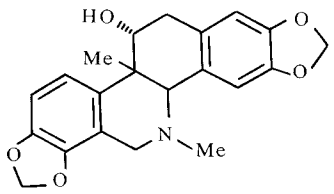


148 2, 3 - Dihydromonocrotaline

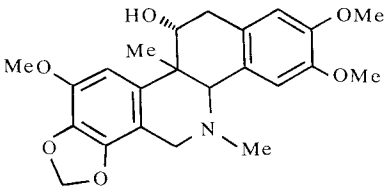
B. Corydalis Alkaloids

A new minor alkaloid, ambinine ($C_{23}H_{27}NO_6$), was isolated from the bulbs of *C. ambigua* (Pall.) Cham et Schlecht, which grows in Northeastern China (*72*). Based on spectral data, and by comparison of data of the derivatives with that of known benzophenathridine alkaloid corynoline (**149**), the structure of ambinine was assigned as (**150**).

According to preliminary pharmacological tests, ambinine has analgesic, sedative, and antispasmodic activities.



149 Corynoline

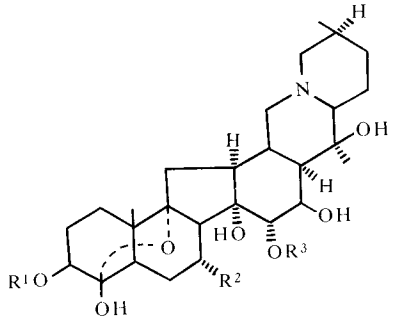


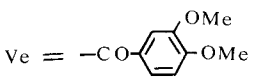
150 Ambinine

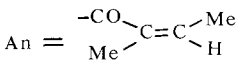
C. Veratrum Alkaloids

Liang and Sun (*73, 74, 75*) have studied the alkaloids of *V. stenophyllum* Diels. Two new bases, stenophylline-A (**151**) and stenophylline-B (**152**), along with veratroylzygadenine (**153**), angeloylzygadenine (**154**), zygadenine (**155**), jervine (**156**), and β-chaconine (**157**) were isolated. The structures of (**151**) and (**152**) were assigned based on the spectral data (IR, ^{1}H-NMR, and MS).

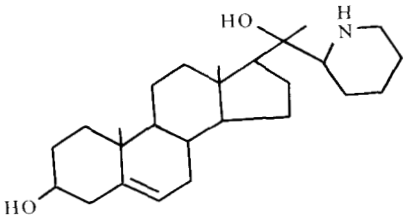
Veratroylzygadenine and stenophylline-A have been proved to have significant activity in lowering blood pressure and heart rate in anesthetized cats and dogs.



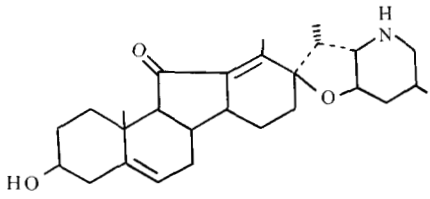




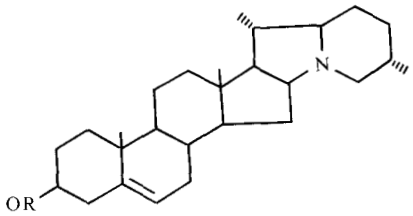
151	Stenophylline - A	R^1 = An R^2 = OH R^3 = An
153	Veratroylzygadenine	R^1 = Ve R^2 = R^3 = H
154	Angeloylzygadenine	R^1 = An R^2 = R^3 = H
155	Zygadenine	R^1 = R^2 = R^3 = H

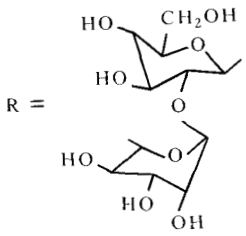


152 Stenophylline - B



156 Jervine





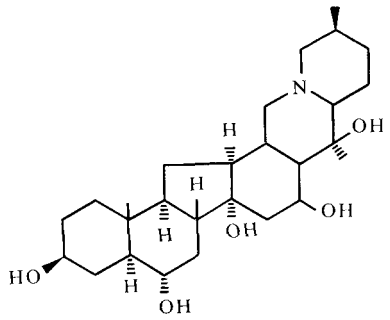
157 β - Chaconine

D. Fritillaria Alkaloids

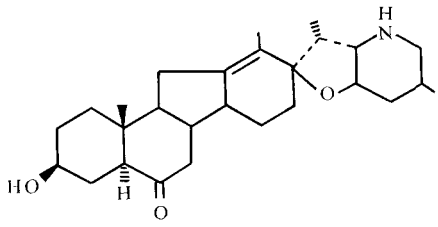
There are about 60 plant species of the genus *Fritillaria* in the world, with 20 plant species and 2 varieties growing in China (*76*). The bulbs of some *Fritillaria* plants named "Bei-mu" were used for the treatment of acute bronchitis, pneumonia, cough with thick phlegm, and scrofula in traditional Chinese medicine. Xu *et al.* (*77*) have reported on studies of *F. ussuriensis* Maxim. Two new alkaloids, pingpeimine-A (**158,** $C_{27}H_{41}NO_3$, mp 258–260°C and pingpeimine-B ($C_{27}H_{45}NO_5$, mp 255–259°C), along with known alkaloids peimisine (**159**) and sipeimine (**160**) were isolated. Based on chemical and spectral data, the structure of pingpeimine-A was assigned as (**158**). The structure of pingpeimine-B is to be investigated. It was shown that pingpeimine-A had expectorant and blood-pressure lowering activities.

Liu *et al.* (*78*) reported on studies of the bulbs of *F. walujewii* Regel, growing in the Xinjiang region of China. A new alkaloid named sinpeinine-A (**161**) was isolated. From chemical and spectroscopic analyses, the structure of sinpeinine-A was established as 3-hydroxy-5,14,17-cevanine-6-one.

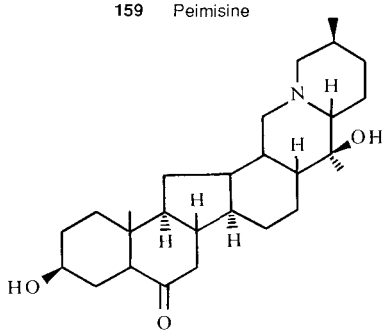
Wu and Pu (*79*) isolated a new base named hupeheninoside (**162,** $C_{33}H_{55}NO$) from the bulbs of *F. hupehensis* Hsiao et K. C. Hsia. On the basis of IR, ^{1}H-NMR, FD-, and El-mass spectra of hupeheninoside and the products of its transformation, the structure and configuration of hupeheninoside was established as 5α, 14α-cevanine-6α-hydroxyl-3α-β-D-glucoside.



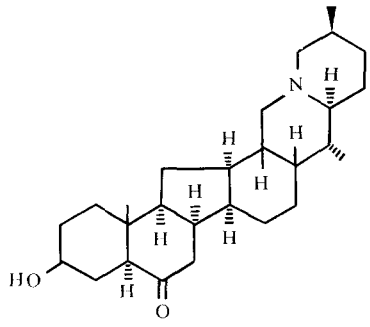
158 Pingpeimine - A



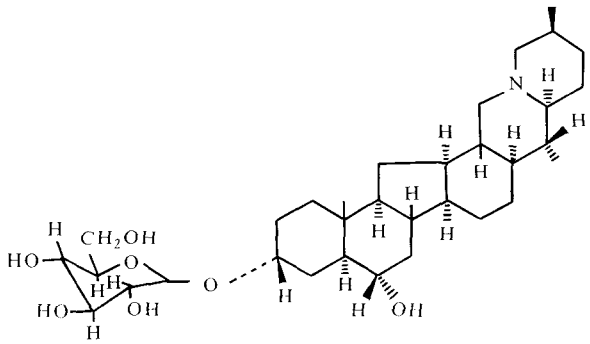
159 Peimisine



160 Sipeimine



161 Sinpeinine - A



162 Hupeninoside

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CHAPTER 4

N-ACYLIMINIUM IONS AS INTERMEDIATES IN ALKALOID SYNTHESIS

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I. Introduction

Since the early developments in the field of alkaloid synthesis, the chemistry of the iminium ion intermediate has been of pivotal significance (*1*). Iminium ions bearing alkyl substituents have been most frequently used, but their applicability is limited, chiefly due to their low electrophilic reactivity. This problem is quite easily remedied through the introduction of a carbonyl substituent onto nitrogen. The electron-withdrawing effect of this carbonyl group enhances considerably the electrophilic reactivity of the iminium ion, thus widening the scope of the iminium methodology. Other occasional advantages of the *N*-acyliminium compared to the ordinary iminium ion involve the greater stability of the direct precursor and the reaction product of the former species. Comprehensive reviews (*2–4*) have recently appeared on the generation of the *N*-acyliminium ion intermediate (**1**) and its applications in organic synthesis, especially in the formation of carbon–carbon bonds (amidoalkylation). In this chapter, the role which the *N*-acyliminium intermediate has played in the laboratory synthesis of alkaloids will be reviewed, in particular with respect to carbon–carbon bond formation. The discussion will be confined to syntheses passing through intermediates **1** with R^1, R^2, and R^3 hydrogen or a carbon substituent, while R^4 may be any substituent (although not always correct, the term *N*-acyliminium ion will be used for any substituent R^4 for the sake of convenience). The most emphasis will be laid on the *N*-acyliminium ion reaction itself, but the preparation of the starting materials for this key reaction, as well as further reactions of the products, will also receive appropriate attention. The order of the discussion will roughly follow the structural classification of alkaloids as used by Hesse (*5*).

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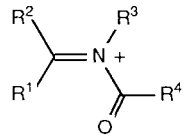

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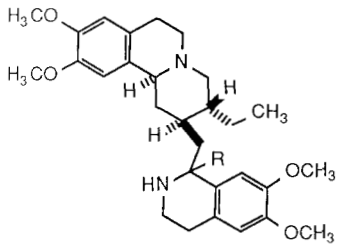
II. Isoquinoline Alkaloids

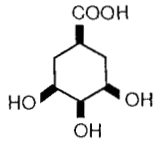
A. Ipecac Alkaloids

Owing to its important biological activity, (−)-emetine (**2**), as a representative of the ipecac(uanha) alkaloids (*6*), has received extensive attention from synthetic chemists. The first employment of *N*-acyliminium chemistry in this field dates back to 1959, when Burgstahler and Bithos (*7, 8*) reported the total synthesis of racemic emetine starting from hexahydrogallic acid (**3**). The carboxylic acid function in **3** was elaborated into the side chain of **4** by using Arndt–Eistert chemistry with 1-diazopropane and homoveratrylamine. Triol **4** was oxidized with periodic acid to a mixture which consisted mainly of lactol lactam **5,** along with the open hydroxy lactam aldehyde, both probably as mixtures of stereoisomers. Treatment of this crude mixture with aqueous phosphoric acid in DME at 75–80°C for 0.5 hr led to the formation of *N*-acyliminium intermediates which cyclized to aldehydes **6.** In order to analyze this product mixture it was oxidized with Ag_2O in aqueous ethanol to the corresponding acids (total yield of crystalline material from **4** was 44–58%). Careful recrystallization eventually led to the recovery of three pure acid isomers **7a** (11%), **7b** (18%), and **7c** (6%). The stereochemistry of **7a** was readily established, since this compound yielded emetine after the following reactions: a) esterification (CH_2N_2), b) reaction with homoveratrylamine, c) Bischler–Napieralski cyclization ($POCl_3$, 85°C) to **8,** and d) reduction of lactam and imine functions ($LiAlH_4$) to a mixture of emetine (**2**) and isometine (**9**). Burgstahler and Bithos (*7, 8*) did not determine the stereochemistry of **7b** (see below) and **7c.**

Almost 20 years later, Takano and co-workers (*9–11*) published an approach to the ipecac alkaloids which is very similar to the above synthesis with respect to the crucial *N*-acyliminium ion cyclization reaction. However, Takano *et al.* aimed at the synthesis of enantiomerically pure (−)-emetine, using norcamphor (**10**) as optically active starting material. To this end, norcamphor was transformed into acid **13** by way of **11** and **12.** Exposure of **13** to methyl iodide in refluxing acetonitrile led to a 1:2 mixture of **7a** and **7b** in 90% yield. In this interesting reaction, cleavage of the thioacetal was immediately followed by *N*-acyliminium ion formation and cyclization. Furthermore, it was found that this



2 R = ▬ H emetine
9 R = ≡ H isoemetine



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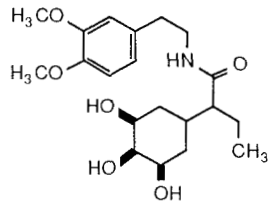

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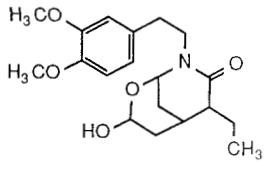

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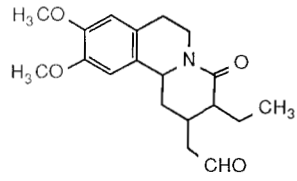

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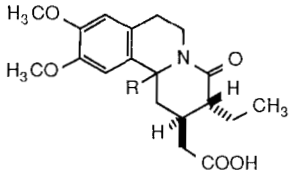
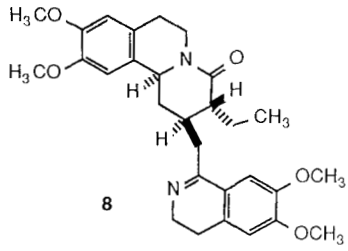


7a R = ≡ H
7b R = ▬ H



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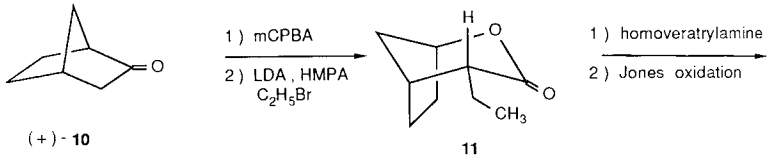
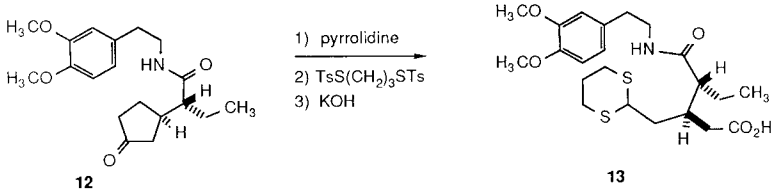
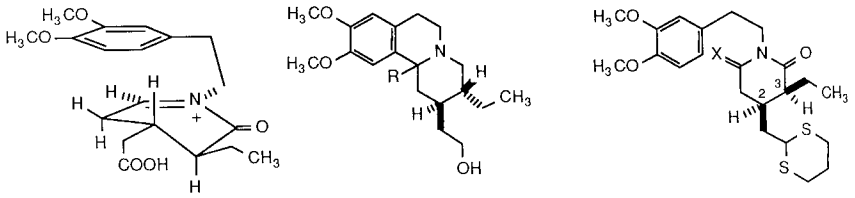
reaction proceeded more slowly at room temperature, but then yielded solely **7b.** Since the products were not interconvertible under the reaction conditions, the product ratio is determined in a kinetically controlled cyclization reaction. According to expectation, the nucleophile preferred to approach the *N*-acyliminium ion in its most favorable half-chair conformation **14** in a trans fashion with respect to the CH_2COOH substituent. Esterification (CH_3OH, HCl) and reduction ($LiAlH_4$) of **7a** and **7b** led to racemic protoemetinol (**15a**) and its isomer (**15b**), respectively. The epimeric relationship between **15a** and **15b** was proven by conversion of **15b** into **15a** via the sequence (*12*): a) acetylation, b) iminium ion formation ($Hg(OAc)_2$), c) iminium ion reduction ($NaBH_4$), and d) deacetylation. This work of Takano *et al.* (*9*) has established the stereochemistry of the



(+)-**10** **11**

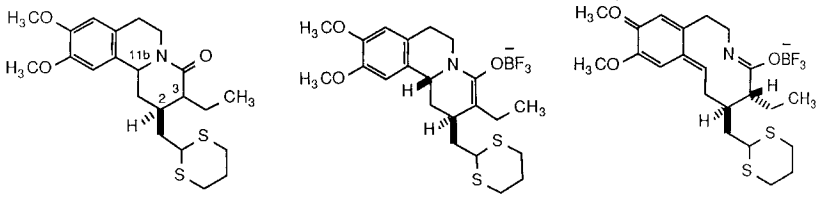


12 **13**



14 **15a** R = α-H protoemetinol
15b R = β-H

17 X = O
18 X = H, OEt



19a C3 β-H ; C11b α-H
19b C3 β-H ; C11b β-H
19c C3 α-H ; C11b β-H

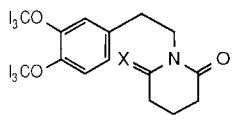
20a **20b**

second product (**7b**) of the key cyclization reaction of Burgstahler and Bithos (*8*). Thus, this synthesis shows that, in principle, (−)-emetine can be prepared rather efficiently from (+)-norcamphor ((+)-**10**).

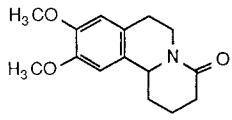
Takano *et al.* have also shown how (−)-norcamphor can be used for the synthesis of (−)-emetine (*2*). Heating of the enantiomer of **13** at 180°C produced cyclic imide **17.** This imide was reduced to ethoxy lactam **18** ($NaBH_4$, EtOH,

HCl), which, without purification, was refluxed in benzene in the presence of a catalytic amount of *p*-toluenesulfonic acid, giving lactams **19a, 19b,** and **19c** in about equal amounts in 68% yield from **17.** Formation of only these products indicated that the $NaBH_4$ reduction was completely regioselective. The cyclization results further showed that epimerization occurred at C3, most probably before cyclization, since the products were stable under the reaction conditions. Due to this epimerization, **19a** already had the stereochemistry present in (−)-emetine (*2*). The products **19b** and **19c** could be converted into the correct stereoisomer required for the synthesis of (−)-emetine through epimerization at C3 and C11b. For instance, treatment of **19c** with $BF_3{\cdot}OEt_2$ at room temperature gave quantitative conversion into **19a,** presumably via intermediates **20a** and **20b.** Thus, (−)−**10** is, in principle, also a suitable starting material for the synthesis of (−)-emetine.

Kametani and co-workers have completed the total synthesis of racemic emetine (**2**) by using an alternative *N*-acyliminium ion reaction for closure of the *B*-ring (*13, 14*). Their precursor **22** was readily made from homoveratrylamine and glutaric anhydride via reduction of imide **21** ($NaBH_4$, EtOH, HCl). When treated with *p*-toluenesulfonic acid in refluxing benzene, **22** cyclized to give **23** in nearly quantitative yield. The most important of the remaining steps towards emetine (**2**) was the Michael addition of dihydroisoquinoline **24** to acceptor **25,** synthesized in four steps from **23.** This Michael reaction gave, in 56% yield, a single product **26** with the undesired stereochemistry. Ethylation at C3 was followed by ester hydrolysis, decarboxylation, amide reduction, and imine reduction to furnish **27** as a mixture of isomers at C1′. The stereochemistry at C11b was then corrected (*12*) through iminium ion formation ($Hg(OAc)_2$) and reduction



21 X = O
22 X = H, OEt



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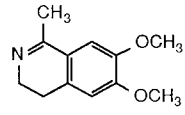

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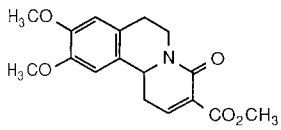

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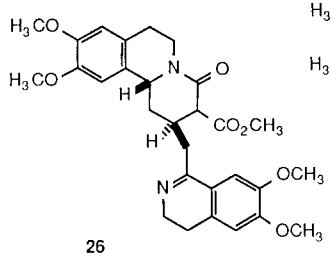

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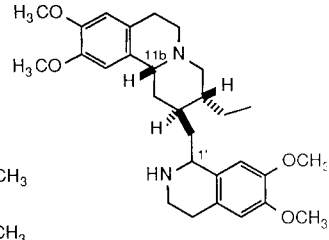

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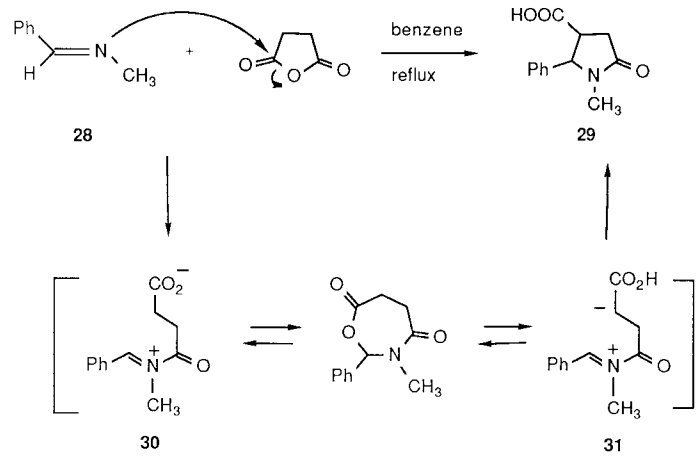
($NaBH_4$), giving a mixture of racemic emetine (**2**) and isometine (**9**), which was separated using chromatography.

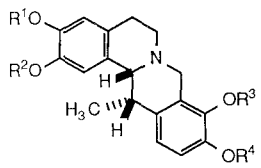
B. Protoberberine Alkaloids

The discovery by Castagnoli in 1969 (*15*), that succinic anhydride reacts with imines (for example, **28**) to form pyrrolidinones (for example, **29**), laid the basis for the synthesis of various protoberberine and benzophenanthridine alkaloids by Cushman and co-workers. Although the mechanism of this reaction has not been thoroughly investigated, a reasonable rationale calls for *N*-acyliminium ions **30** and **31** as intermediates (*16, 17*). The successful use of homophthalic anhydrides

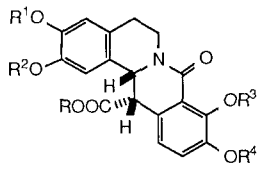


in this process paved the way for the synthesis of protoberberine alkaloids (*18, 19*). The key step in the synthesis of corydaline (**32**), was the reaction of dihydroisoquinoline **33** with homophthalic anhydride **34** (*20*). This reaction proceeded readily at room temperature in chloroform, and furnished a 1:3 mixture of **35** and its *trans*-stereoisomer in high yield. Reflux of this mixture in acetic acid led to complete formation of the *cis* isomer **35.** It was suggested that the *trans* isomer is less stable than the *cis* isomer because of nonbonded interaction between the pseudoequatorial carboxyl group and the *A*-ring in the *trans* isomer. Conversion of **35** into its methyl ester (CH_2N_2), followed by reduction with $LiAlH_4$ yielded the corresponding alcohol, which by means of methane sulfonylation and reduction ($NaBH_4$, EtOH, reflux), was transformed into corydaline (**32**).

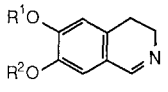
By using dihydroisoquinoline **36** as reaction partner for **34,** another series of natural products was obtained (*21*). In the same way as described in the previous paragraph, thalictricavine (**37**) was synthesized from **34** and **36** in high yield via



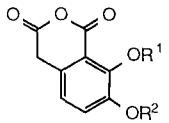
32 $R^1 = R^2 = R^3 = R^4 = CH_3$ corydaline
37 $R^1, R^2 = CH_2$; $R^3 = R^4 = CH_3$ thalictricavine
47 $R^1 = R^2 = CH_3$; $R^3, R^4 = CH_2$ cavidine



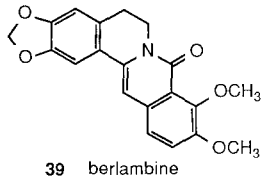
35 $R^1 = R^2 = R^3 = R^4 = CH_3$; R = H
38 $R^1, R^2 = CH_2$; $R^3 = R^4 = CH_3$; R = H
46 $R^1 = R^2 = CH_3$; $R^3, R^4 = CH_2$; $R = CH_3$



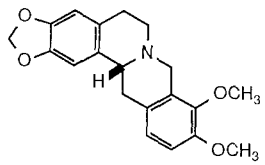
33 $R^1 = R^2 = CH_3$
36 $R^1, R^2 = CH_2$



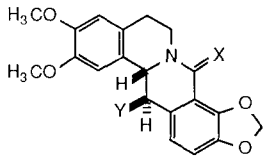
34 $R^1 = R^2 = CH_3$
41 $R^1, R^2 = CH_2$



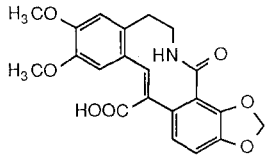
39 berlambine



40 canadine



42 X = O ; Y = COOH
43 X = H_2 ; Y = CH_3 thalictrifoline
45 X = O ; Y = $COOCH_3$



44

acid **38.** Oxidative decarboxylation of **38** ($Pb(OAc)_4$, $Cu(OAc)_2$, KOAc, HOAc, DMF, 100°C) furnished berlambine (**39**) in 65% yield. Reduction of **39** (AlH_3, ether) produced canadine (**40**) in 63% yield. It was found later (*22, 23*) that the enantiomers of acid **38** could be easily separated by means of recrystallization of the strychnine salt. In this manner, enantiomerically pure **37** and enantiomerically enriched **40** (through thermal decarboxylation at 240°C, accompanied by racemization) were available, and the absolute configuration of the natural product could be established.

Reaction of homophthalic anhydride **41** with imine **33** gave *trans*-acid **42** in 78% after recrystallization (*23, 24*). This acid was also readily resolved by using strychnine as a chiral base. In the same manner in which **35** was transformed into **32,** resolved acid **42** was converted into enantiomerically pure thalictrifoline **43.** Interestingly, acid **42** underwent complete racemization during reflux in acetic acid, presumably through the intermediacy of seco acid **44.** On the other hand,

ester **45** could be epimerized by using NaOMe in methanol to *cis*-ester **46** with complete retention of optical activity. Enantiomerically pure cavidine **47** was synthesized from **46.**

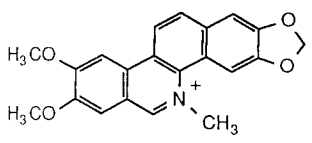
C. Benzophenanthridine Alkaloids

The reaction of imines with anhydrides, discussed in the previous section, has also been shown to be very useful for the synthesis of benzophenanthridine alkaloids (*25*). Nearly all of the work in this field has been performed by Cushman and co-workers. Nitidine (**48**) was the first alkaloid of this class that was synthesized using *N*-acyliminium ion chemistry (*26*). Reaction of homophthalic anhydride **49** with imine **50** occurred readily in chloroform at room temperature to produce a mixture of *cis*-acid **51** (49%) and *trans*-acid **52** (39%), which could easily be separated by recrystallization. In refluxing, acetic acid **51** changed completely into the more stable *trans*-acid **52.** Remarkably, the vicinal coupling constant in the *trans*-acid (J_{AB} = O Hz) is much smaller than in the *cis*-acid (J_{AB} = 6 Hz). This is in agreement with a preferred conformation of **52** in which the carboxyl and the aromatic substituent occupy pseudoaxial orientations. Arndt–Eistert homologation of **52,** followed by polyphosphoric acid, induced Friedel–Crafts cyclization and yielded ketone **53.** This keto-amide **53** was reduced with $LiAlH_4$ and the resultant amino alcohol dehydrated and dehydrogenated with palladium on charcoal to afford the natural product **48,** isolated as chloride.

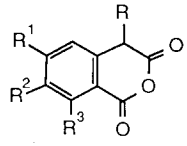
Since nitidine (**48**) displays antileukemic activity, but is very toxic, attention has been paid to the synthesis of analogues, which possibly would show a more favorable balance between these properties (*27, 28*). Treatment of **51** with polyphosphoric acid at 70–80°C furnished ketone **54.** This ketone was converted to the indenoisoquinoline analogue **55** of nitidine in the same way as **53** was converted to **48.** The biological properties of **55** did not deviate much from those of **48.**

The key step in the total synthesis of chelidonine (**56**) was the reaction of imine **50** with homophthalic anhydride **57** (*29, 30*). The best yield (62% of crystalline material) of *cis*-isomer **58,** at the expense of the more stable *trans*-isomer, was obtained when this reaction was carried out in refluxing acetonitrile. *Cis* acid **58** was converted into diazoketone **59** via the acid chloride in 50% overall yield. Treatment of **59** with trifluoroacetic acid for 1 min at 0°C gave the ketone **60** in 19% yield. Reduction of **60** with $LiAlH_4$ proceeded stereoselectively to produce the natural product **56** in 93% yield.

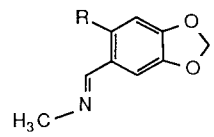
The next synthetic goal of Cushman *et al.* was corynoline (**61**) and congeners (*31–33*). The reaction of imine **50** with homophthalic anhydride **62** proceeded smoothly at room temperature, despite the presence of the methyl group, and furnished a mixture of **63** and **64** in quantitative yield. The ratio of **63** and **64**



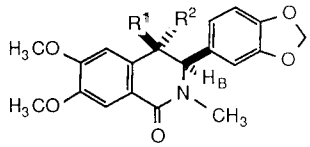
48 nitidine



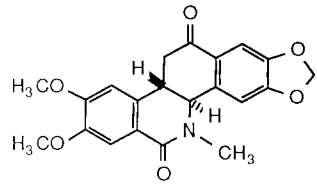
49 $R^1 = R^2 = OCH_3$; $R = R^3 = H$
57 $R = R^1 = H$, $R^2, R^3 = OCH_2O$
62 $R = CH_3$; $R^1 = H$, $R^2, R^3 = OCH_2O$



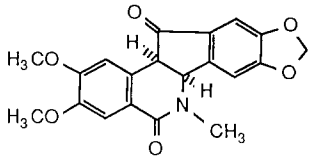
50 R = H
69 $R = CH_2CN$



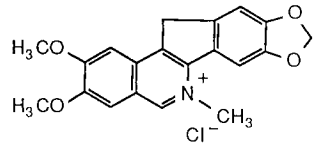
51 $R^1 = COOH$, $R^2 = H_A$
52 $R^1 = H_A$; $R^2 = COOH$



53

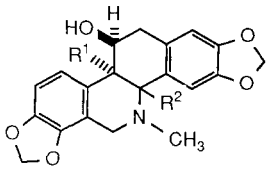


54

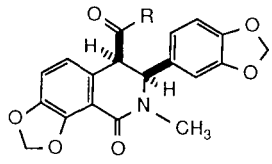


55

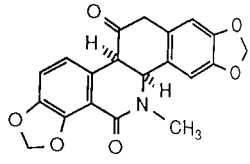
varied from almost 1:1 in methanol as reaction solvent to 1:9 in a very apolar solvent like benzene. The origin of this solvent effect is unclear. The two isomers were easily separable due to the large difference in solubility of their sodium salts in water. From **63,** corynoline **61** was synthesized in 27% yield in the same manner as described for the transformation of **58** into **56.** A similar route for the synthesis of epicorynoline (**65**) from **64** failed, since $LiAlH_4$ reduction of the intermediate ketone furnished the wrong alcohol stereoisomer, and all attempts to isomerize this alcohol were in vain. The following route, however, did lead to **65.** Arndt–Eistert homologation of **64,** followed by Friedel–Crafts ring closure, yielded ketone **66.** Reduction of this ketone ($NaBH_4$) and dehydration of the resulting alcohol, gave an olefin which was epoxidized stereoselectively to **67.** Reduction of this epoxide with $LiAlH_4$ furnished epicorynoline (**65**) in 24% overall yield from **64.** Very recently, Cushman and Chen have published an asymmetric synthesis of corynoline (**61**) by using as the imine component a modification of **50,** which has, instead of methyl, an asymmetric substituent on nitrogen (*33a*).



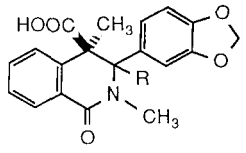
56 $R^1 = H$; R^2 = ⫼ H chelidonine
61 $R^1 = CH_3$; R^2 = ⫼ H corynoline
65 $R^1 = CH_3$; R^2 = ▬ H epicorynoline



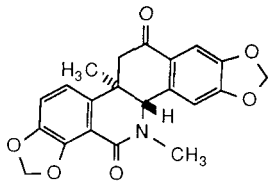
58 R = OH
59 R = CHN_2



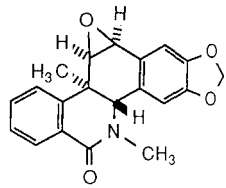
60



63 R = ⫼ H
64 R = ▬ H



66

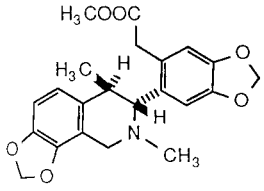


67

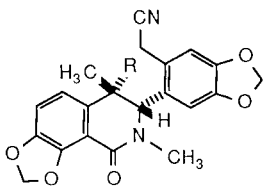
Corydalic acid methyl ester **68** is a natural product which is presumably derived from an intermediate in the biosynthesis of benzophenanthridine alkaloids from protoberberine alkaloids. The key step in the laboratory synthesis of **68** by Cushman and Wong, is the reaction of imine **69** with homophthalic anhydride **62,** giving **70** and its stereoisomer as 1:2 mixture, in 94% yield (*34*). Decarboxylation of this mixture at 160°C in DMSO provided **71** and its stereoisomer as a 5:2 mixture, which was separated using chromatography. Conversion of the cyano function into a methyl ester (HCl, CH_3OH), and reduction of the amide (a. $POCl_3$, 60°C; b. $NaBH_4$, EtOH), led to corydalic acid methyl ester (**68**).

From this and the previous section, it has become evident that the reaction of imines with homophthalic anhydrides is a powerful method for the synthesis of two classes of alkaloids, elegantly elaborated by Cushman and co-workers. Although one can hardly imagine a mechanism which does not involve *N*-acyliminium ions as intermediates, there is still some mystery around this reaction. It appears to proceed well in many solvents. More mechanistic work in this field is necessary to explain the fine details of this reaction, in particular, the solvent-dependent isomer ratios (*34a*). A possible rationale would be to involve a 1,5 dipolar cyclization mechanism according to structure **75a.**

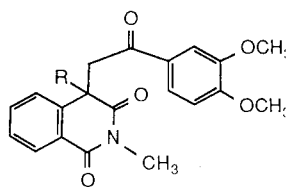
Iida and co-workers have described a different approach to the basic skeleton of corynoline (**61**), which also involves *N*-acyliminium chemistry (*35, 36*). Alkylation reactions on *N*-methylhomophthalimide furnished imides **72** and **73,** respectively. Reduction of **73** with $NaBH_4$ occurred with high regioselectivity,



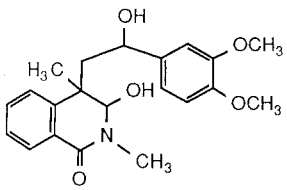
68



70 R = COOH
71 R = H



72 R = H
73 R = CH_3



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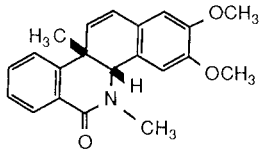
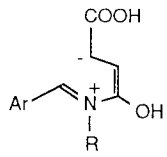


75

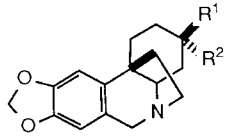


75a

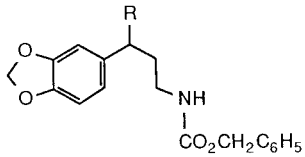
giving dihydroxy lactam **74** in 90% yield. After treatment with hydrochloric or *p*-toluenesulfonic acid in refluxing benzene, **74** underwent ring closure with concomitant elimination of the benzylic hydroxyl function, producing **75** as a single isomer in 90% yield. Compounds of this type have been transformed into benzophenanthridine alkaloids by Ninomiya *et al.* (*37, 38*).

D. Amaryllidaceous Alkaloids

Plants within the family of the Amaryllidaceae produce a large number of alkaloids of wide structural variety (*39*). Consequently, synthetic activity in this field has been intense, but only limited use has been made of *N*-acyliminium ions as intermediates. In this section only the work of Sánchez and co-workers will be treated. The synthesis of mesembrine will be dealt with in Section IVA. Elwesine (**76**) and epielwesine (**77**) have been synthesized by two routes, which involve quite different *N*-acyliminium reactions. The pertinent chemistry of the first route to be discussed (*40*) begins with nitrile **78.** Reduction of **78** with diisobutylaluminum hydride, followed by treatment with aqueous HCl, furnished hydroxy carbamate **79.** The reaction of **79** with methyl vinyl ketone failed under acid catalysis, giving only elimination of water. On the other hand, basic catalysis (Triton-B, THF 0°C) led to the formation of adduct **80,** most likely through the intermediacy of the enolate anion of the aldehyde tautomer of **79.** Adduct **80** was not isolated but treated with methanolic HCl, inducing *N*-acyliminium ion cyclization to **81** in 46% overall yield. Three steps—ketone reduction, removal

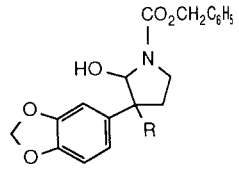


76 $R^1 = H$; $R^2 = OH$ elwesine
77 $R^1 = OH$; $R^2 = H$ epielwesine
85 R^1 , $R^2 = O$

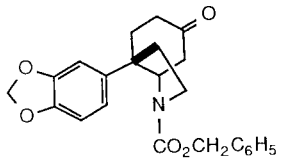


78 R = CN

82 R = 



79 R = H
80 $R = CH_2CH_2COCH_3$



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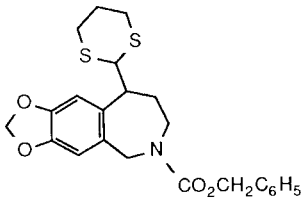

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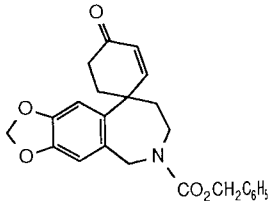
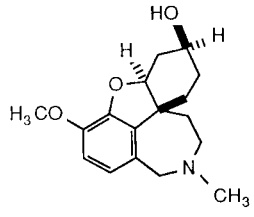
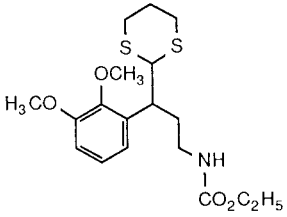


84

of the *N*-protecting group, and Pictet–Spengler cyclization—furnished the natural product **76** and **77.** The key reactions of the second route (*41*) commence with dithiane **82.** Base (NaOH) catalyzed reaction of **82** with aqueous formaldehyde gave the *N*-hydroxymethyl compound, which after treatment with a catalytic amount of *p*-toluenesulfonic acid in refluxing benzene (Dean–Stark apparatus),



86 lycoramine



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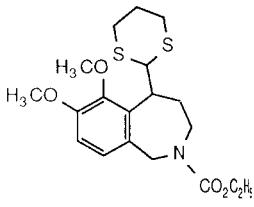

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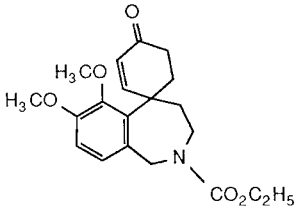

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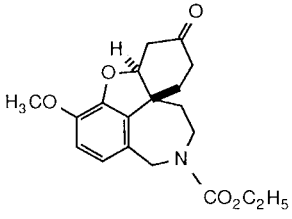


90

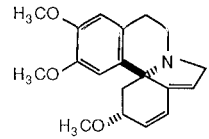
cyclized to hydrobenzazepine **83** in 95% overall yield. This process constitutes a nice example of an intramolecular Tscherniac–Einhorn reaction (*42*). The remaining steps towards the natural products **76** and **77** included spiroannulation to **84** (72% yield from **83**) and an interesting one-pot *N*-deprotection conjugate addition reaction of **84** to **85** (92% yield).

A similar Tscherniac–Einhorn reaction was applied in the total synthesis of lycoramine (**86**) (*43*). Treatment of **87** with aqueous formaldehyde, and heating of the resulting *N*-hydroxymethyl compound with a catalytic amount of *p*-toluenesulfonic acid in refluxing benzene, furnished tetrahydrobenzazepine **88** in 90% overall yield. Aldehyde deprotection and spiroannulation produced enone **89** in 53% overall yield. Treatment of **89** with ethyl sulfide in the presence of $AlCl_3$ gave rise to formation of the lycoramine skeleton **90** in 75% yield. Reduction of **90** with $LiAlH_4$ completed the synthesis of lycoramine **86.**

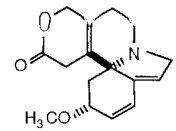
E. Erythrina Alkaloids

The early work in the field of the erythrina alkaloids (*44*) by Belleau (*45*), Boekelheide *et al.* (*46*), and Mondon (*47*) has been of fundamental significance for the development of *N*-acyliminium chemistry and the establishment of its synthetic utility. Erysotrine (**91**) and β-erythroidine (**92**) exemplify the most important structural types of erythrina alkaloids. The basic skeleton **93** of erysotrine could not be synthesized from hexahydroindole **94** through cyclization of iminium salt **95** (*45–47*). Belleau thus reasoned that a more electrophilic iminium ion was necessary, and attempted a cyclization reaction in which *N*-acyliminium ion **96** should serve as reactive intermediate. This reasoning was fully correct, since heating of amide **97** with phosphorus pentoxide and phosphoric acid at 100°C for 3 hr yielded crystalline amide **98** in 71% yield (*45, 46, 48*). Reduction with $LiAlH_4$ furnished the desired amine **93.**

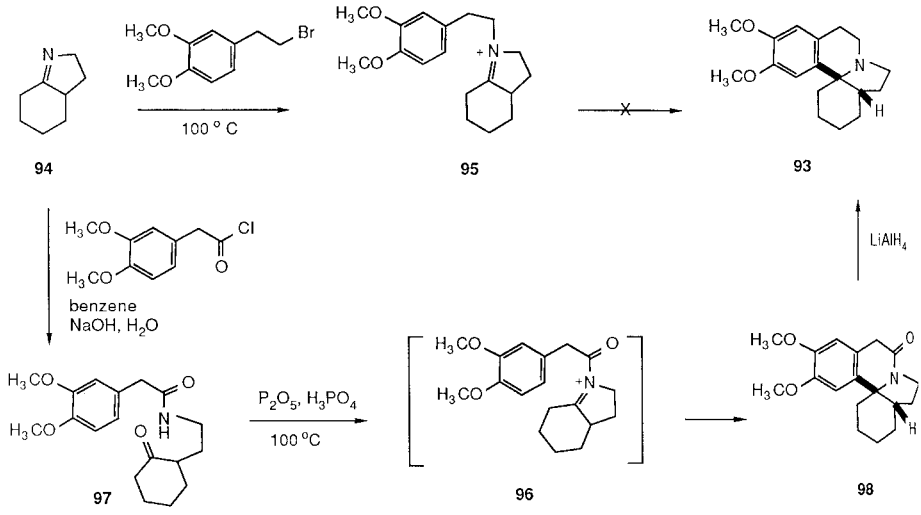
Mondon *et al.* used a different approach to synthesize **93** (*47–49*). Heating acid **99** with homoveratrylamine in the presence of an acidic catalyst at 180°C produced amide **101** in 72% yield, most likely through the intermediacy of *N*-acyliminium ion **100.** Reduction of **101** with $LiAlH_4$ gave the same amine **93** as Belleau obtained. Boekelheide *et al.* (*46*) have compared the strategies of Belleau and Mondon and found that the cyclization via **100** is a more facile process than via **96.** This fact can be understood by realizing that the six-membered cyclic transition state for the reaction **96** → **98** is more strained (only one sp^3 carbon in the ring), than for the reaction **100** → **101** (two sp^3 carbons in the ring). An additional advantage of the Mondon strategy in comparison with the Belleau strategy is the greater accessibility of starting materials. Therefore, the former is to be preferred for the synthesis of erythrina alkaloids. The cyclization reactions were stereoselective, giving only products with the cis ring junction in the hydrooxindole moiety (*48*). Belleau (*50*) and Boekelheide and co-workers (*51*) have



91 erysotrine



92 β-erythroidine

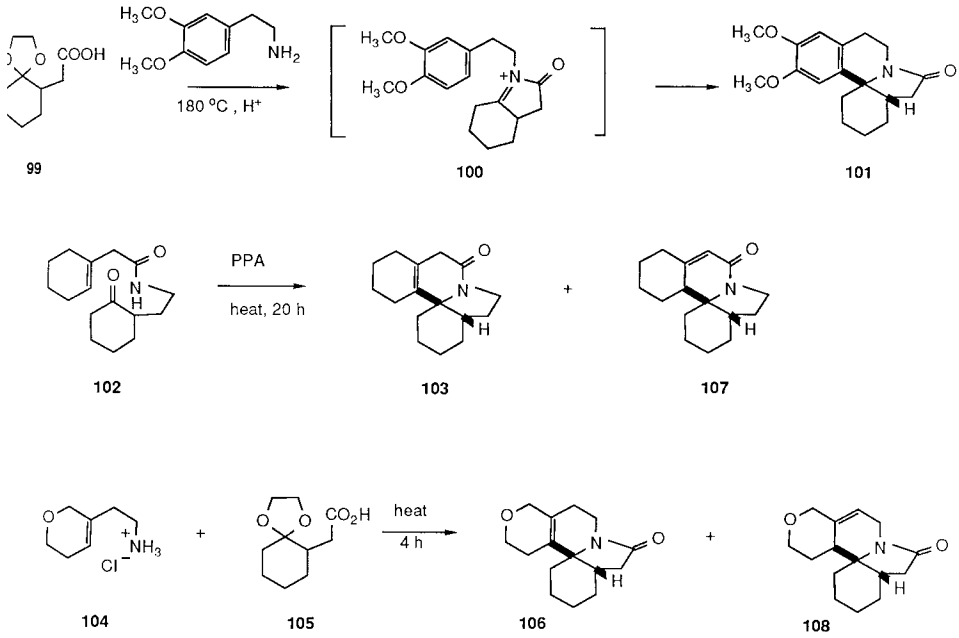


94 95 93

97 96 98

also employed simple olefins as nucleophiles in order to synthesize the skeleton of erythroidine **92.** Heating amide **102** in polyphosphoric acid produced tetracycle **103** in good yield (*50*). Ammonium salt **104** and acid **105** were coupled at high temperature, yielding amide **106** (*51, 52*). Mondon and Neffgen have reinvestigated these reactions and found that the products **103** and **106** were produced as mixtures with the double-bond isomers **107** and **108,** respectively (*53*).

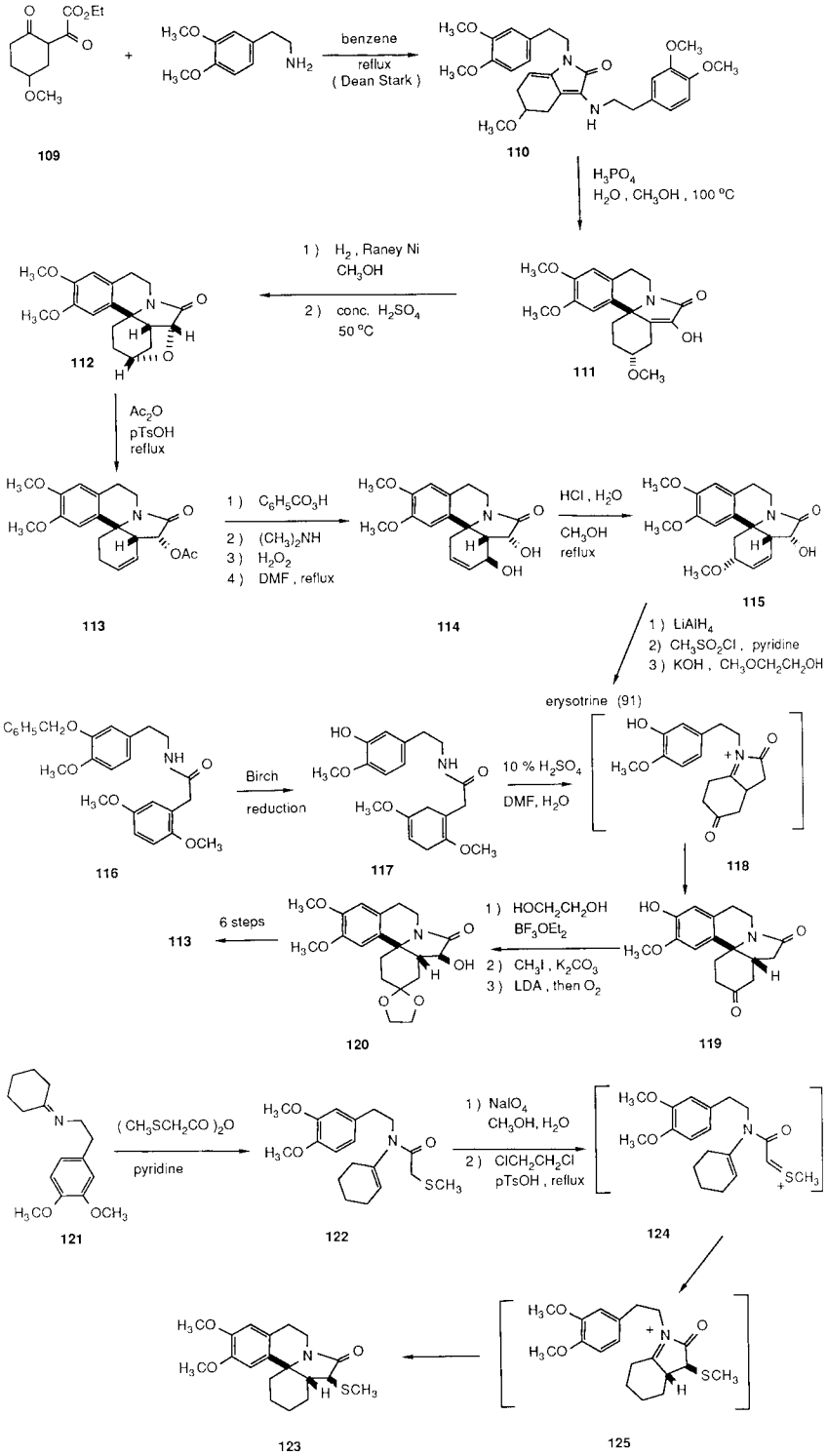
The above studies constituted the basis for the first total synthesis of erysotrine (**91**) by Mondon and co-workers in 1964 (*54–56*). Condensation of 2 equiv of homoveratrylamine with diketoester **109** furnished oxindole **110** in 69%. When this product was heated at 100°C with dilute phosphoric acid, both *N*-acyliminium ion cyclization, and hydrolysis of the enamine occurred, producing tetracycle **111** in 74% yield. Although **111** already has the correct carbon skeleton, it took many additional steps to arrive at the natural product **91.** Hydrogenation of **111** in the presence of Raney-nickel occurred from the β-face to give the dihydro-compound in 90% yield. When this product was treated for 30 min with concentrated sulfuric acid at 50°C, cyclization to ether **112** took place in



78% yield. This ether was cleaved in refluxing acetic anhydride in the presence of *p*-toluenesulfonic acid. Crystallization of the product mixture yielded olefin **113** in 49% yield. Epoxidation of this olefin occurred selectively from the β-face in 37% crystallized yield. Dimethylamine then regioselectively opened the epoxide and cleaved the acetate to give a dihydroxyamine (90%) which, on Cope elimination of the derived *N*-oxide, furnished olefin **114** (62%). Methanolysis of **114** was a troublesome step in the synthesis, and furnished **115** in 20% yield after tedious chromatographic separation. Reduction, mesylate formation, and elimination then produced erysotrine (**91**) in 24% from **115.**

More recently the Mondon approach to the erythrinane skeleton has been modified by various research groups, to simplify the total synthesis of erythrina alkaloids and to facilitate the preparation of analogues (*57–65*). In 1976, Haruna and Ito (*62*) published a formal synthesis of erysotrine (**91**), beginning with amide **116.** Birch reduction furnished **117,** which, when treated with 10% aqueous H_2SO_4 in DMF, cyclized to erythrinane skeleton **119** via *N*-acyliminium ion **118** in 90% overall yield. After protecting the ketone and phenolic methylation, the lithium enolate of the lactam was oxidized with molecular oxygen to produce **120** in 67% yield from **119.** After six subsequent steps (22% overall), **113** was obtained, which had been transformed into **91** by Mondon and Nestler (*56*).

Recently, an interesting double cyclization reaction was reported, leading to the erythrinane skeleton (*65*). Treatment of imine **121** with the anhydride of


benzene
reflux
(Dean Stark)
109
110
H_3PO_4
H_2O , CH_3OH , 100 °C
1) H_2 , Raney Ni
CH_3OH
2) conc. H_2SO_4
50 °C
111
112
Ac_2O
pTsOH
reflux
1) $C_6H_5CO_3H$
2) $(CH_3)_2NH$
3) H_2O_2
4) DMF , reflux
HCl , H_2O
CH_3OH
reflux
113
114
115
1) $LiAlH_4$
2) CH_3SO_2Cl , pyridine
3) KOH , $CH_3OCH_2CH_2OH$
erysotrine (91)
Birch
reduction
10 % H_2SO_4
DMF, H_2O
116
117
118
1) $HOCH_2CH_2OH$
BF_3OEt_2
2) CH_3I , K_2CO_3
3) LDA , then O_2
6 steps
113
120
119
(CH_3SCH_2CO)$_2O$
pyridine
121
122
1) $NaIO_4$
CH_3OH, H_2O
2) $ClCH_2CH_2Cl$
pTsOH , reflux
124
125
123

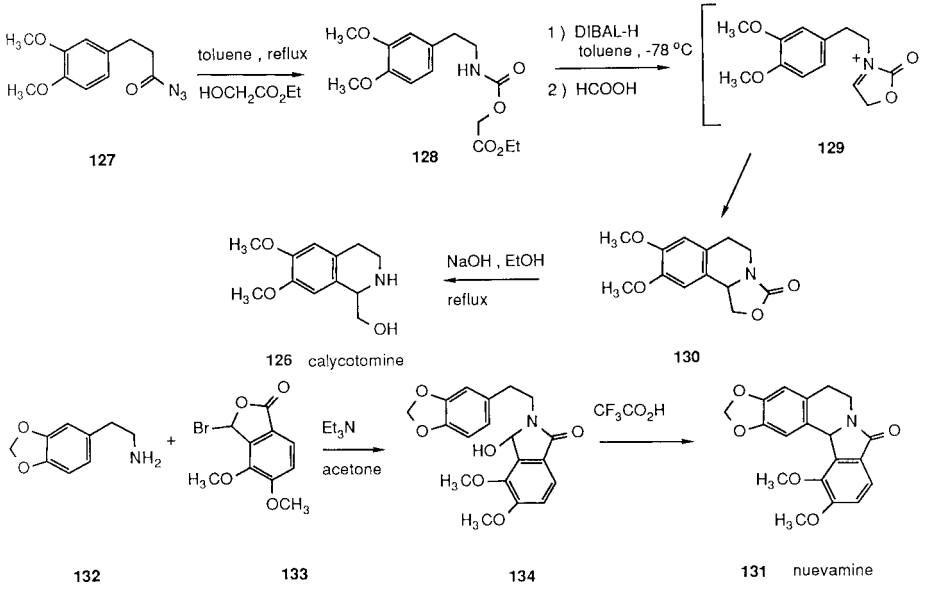
α-(methylthio)acetic acid gave enamide **122** in 57% yield from cyclohexanone and homoveratrylamine. The sulfoxide of **122** was refluxed in 1,2-dichloroethane in the presence of *p*-toluenesulfonic acid, producing tetracycle **123** in 50% from **122.** Most likely this double cyclization reaction proceeds via thionium ion **124** and *N*-acyliminium ion **125.**

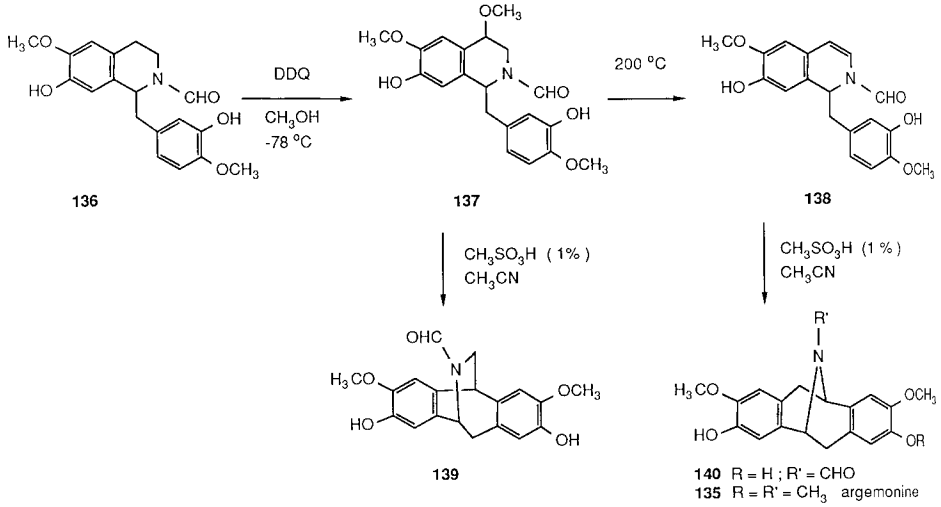
F. Remaining Isoquinoline Alkaloids

The simple isoquinoline alkaloid calycotomine (**126**) has been prepared from acylazide **127** by first employing a Curtius rearrangement in the presence of ethyl glycolate to carbamate **128** in 72% yield. The ester function was then reduced with diisobutylaminium hydride in toluene at −70°C and the crude product stirred in formic acid for 14 hr, giving **130** in 70% yield via oxaacyliminium ion **129.** Hydrolysis of **130** furnished calycotomine (*66*).

The structure of nuevamine was revised to **131** on the basis of a total synthesis from amine **132** and lactone **133** (*67*). Condensation of the latter two compounds gave **134** (55%), which cyclized in trifluoroacetic acid in quantitative yield to nuevamine (**131**).

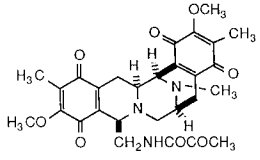
Synthetic work directed to the pavine alkaloids (e.g. argemonine **135**) has furnished interesting *N*-acyliminium ion chemistry (*68*). Oxidation of *N*-formyl-*N*-norreticuline (**136**) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in methanol at −78°C, furnished methoxy derivative **137** in 75% yield.



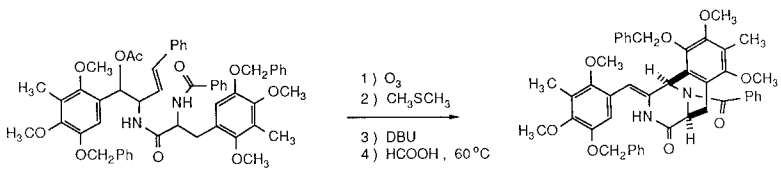


Elimination of methanol occurred at 200°C, generating olefin **138** (87%). Both compounds **137** and **138** were subjected to treatment with 1% methanesulfonic acid in acetonitrile. From **137,** only isopavinan **139** was obtained (64% yield) through the intermediacy of a benzylic cation. Interestingly, **138** produced pavinan **140** as the sole product in 75% yield. This result indicates that the *N*-acyliminium ion in this system is more stable than the benzylic cation. From **140,** argemonine (**135**) was obtained in two steps in 55% overall yield.

In the total synthesis of saframycin B (**141**), a similar type of reaction was utilized (*69*). Ozonolysis of **142** followed by reduction with dimethyl sulfide,



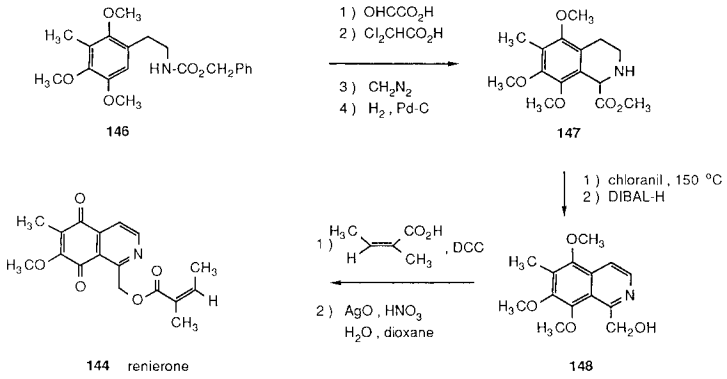
141 saframycin B



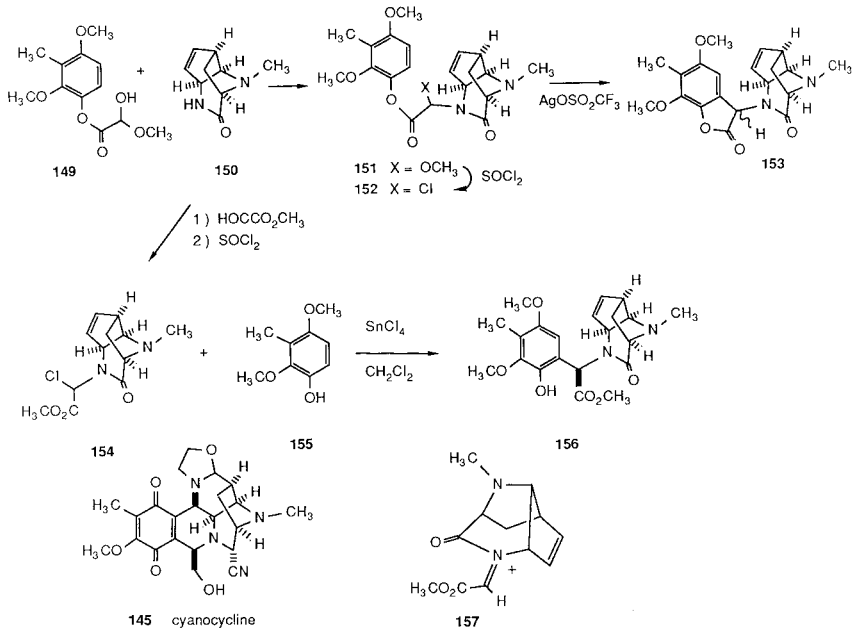
142 **143**

gave a mixture of unstable aldehydes. After elimination of acetic acid with DBU, the mixture was heated at 60°C in formic acid for 20 min. This caused the formation of an *N*-acyliminium intermediate, which cyclized to a single product **143** in 74% overall yield from **142.**

N-acyliminium ion chemistry has also been crucial in the total synthesis of two other isoquinolinoquinones, namely renierone (**144**) and cyanocycline (**145**). Treatment of carbamate **146** with glyoxylic acid gave an adduct, which was stirred with dichloroacetic acid for 16 hr at room temperature (*70*). Subsequent esterification and hydrogenolysis created methyl ester **147** in 80% overall yield from **146.** The remaining steps of the synthesis of renierone (**144**) included dehydrogenation to a highly substituted isoquinoline by using tetrachlorobenzoquinone, ester reduction to **148,** esterification with angelic acid, and oxidation to the quinone (*70*). In the synthesis of cyanocycline (**145**), the *N*-acyliminium cyclization of **152** was anticipated to be a promising pathway (*71, 72*). Condensation of **149** with lactam **150** in the presence of molecular sieves gave **151,** which, on treatment with $SOCl_2$, was converted into chloride **152.** Cyclization of **152** occurred on treatment with silver triflate (4 equiv) in CH_2Cl_2 in 58% overall yield to form **150.** However, the stereoselectivity of this process was low, and **153** was obtained as a 3:2 mixture. Then, attention was directed to the intermolecular variant. Lactam **150** was converted into chloride **154** in 96% yield by way of reaction with monomeric methyl glyoxylate and treatment with $SOCl_2$. When chloride **154** was mixed with phenol **155** in the presence of 2.6 equiv of tin tetrachloride in dichloromethane, a mixture of amidoalkylation products was obtained, from which the desired product **156** crystallized in 48% yield. The ratio of **156** and its benzylic epimer was 85 : 15 in the crude product mixture. Thus, the intermolecular reaction proceeded much more satisfactorily than its intramolecular counterpart. The intermediate in the former reaction was proposed to be the *N*-acyliminium ion **157,** which coupled with **155** from the α-face (*71, 72*).



146 147 144 renierone 148



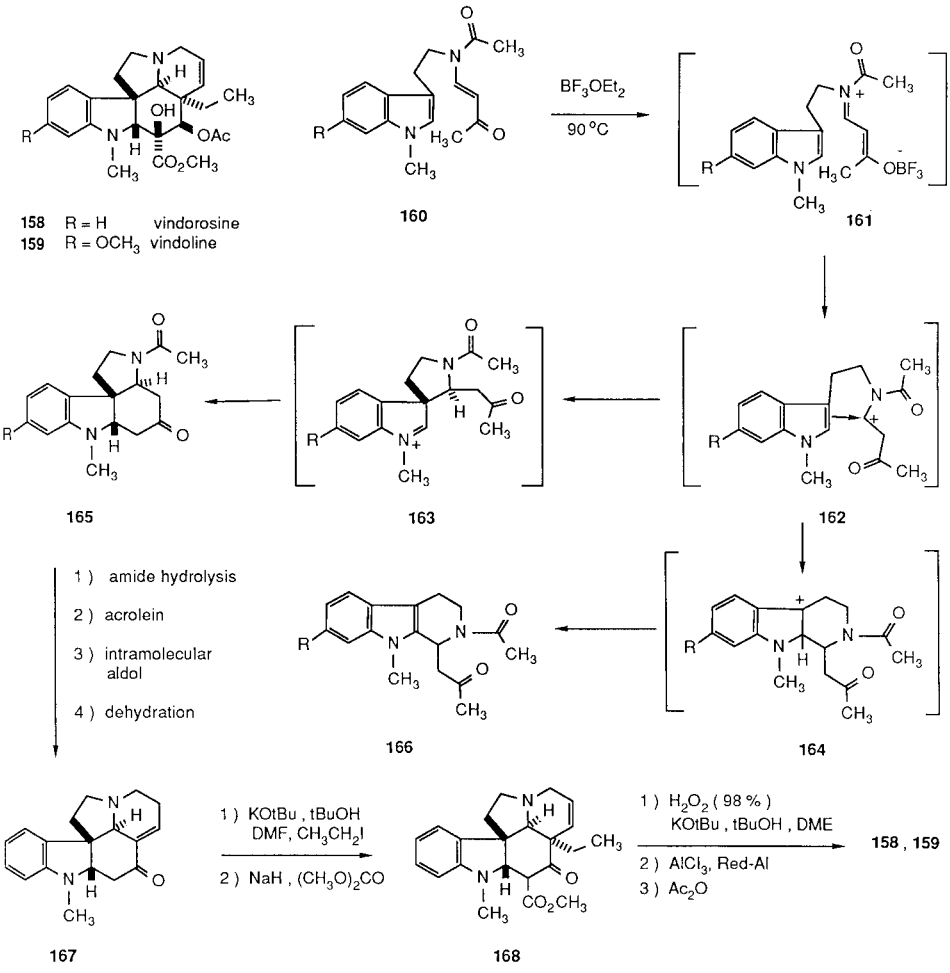
III. Indole Alkaloids

A. Aspidospermine Alkaloids

The aspidospermine alkaloids (*73*) have received extensive attention from synthetic chemists, partly due to the antitumor activity of a few members of this alkaloid class. Vindorosine (**158**) and vindoline (**159**), very important compounds in this respect, were synthesized by Büchi and co-workers in the early 1970s by employing *N*-acyliminium ions as intermediates (*74, 75*). When vinylogous imide **160** was heated for 15–30 min at 90°C in neat boron trifluoride etherate, a mixture of the desired tetracycle **165** and by-product **166** was obtained. For R = H and OTs, the desired **165** was the major product in 38% and 89%, respectively, whereas for R = OCH_3, **165** was formed in only 9% yield. This can be understood to a certain extent by assuming a reaction mechanism is present which proceeds via *N*-acyliminium ion **161,** π-complex **162,** and discrete carbocations **163** and **164.** In the case of R = OCH_3, cation **164** experiences extra stabilization, thus leading to more of **166** at the expense of **165.** It may further be noted that none of the desired tetracycle could be obtained from the corresponding vinylogous amide **160** (NH instead of NAc), again emphasizing

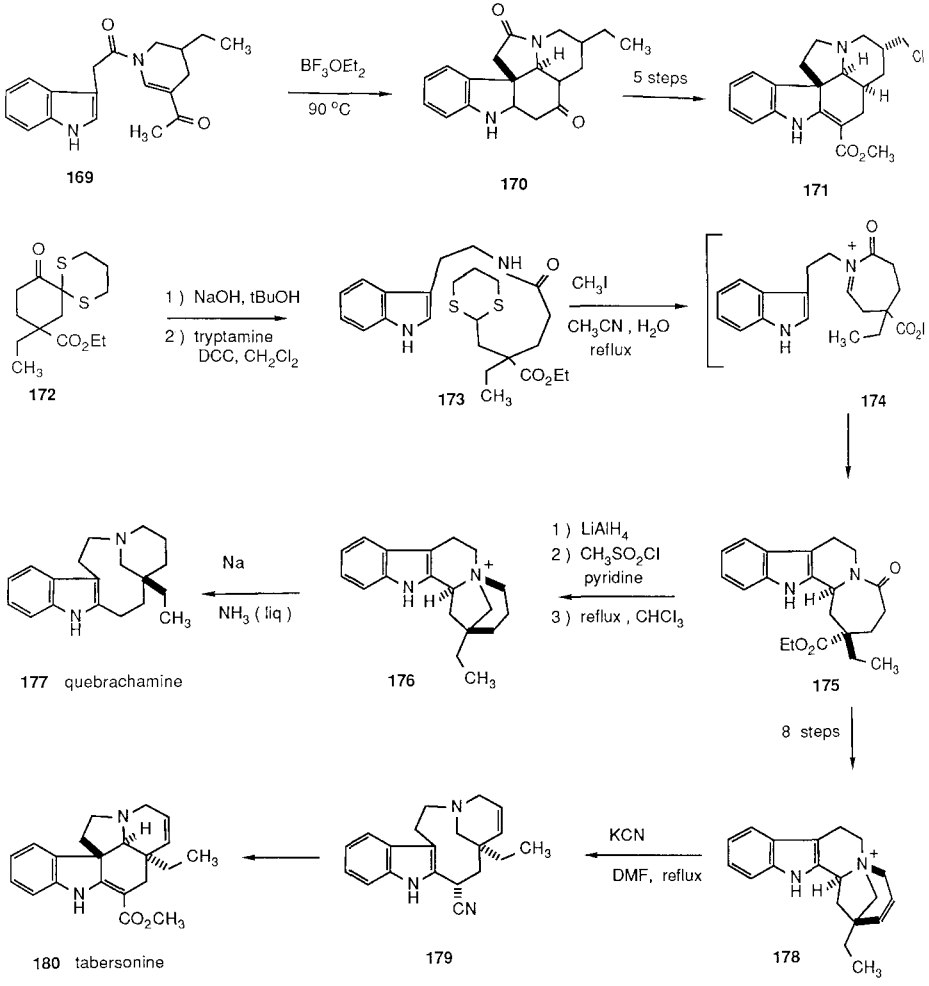
the usefulness of *N*-acyliminium ions as intermediates versus ordinary iminium ions. Compound **165** (R = OTs) could readily be transformed into **165** (R = OCH_3). From tetracycles **165** (R = H) and **165** (R = OCH_3), the natural products **158** and **159,** respectively, were synthesized in a similar manner, in a nine-step sequence (*74, 75*). Most crucial were the ethylation of the dienolate, derived from **167,** and the hydroxylation of **168** by using 98% hydrogen peroxide.

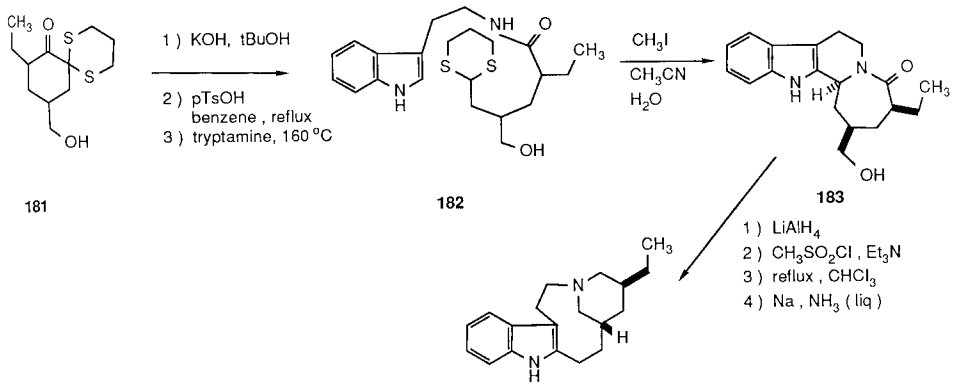
Wenkert and co-workers (*76–79*) have developed a mechanistically similar approach to the aspidospermine skeleton, starting from vinylogous imide **169.** Treatment of this compound with boron trifluoride etherate at 90°C for 20 min



provided a mixture of stereoisomers **170** in 81% yield (*79*). Compound **170** could be transformed in a few steps into 20-epipseudovinc adifformine (**171**).

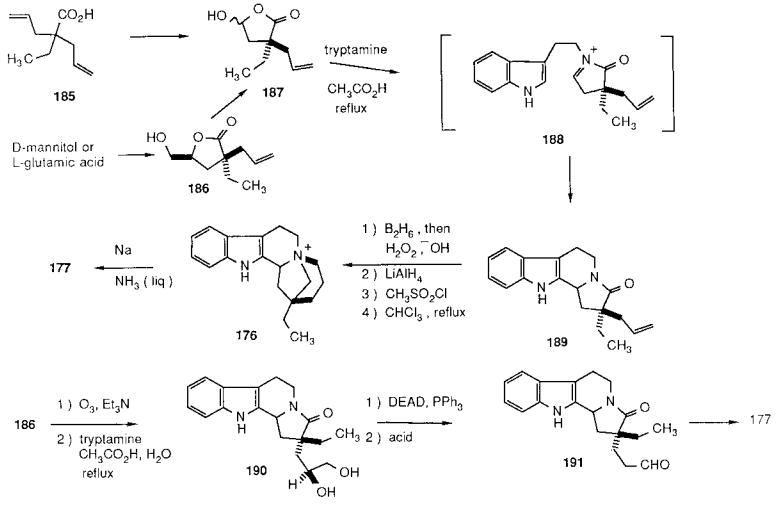
Takano and co-workers have utilized cyclization reactions of seven-membered ring *N*-acyliminium ions of type **174** to synthesize quebrachamine (**177**), tabersonine (**180**), and dihydrocleavamine (**184**) (*80, 81*). Hydroxide-induced ring opening of cyclohexanone **172,** followed by condensation with tryptamine, gave amide **173.** Reflux of this compound in aqueous acetonitrile containing excess methyl iodide led to formation of intermediate **174,** which cyclized to a 6:1 mixture of stereoisomers in 83% yield with **175** as the major product. In a four-step sequence, **175** was transformed via quaternary ammonium salt **176,** into





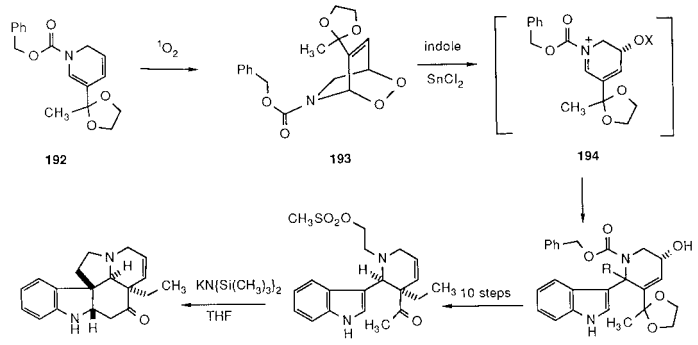
quebrachamine (**177**). Alternatively, **175** could be converted into unsaturated ammonium salt **178,** which provided access to tabersonine (**180**) (*80*). In a similar series of reactions, cyclohexanone **181** served as starting material for the synthesis of dihydrocleavamine (**184**) (*81*). Treatment of **182** with excess methyl iodide in aqueous acetonitrile at room temperature furnished a mixture of cyclization products with **183** as major isomer, formed in 39% yield.

Cyclization reactions involving five-membered ring *N*-acyliminium ions of type **188** have been employed by Takano and co-workers for the synthesis of racemic, (+)-, and (−)-quebrachamine (**177**) (*82–85*). Racemic **187,** obtained from diallylbutyric acid **185,** was condensed with tryptamine in refluxing acetic acid to give tetracycle **189** as a 1 : 1 mixture in 74% yield. By means of a five-



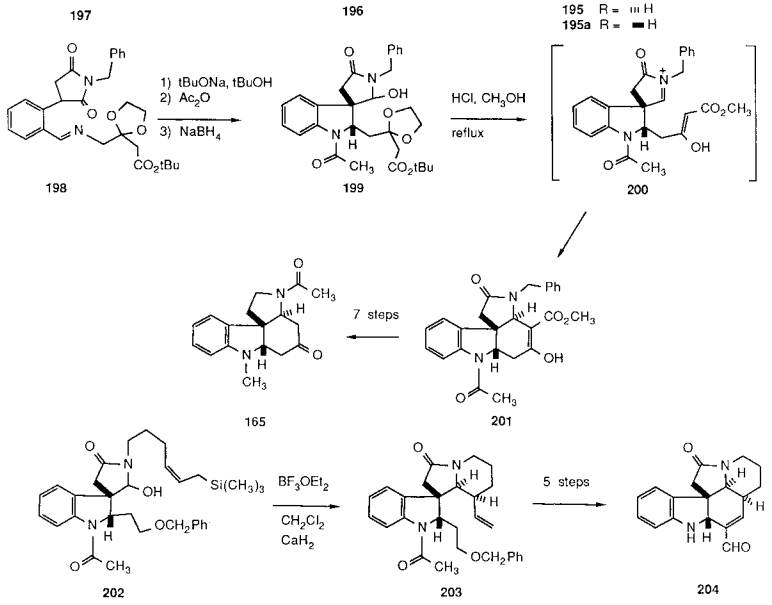
step sequence, **189** was readily transformed into racemic quebrachamine **177** (*85*). The same reactions were performed starting from enantiomerically pure **187,** obtainable from L-glutamic acid or D-mannitol, which led to (+)-quebrachamine (*83*). (−)-Quebrachamine was obtained from the same chiral starting materials by carrying out the crucial *N*-acyliminium ion cyclization with the aldehyde obtained after ozonolysis of **186.** Reaction with tryptamine in refluxing aqueous acetic acid produced a 1 : 1 mixture of **190** in about 50% from **186.** Rearrangement of the epoxide derived from diol **190** furnished aldehyde **191,** which, via the standard procedure, was converted into (−)-quebrachamine (*84*).

Natsume and co-workers have developed an interesting approach toward the aspidospermine skeleton starting from dihydropyridine **192** (*86, 87*). Singlet oxygen addition to **192,** followed by treatment with indole in the presence of stannous chloride furnished **195** in 57% yield. This remarkable sequence, in which *N*-acyliminium ion **194** probably occurs as intermediate, showed a surprisingly high regio- and stereoselectivity, as **195a** was the only by-product, formed in 2% yield. In 10 steps, **195** was transformed into **196,** which on treatment with base underwent two sequential cyclization reactions to produce the aspidospermine skeleton **197.**



Veenstra and Speckamp have developed a formal synthesis of vindorosine (**158**), in which an enol serves as nucleophile in an *N*-acyliminium ion cyclization reaction (*88*). The precursor **199** for this key cyclization reaction was obtained by means of a stereoselective spirocyclization of **198,** followed by *N*-acetylation and imide reduction to a 2 : 1 mixture of **199** and its regioisomer. Treatment of **199** with HCl in refluxing methanol for 30 min furnished **201** in 70% yield, through the intermediacy of **200.** Compound **201** was converted into known ketone **165,** which had been transformed in vindorosine (*74, 88*). In a different approach (*89*), tricycle **202** was synthesized in a similar fashion as **199.** Cyclization of **202** occurred with the addition of boron trifluoride etherate in

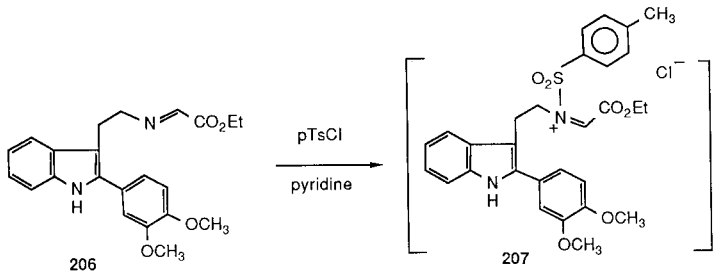
dichloromethane containing calcium hydride to produce a single stereoisomer **203** in 65% yield. In a five-step sequence **203** was transformed into pentacycle **204** (*89*).

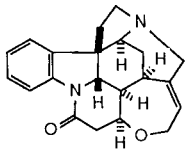


B. STRYCHNOS ALKALOIDS

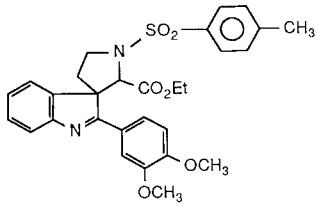
In the only, and outstanding, total synthesis of strychnine (**205**), by Woodward and co-workers (*90*), one of the crucial carbon–carbon bond formation steps is analogous to the topic discussed here. The Schiff base **206,** obtained through condensation of 2-veratryltryptamine with ethyl glyoxylate, underwent ring closure to **208** in 64% yield following treatment with *p*-toluenesulfonyl chloride in pyridine. *N*-Sulfonyliminium ion **207** probably serves as intermediate in this reaction. Its formation is related to the general reaction of imines with acyl chlorides, which is known to generate the *N*-acyliminium intermediate (*91*).

A promising synthetic approach to strychnine was published by van Tamelen *et al.* in 1960 (*92*). Hydroxylation and diol cleavage of **209** gave hydroxy amide **210.** When heating this compound in buffered aqueous acetic acid, *N*-acyliminium ion (**211**) formation occurred, followed by double cyclization via indolenium ion **212** to pentacycle **213.** An interesting aspect of this *N*-acyliminium cyclization is the fact that only the product arising from reaction at the indole β-carbon atom is obtained. It has been argued that this outcome is determined by





205 strychnine



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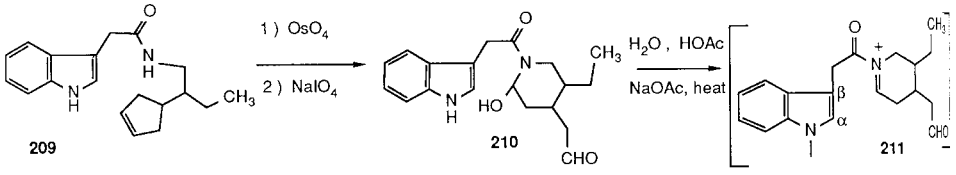
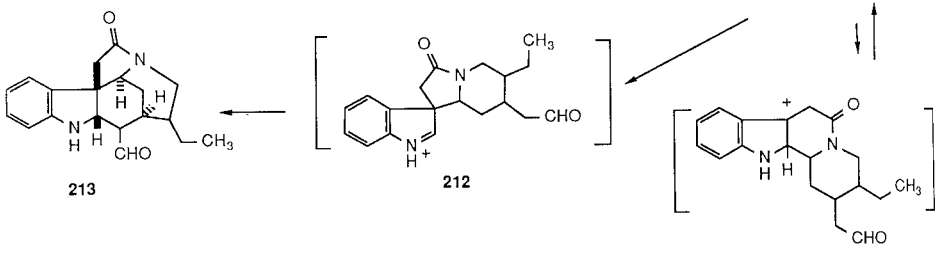
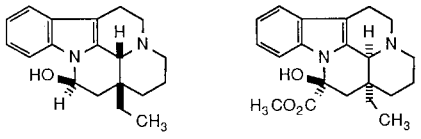


209 210 211



213 212 214

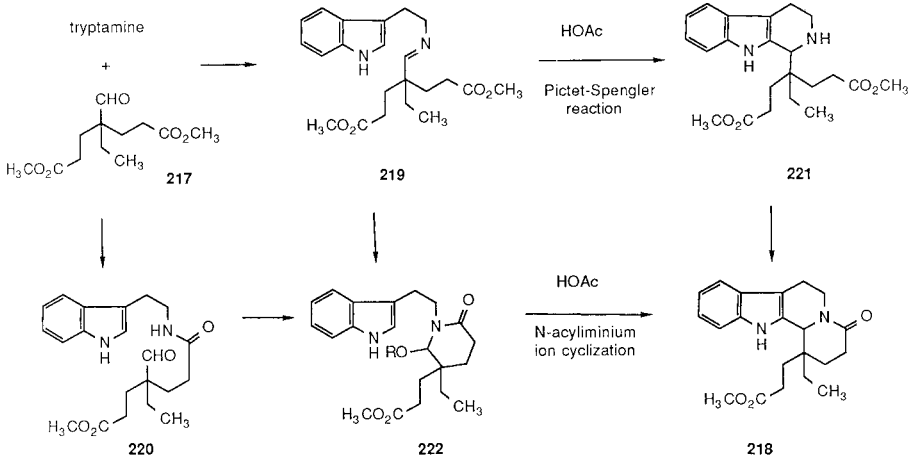


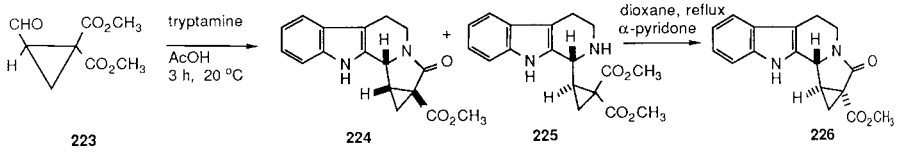
215 eburnamine 216 vincamine

thermodynamics, i.e., the γ-lactam ring in **212** is more favorable than the δ-lactam ring in **214** (*93*).

C. Eburnamine Alkaloids

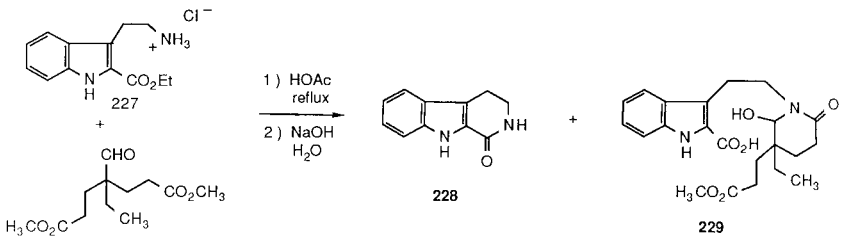
The best-known natural products of the class of eburnamine alkaloids (*94*) are eburnamine itself (**215**) and vincamine (**216**). The first total synthesis of vincamine was achieved by Kuehne in 1964 (*95*). The key step in this synthesis is the condensation of tryptamine with diester aldehyde **217** to tetracyclic lactam **218.** The mechanism of this transformation is an interesting point of discussion in this context. The first step can either be formation of imine **219** or amide **220.** The imine **219** may then undergo an ordinary Pictet–Spengler cyclization by means of its iminium salt to secondary amine **221,** which cyclizes to lactam **218.** Alternatively, imine **219** may give an intramolecular imine acylation to hydroxy or methoxy lactam **222.** This compound arises from **220** by way of a simple intramolecular reaction of the aldehyde. Next, conversion of **222** into **218** occurs through the intermediacy of an *N*-acyliminium ion. The actual mechanism of the conversion **217** → **218** is unknown, but intuitively, the path by means of **219** and **221** looks the most probable. Imine formation occurs under milder conditions than amide formation, and the high nucleophilicity of the indole ring renders the Pictet–Spengler cyclization an easy reaction. Evidence is obtained from the work of Becker *et al.* (*96*), which involves the condensation of tryptamine with diester aldehyde **223.** Under very mild conditions (3 hr at room temperature in acetic acid), a 1 : 4 mixture of **224** and **225** is obtained in quantitative yield. In refluxing dioxane in the presence of α-pyridone, **225** cyclized quantitatively into **226.**





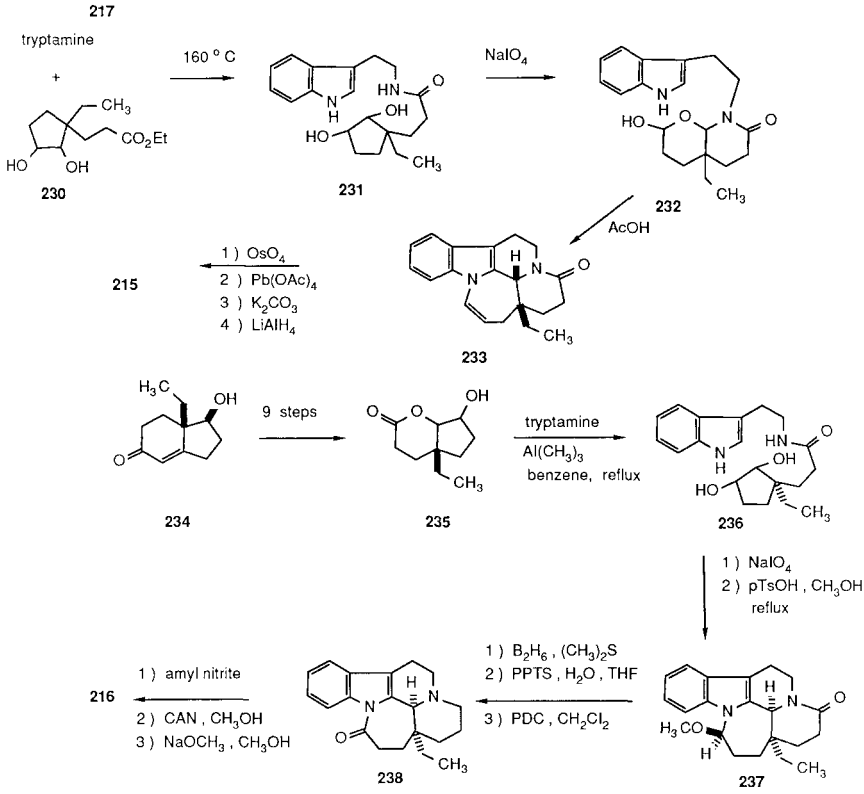
Thus, in the case of aldehyde **223,** the formation of **226** certainly proceeds by means of an ordinary Pictet–Spengler cyclization. Therefore, condensation of tryptamine with ester aldehydes leading to lactams of type **218, 224,** and **226** will not be further discussed in this review, although this method has frequently been utilized, especially in the (hetero)yohimbane field. Only those syntheses which certainly involve *N*-acyliminium intermediates will be discussed here.

Szántay and co-workers have performed Kuehne's reaction (*95*) with tryptamine derivative **227,** which cannot give ring closure on the indole nucleus (*97*). After refluxing for 120 hr in acetic acid, followed by mild saponification, a mixture of the unwanted by-product **228** (45%) and the *N*-acyliminium ion precursor **229** (36%) was obtained. When **229** was refluxed in acetic acid for 20 hr, decarboxylation occurred, followed by *N*-acyliminium ion cyclization to tetracycle **218** as a 1 : 1 mixture of stereoisomers (*97*). Kuehne has transformed **218** into vincamine (**216**) in a few steps (*95*).



Eburnamine (**215**) has been synthesized by Gibson and Saxton (*98*) by utilizing the van Tamelen (*92*) procedure for the preparation of the *N*-acyliminium ion precursor. Thus, condensation of tryptamine with diol **230** produced amide **231,** which was subjected to diol cleavage, providing **232** as a mixture of stereoisomers. After heating this crude mixture in acetic acid at 55–60°C for 65 hr, ring closure took place to produce **233** and its epimer in 57% yield from **231** as a nearly 1 : 1 mixture, which was separated using chromatography. Pure epimer **233** was transformed in four steps into eburnamine.

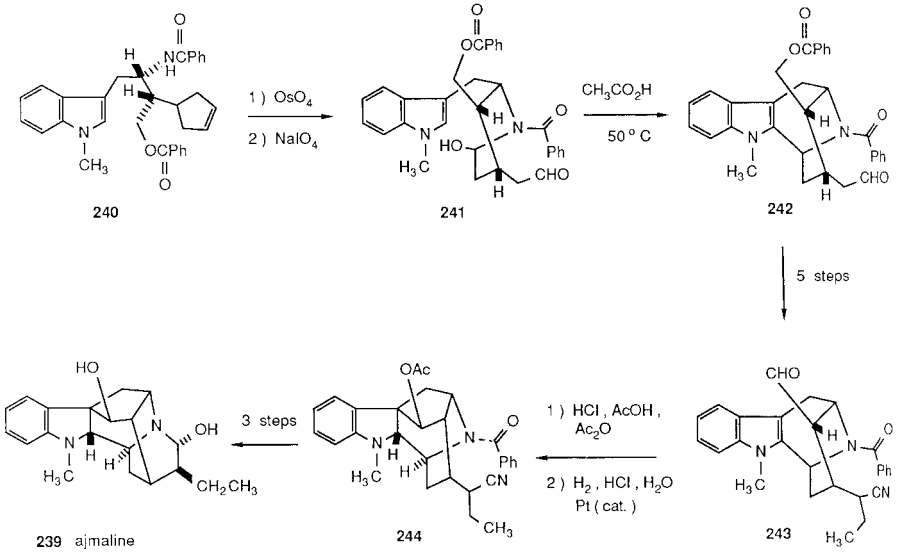
Enantiomerically pure (+)-vincamine (**216**) was prepared by Takano and coworkers (*99*) by using the same *N*-acyliminium ion cyclization reaction as Gibson and Saxton (*98*), but starting from enantiomerically pure and readily available enone **234.** This was converted in nine steps into lactone **235,** which on



condensation with tryptamine, yielded diol **236.** Diol cleavage and *N*-acyliminium ion cyclization induced by *p*-toluenesulfonic acid in methanol furnished **237** (34%) and its epimer (28%). From **237,** another six steps were required to arrive by means of **238** at (+)-vincamine (*99, 100*).

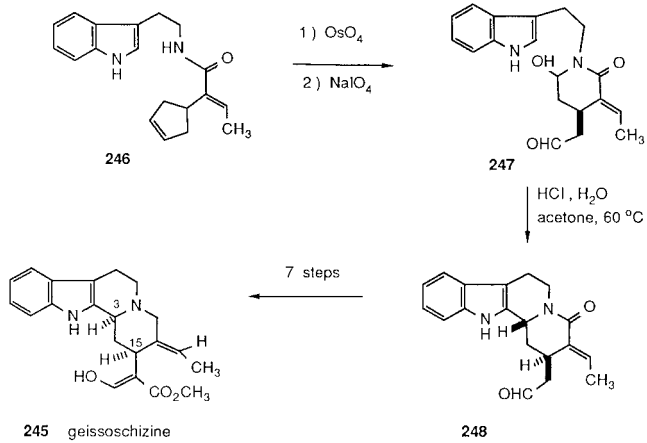
D. Heteroyohimbane Alkaloids

The first total synthesis of ajmaline (**239**) was achieved by Masamune and coworkers in 1967 (*101*), by utilizing van Tamelen's methodology for the synthesis of an *N*-acyliminium ion, starting with oxidative cleavage of a cycloalkene double bond (*102*). Treatment of cyclopentene **240** with osmium tetroxide, followed by sodium metaperiodate, gave the *N*-acyliminium precursor **241** in quantitative yield. When **241** was warmed in acetic acid at 50°C for 1 hr, tetracyclic aldehyde **242** was obtained in 40% yield. This β-carboline synthesis is different from most other synthetic routes to β-carbolines involving *N*-acyliminium ion chemistry. Usually, the amide carbonyl function is endocyclic, whereas in this



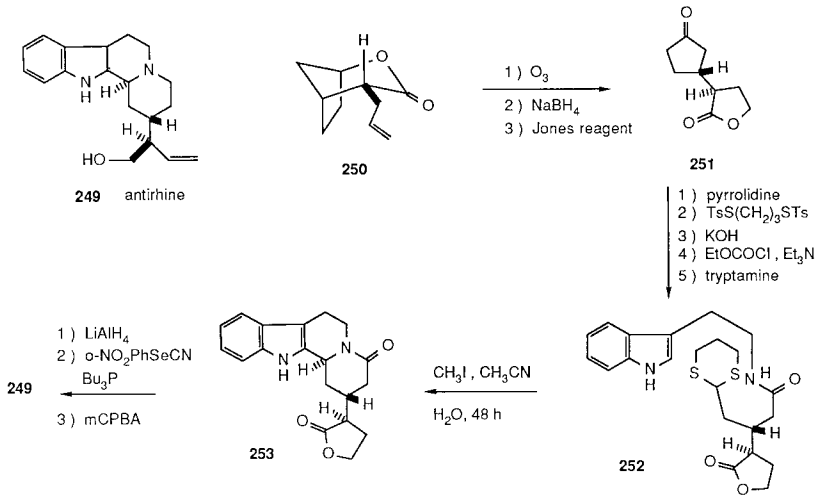
synthesis, the carbonyl group is exocyclic. The next important ring closure reaction was performed with aldehyde **243,** which after acid treatment followed by hydrogenation was converted into pentacycle **244.** The synthesis of ajmaline was completed through the removal of the protecting groups and the closure of the sixth ring (*101*).

The synthesis of geissoschizine (**245**) by Yamada *et al.* (*102*), proceeded by way of oxidative cleavage of the double bond of cyclopentene **246,** followed by *N*-acyliminium ion cyclization. Thus, successive treatment of **246** with osmium



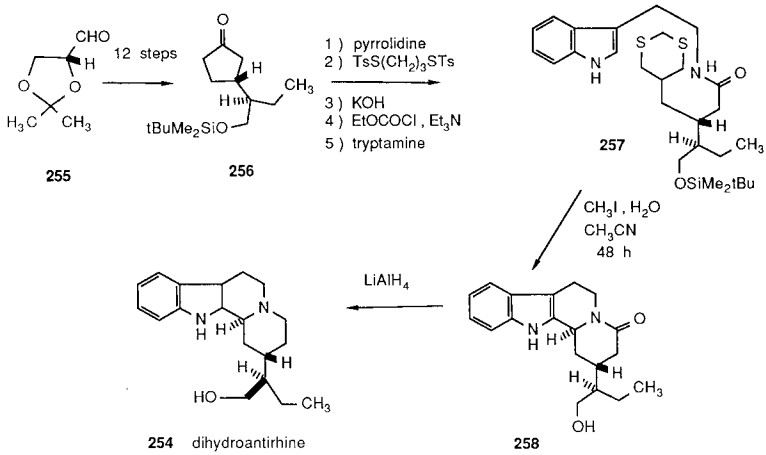
tetroxide and metaperiodate furnished hydroxy lactam **247,** which cyclized, after stirring for 30 min at 60°C in acetone/0.05N HCl, to tetracycle **248** in 62% yield from **246.** Seven more steps, involving elaboration of the aldehydic side chain, reduction of the amide carbonyl group, and epimerization at C3 provided racemic geissoschizine (*102*).

Antirhine (**249**) was synthesized by Takano *et al.* from racemic norcamphor (*103*). In a similar fashion as shown for Takano's emetine synthesis (*11*), norcamphor was transformed into **250** and then, by way of **251,** into *N*-acyliminium ion precursor **252.** When **252** was treated with methyl iodide in aqueous acetonitrile at room temperature for 48 hr, hydrolysis of the dithiane occurred, followed by *N*-acyliminium ion formation and ring closure, to furnish **253** in 37% yield. Reduction with $LiAlH_4$ and elimination of the least-hindered hydroxyl function led to racemic antirhine. Since norcamphor is available in optically active form, the synthesis of natural antirhine is now possible (*103*).

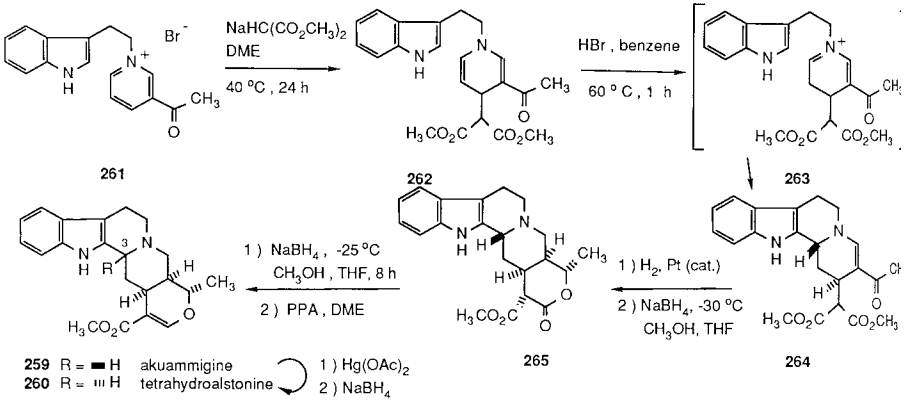


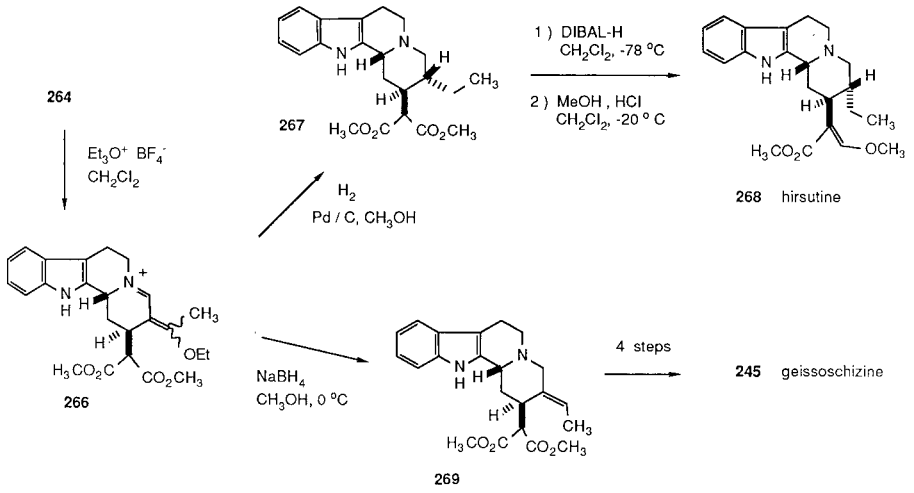
Kametani *et al.* have achieved the synthesis of one enantiomer of dihydroantirhine (**254**) from (*R*)-1,2-isopropylideneglyceraldehyde (**255**) (*104*). From **256,** obtained in 12 steps from **255,** this synthesis proceeded in exactly the same manner as Takano's synthesis (*103*). The *N*-acyliminium ion cyclization of **257** to **258** occurred in an unspecified yield. $LiAlH_4$ reduction of **258** resulted in optically active dihydroantirhine (*104*). Recently, this methodology was applied also to the synthesis of (−)-antirhine (**249**) (*105*).

Wenkert and co-workers have developed a synthetic route to various heteroyohimbane alkaloids (*106–108*), based on the addition of the malonate anion to pyridinium salt **261,** producing dihydropyridine **262,** and subsequent acid-in-



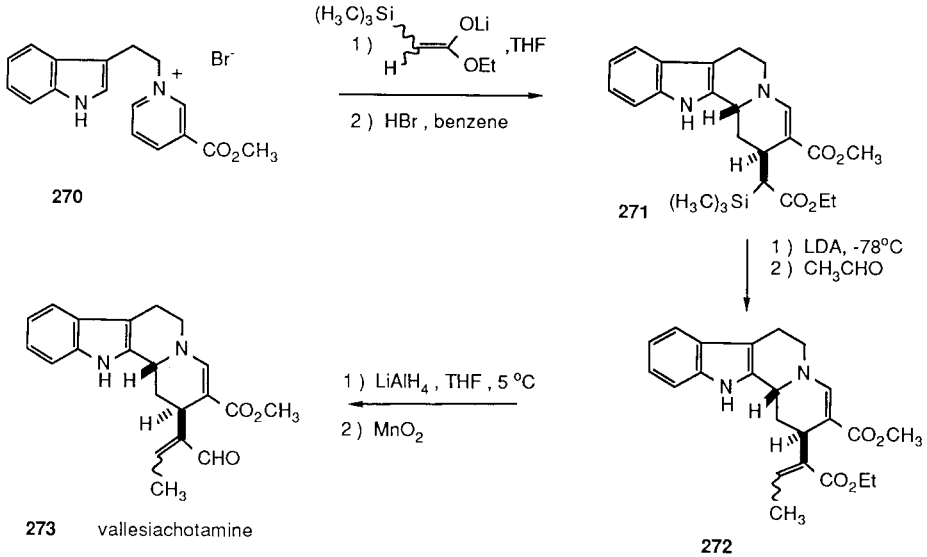
duced ring closure to tetracycle **264.** This cyclization proceeds most likely through the intermediacy of vinylogous *N*-acyliminium ion **263.** The overall yield of **264** was around 28% from **261.** Vinylogous amide **264** appeared to be a convenient starting material for various alkaloids, such as akuammigine (**259**), tetrahydroalstonine (**260**), hirsutine (**268**), and geissoschizine (**245**). Thus, hydrogenation of **264** occurred primarily from the α-face, and mild $NaBH_4$ reduction of the product gave **265** in 33% overall yield. Reduction of this lactone to the lactol, followed by polyphosphoric acid-mediated dehydration created akuammigine (**259**) (*107*). Epimerization at C3, by way of the iminium salt, furnished tetrahydroalstonine (**260**) (*107*). Treatment of **264** with triethyloxonium tetrafluoroborate led to salt **266** which, following hydrogenation, provided **267** in 74% yield from **264.** Reduction to the aldehyde, followed by methanolysis,





furnished hirsutine (**268**) (*108*). $NaBH_4$ reduction of salt **266** gave **269,** which could be converted into geissoschizine (**245**) (*108*).

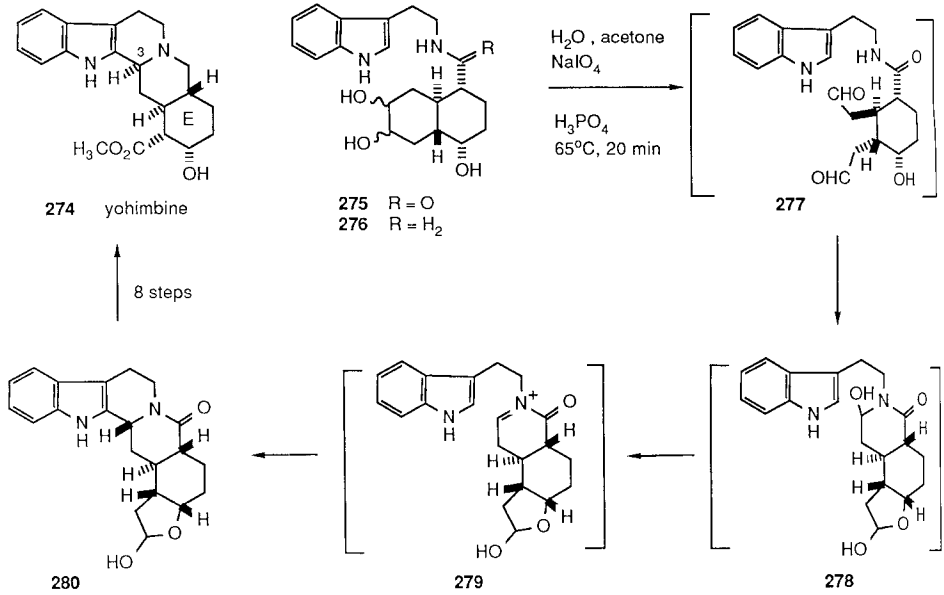
Recently, vallesiachotamine (**273**) was synthesized along similar lines (*109*). Addition of the lithium enolate of methyl trimethylsilylacetate to pyridinium salt **270,** followed by ring closure with HBr-furnished **271** in 47%. Peterson olefination, selective ester reduction, and MnO_2 oxidation then gave the natural product (*109*).



It should be noted here that all cyclization reactions leading to the β-carboline skeleton discussed here (**247** → **248**, **252** → **253**, **257** → **258**, **262** → **264**, and **270** → **271**) show the same stereochemical course. The indole nucleophile always attacks the *N*-acyliminium ion trans with respect to the C15 substituent (see **245** for numbering).

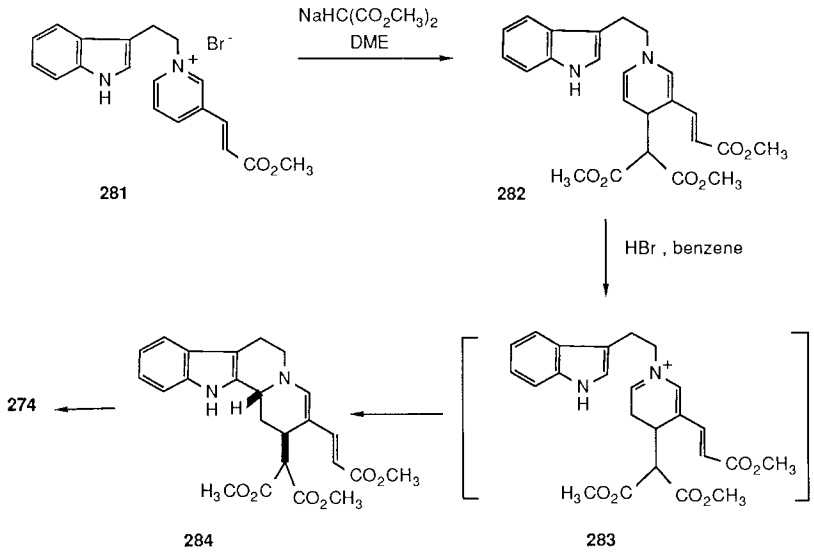
E. Yohimbane Alkaloids

The total synthesis of yohimbine (**274**) by van Tamelen and co-workers in 1958 (*110*) constitutes the first successful application of *N*-acyliminium chemistry in natural product synthesis. Triol amide **275** served as the precursor for the crucial cyclization reaction. Treatment of **275** with sodium periodate, immediately followed by phosphoric acid at 60–70°C for 20 min in a water–acetone mixture, furnished hexacyclic **280** in 60% yield after crystallization. This remarkable process most likely proceeds by way of dialdehyde **277,** hydroxy lactam **278,** and *N*-acyliminium ion **279.** The stereochemistry of **280** is the result of axial addition to the preferred chair-like conformation of the iminium moiety. It is interesting to note that the original plan of van Tamelen *et al.* envisioned the use of an ordinary Pictet–Spengler cyclization starting from amine **276.** However, this amine appeared to be inaccessible by synthesis, thus inducing van Tamelen's pioneering studies in the field of *N*-acyliminium chemistry. The remaining steps of the yohimbane synthesis from **280** involved eight steps, including in-



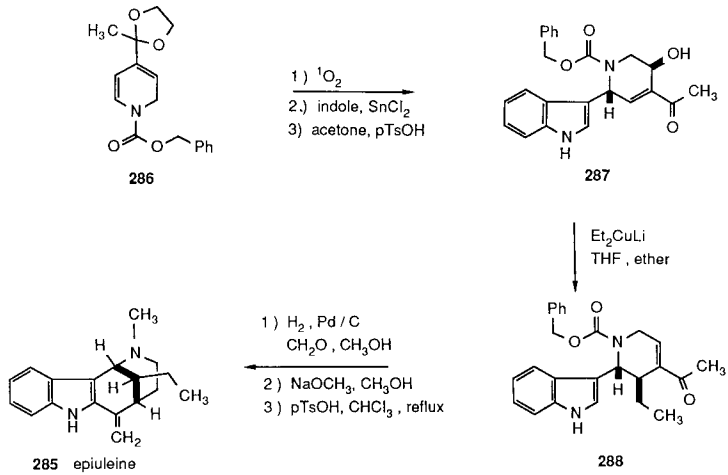
stallation of the correct substituent in the E-ring by way of double-bond cleavage of a dihydrofuran, removal of the amide carbonyl, and isomerization at C3 (*110*).

About 20 years later, Wenkert and co-workers described a synthesis of yohimbine (**274**) and cogeners, in which vinylogous *N*-acyliminium ion **283** was the key intermediate (*111*). Addition of dimethyl sodiomalonate to pyridinium salt **281** in DME led to 1,4-dihydropyridine **282.** This product was not isolated, but directly treated with a saturated solution of HBr in benzene to furnish tetracycle **284** via **283** in 18% overall yield from **281.** Triester **284** could be converted in a few steps (including hydrogenation, decarbomethoxylation, and Dieckman cyclization of the E ring) into yohimbine and its various natural stereoisomers (*111, 112*).



F. Remaining Indole Alkaloids

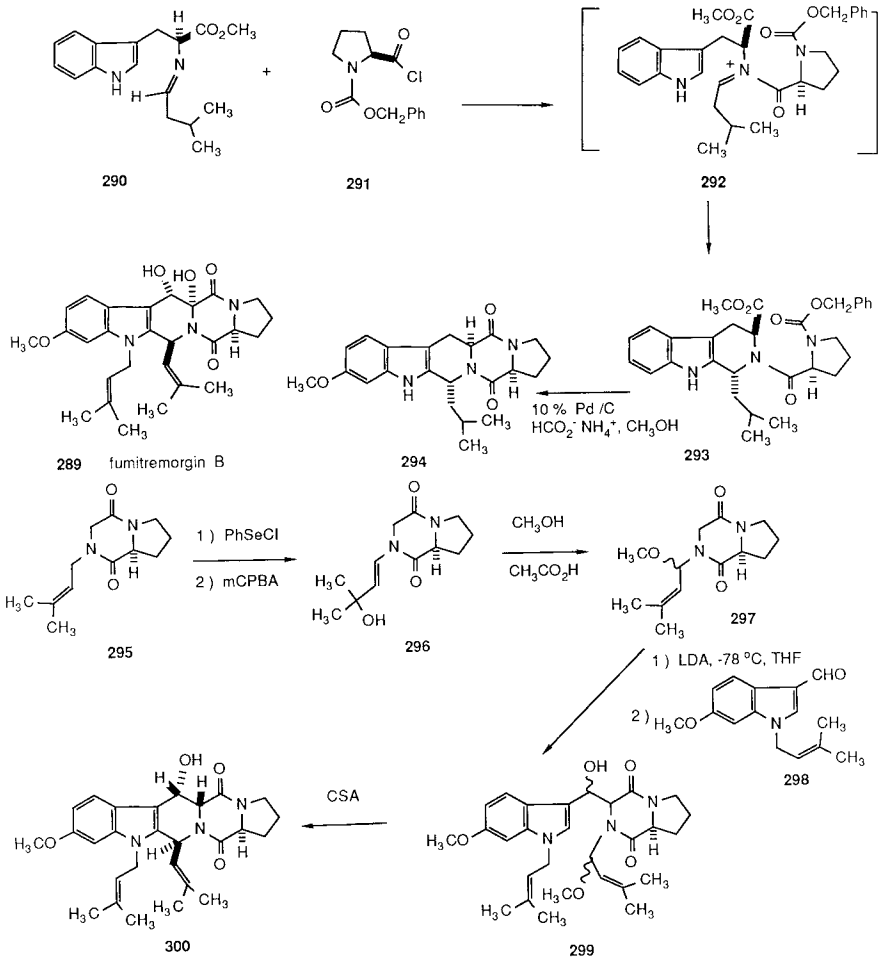
Natsume and Kitagawa have reported the synthesis of 3-epiuleine (**285**) from dihydropyridine derivative **286** (*86, 113*). In a similar fashion as was discussed in Section III,A, cycloaddition of singlet oxygen to **286,** followed by treatment with indole in the presence of $SnCl_2$, and acetal hydrolysis provided **287** as a single stereoisomer in good yield. Treatment of **287** with lithium diethylcuprate gave rise to an addition–elimination sequence furnishing enone **288,** once again as a single isomer in 60% yield. Three more steps (hydrogenation in the presence of formaldehyde, isomerization of the acetyl group, and ring closure) were required to complete an elegant synthesis of 3-epiuleine (**285**) (*113*).



286 287 288 285 epiuleine

The skeleton of fumitremorgin-B (**289**) has recently been synthesized by two Japanese research groups by means of *N*-acyliminium ion chemistry. Nakagawa *et al.* (*114*) treated the imine **290,** derived from L-tryptophan and isovaleraldehyde, with *N*-protected L-prolyl chloride **291** to provide as the major product, the *trans*-disubstituted tetrahydro-β-carboline **293** in 41% yield. The *N*-acyliminium ion **292** is the key intermediate in this reaction, which proceeded without detectable racemization. Interestingly, the corresponding Pictet–Spengler cyclization of **290** was attended with racemization, unless strong acid (trifluoroacetic acid) was used, and created a mixture of stereoisomers with the *cis* product predominating. Hydrogenolysis of **293** furnished pentacycle **294** (*114*). Nakatsuka *et al.* (*115*) used diketopiperazine **295** as starting material and converted it into *N*-acyliminium ion precursor **297** by way of allylic alcohol **296.** Selective deprotonation of **297** at the less-substituted position, followed by addition of indole aldehyde **298,** produced **299** in 59% as a mixture of four stereoisomers. Treatment of this mixture with camphorsulfonic acid in dichloromethane at room temperature provided pentacycle **300** as a single stereoisomer in 80% yield (*115*). Very recently, a slightly modified approach was published (*115a*) which led to the successful synthesis of fumitremorgin-B (**289**). Related *N*-acyliminium cyclizations in this area have been reported by O'Malley and Cava (*115b*).

In earlier work, Nakatsuka *et al.* (*116*) treated the lithium enolate of **301** with indole aldehyde **302** and obtained after dehydration **303** as a 10 : 1 mixture of *Z* and *E* isomers in 85% yield. Hydrogenation of **303,** followed by *N*-acyliminium ion cyclization of **304** occurred uneventfully to give tetrahydro-β-carboline **306** in 92% yield. However, when *Z* (or *E*) **303** was subjected to acidic conditions, ring closure took place at the indole C4-position to give compound **305,** in which has the same skeleton as clavicipitic acid (**307**) in 88% yield. The main reason

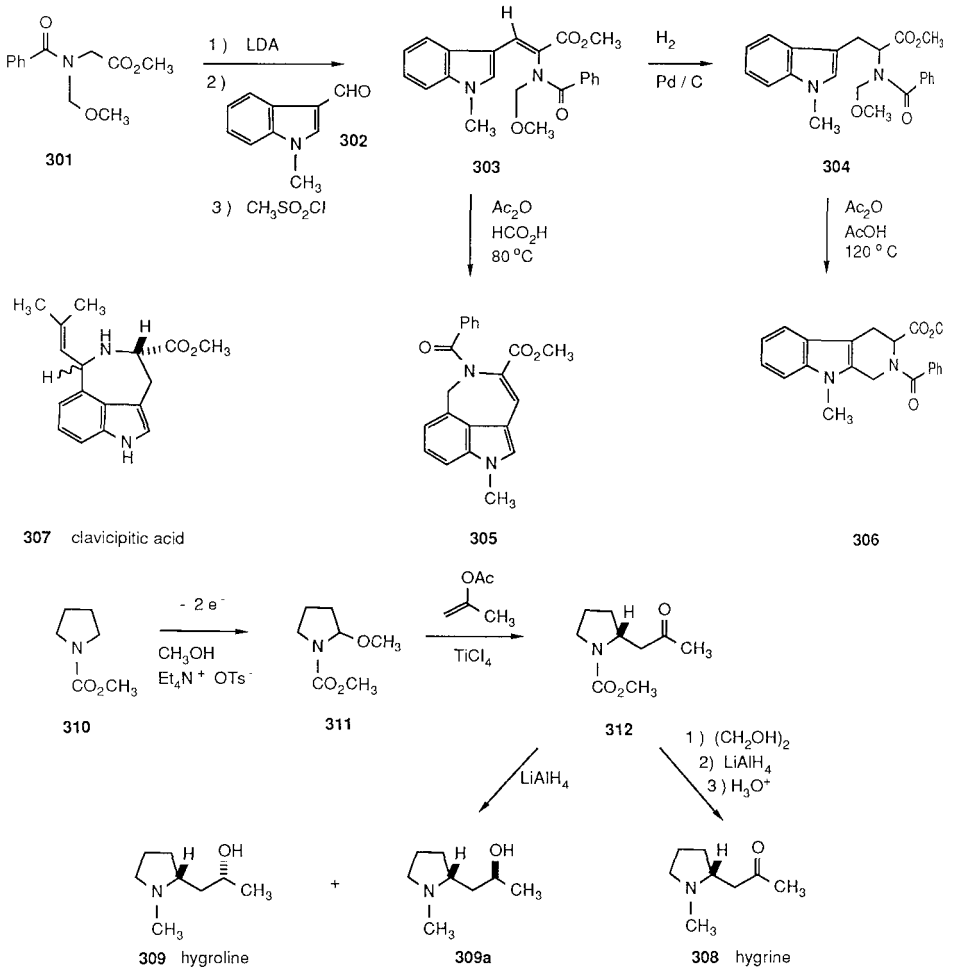


for the cyclization behavior of **303** is probably the reduced nucleophilicity of the indole C2-position as a result of conjugation with the ester function.

IV. Pyrrolidine, Piperidine, and Tropane Alkaloids

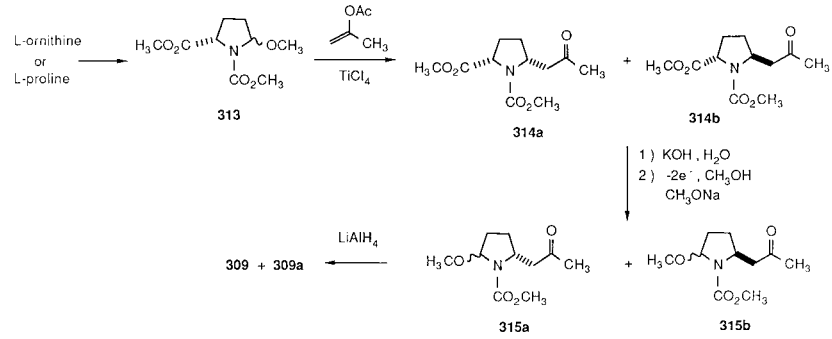
A. Pyrrolidine Alkaloids

The simple pyrrolidine alkaloids (*117*), hygrine (**308**) and hygroline (**309**), have been synthesized by Shono *et al.* (*118*) from pyrrolidine in an expeditious fashion. Anodic oxidation of *N*-carbomethoxypyrrolidine (**310**) furnished the

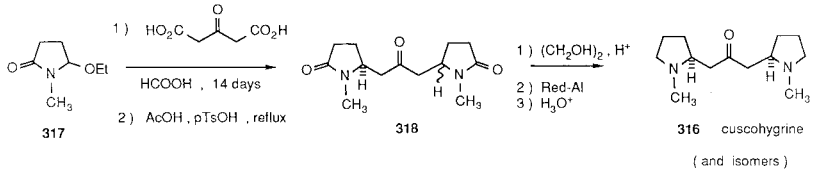


methoxy carbamate **311** in 80% yield. An *N*-acyliminium ion reaction with isopropenyl acetate, induced by $TiCl_4$ in dichloromethane at 0°C, produced ketone **312** in 84% yield, from which **308** and **309** (as a separable 1 : 1 mixture with **309a**) were obtained through standard methods (*117*). Hygroline (**309**) was prepared in optically active form by starting with L-ornithine or L-proline (*119*). From either of these amino acids, methoxy carbamate **313** was obtained as a separable 1 : 1 mixture of isomers in a few steps, including anodic oxidation in methanol. Reaction with isopropenyl acetate, as described before, furnished in 85% yield a 7 : 3 mixture of **314a** and **314b.** This ratio appeared to be independent of the stereochemistry of the starting material, thus indicating a genuine

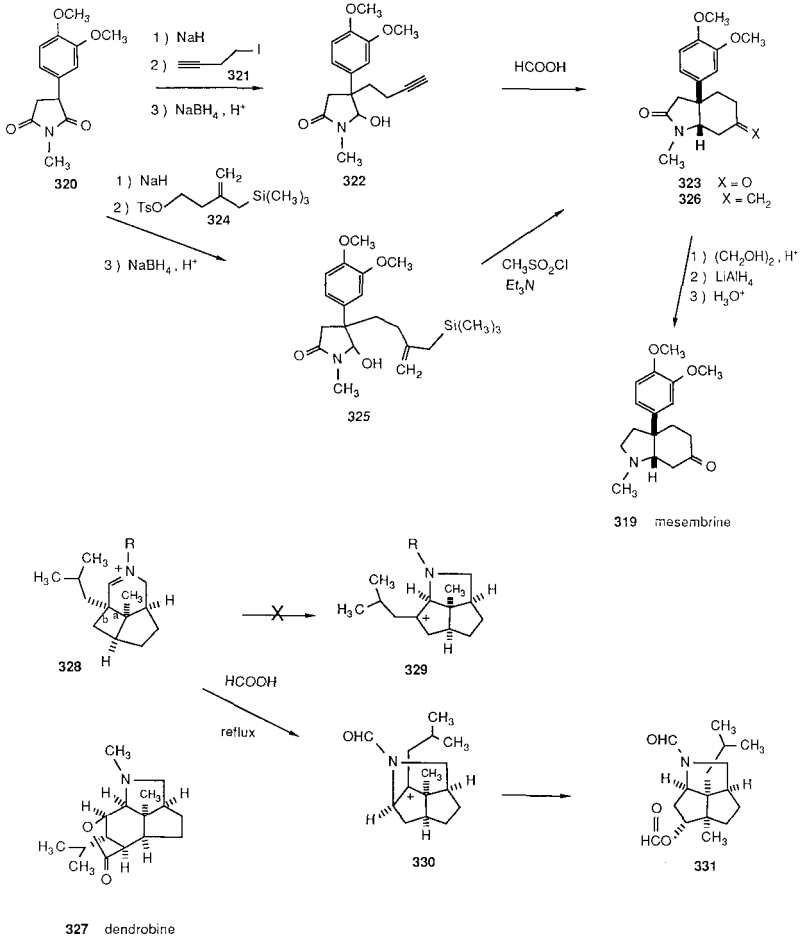
S_N1-type mechanism. Interestingly, the *cis*-substituted pyrrolidine is the major product, but the stereoselectivity is low (40%). The mixture of **314a** and **314b** was saponified and then subjected to anodic decarboxylation to give **315a** and **315b.** Subsequent $LiAlH_4$ reduction furnished **309** and **309a,** both with an enantiomeric purity of around 40% (*119*).



De Boer and Speckamp have synthesized cuscohygrine (**316**) from *N*-methylsuccinimide (*120, 121*). Acid-assisted $NaBH_4$ reduction followed by ethanolysis gave ethoxy lactam **317.** An *N*-acyliminium ion reaction with acetonedicarboxylic acid and subsequent decarboxylation led in 74% yield to a 1 : 1 mixture of racemic and meso ketone **318,** which could be separated via recrystallization. From meso **318,** cuscohygrine (**316**) was obtained by using standard methods.



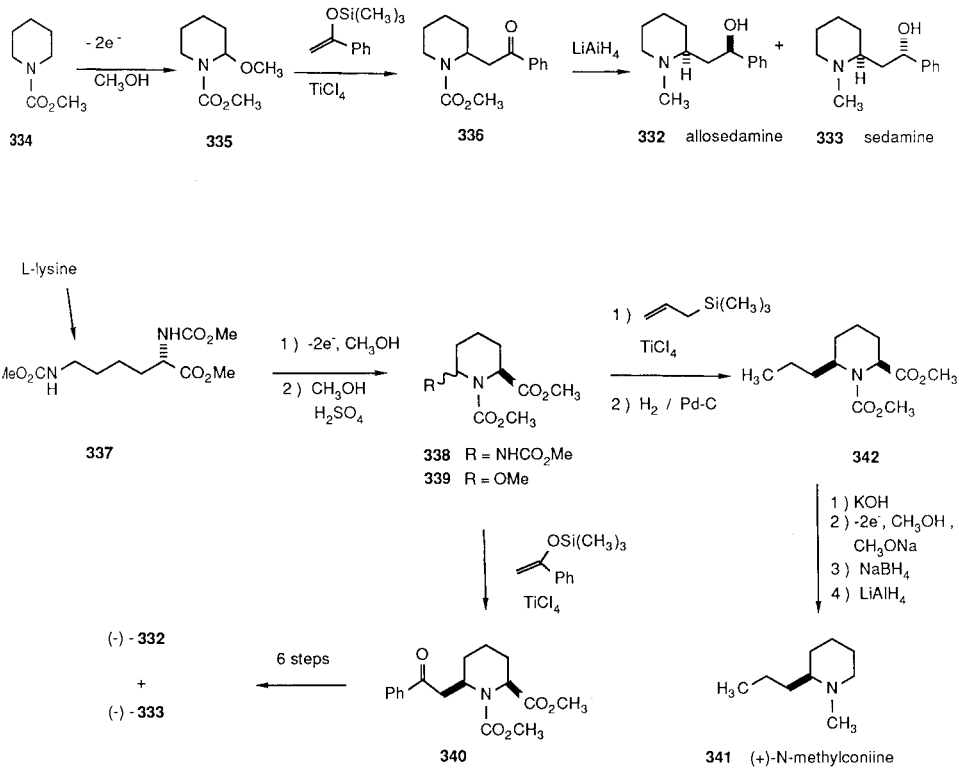
Mesembrine (**319**) has been obtained from imide **320** in two different ways (*122, 123*). Alkylation of **320** with iodobutyne **321** and allyl silane **324,** followed by $NaBH_4$ reduction, led to hydroxy lactams **322** and **325,** respectively. Formic acid-induced cyclization of **322** furnished ketone **323** in 85% yield after 5 days (*122*). Cyclization of **325** was successful by using more neutral conditions to give olefin **326** in 80% yield (60% conversion) (*123*). Ozonolysis of **326** and reductive workup also produced **323,** which was converted into mesembrine by standard methods.



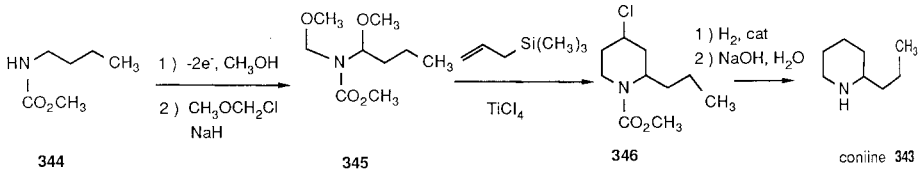
Connolly and Heathcock have published an attempt to synthesize dendrobine (**327**) by using as the key step the iminium variant of the cyclobutylcarbinyl rearrangement (*124*). It appeared that ordinary iminium ion **328** (R = CH_3) was unwilling to rearrange under a variety of conditions. The more reactive *N*-acyliminium ion **328** (R = CHO) rearranged readily in formic acid. Unfortunately, migration of cyclobutane bond *a* to the desired ring system **329** did not take place at all. Exclusive migration of bond *b* occurred to produce **330,** which, in refluxing formic acid by means of two more shifts, led to formate **331** in 55% yield.

B. Piperidine Alkaloids

The piperidine alkaloids (*125*) allosedamine (**332**) and sedamine (**333**) have been synthesized by Shono and co-workers (*118*) from *N*-carbomethoxypiperidine (**334**). Anodic oxidation in methanol afforded **335,** which on Lewis acid-catalyzed reaction with α-trimethylsilyloxystyrene, led to ketone **336** in 70% overall yield. Reduction of **336** with $LiAlH_4$ gave a 1 : 1 mixture of **332** and **333.** The (−)-enantiomers of these alkaloids have been synthesized by Irie *et al.* (*126*) from L-lysine. Anodic oxidation of **337** in methanol produced cyclized carbamate **338,** which on methanolysis gave **339** in 50% overall yield. $TiCl_4$-induced reaction with α-(trimethylsilyl)oxystyrene at −50°C in dichloromethane furnished a 10 : 1 mixture of cis-disubstituted piperidine **340** and its trans diastereomer in 64% yield. The cis isomer **340** was then converted in six steps into enantiomerically pure (−)-allosedamine (**332**) and (−)-sedamine (**333**). Shono *et al.* have synthesized (+)-*N*-methylconiine (**341**) from **339.** Reaction of **339** with allyltrimethylsilane in the presence of $TiCl_4$ in dichloromethane, followed by

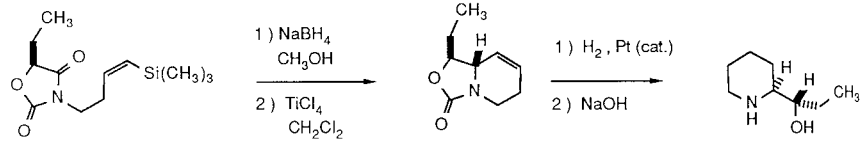


hydrogenation, led to **342** in 68% overall yield. In this *N*-acyliminium ion reaction, exclusive formation of the cis-disubstituted piperidine was observed. By means of successive saponification, anodic decarboxylation, and reduction, ester **342** was transformed into the pure (+)-enantiomer of *N*-methylconiine (**341**) (*119*). Racemic coniine (**343**) has been synthesized from carbamate **344.** Anodic oxidation of **344** in methanol, followed by methoxymethylation of nitrogen, gave **345** in 62% overall yield. An interesting double *N*-acyliminium ion reaction was employed to construct the piperidine ring. Thus, treatment of **345** with allytrimethylsilane and $TiCl_4$ in dichloromethane created **346** as an 85 : 15 mixture of isomers in 75% yield. Two trivial steps completed the synthesis of coniine (**343**) (*127*).



344 345 346 coniine 343

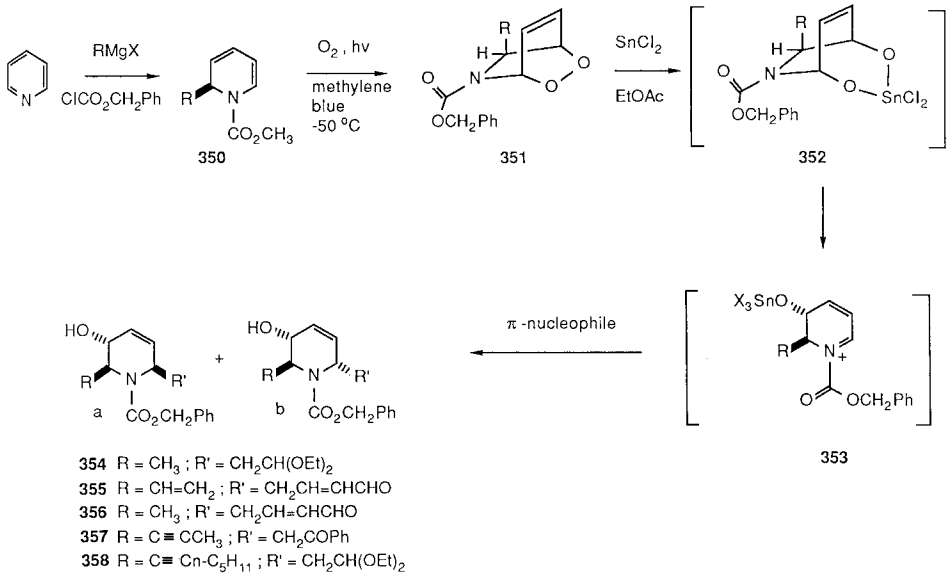
Kano and co-workers have achieved a synthesis of β-conhydrine (**347**) by employing an *N*-acyliminium cyclization reaction with a vinylsilane as π-nucleophile (*128*). Thus, $NaBH_4$ reduction of **348** and treatment of the resulting hydroxy lactam with $TiCl_4$, furnished **349** as a single diastereomer in 70% yield. Hydrogenation and hydrolysis completed the synthesis of β-conhydrine.



348 349 347 β-conhydrine

Natsume and co-workers have developed an elegant route to various piperidine alkaloids through highly selective multifunctionalization of pyridine (*86*). Grignard addition to pyridine, quaternized with benzyl chloroformate, furnished 2-substituted dihydropyridines **350** in high yield. Singlet oxygen cycloaddition to **350** occurred with complete stereoselectivity to give endoperoxides **351,** which were directly treated with a π-nucleophile in the presence of stannous chloride affording the alkylation products **354–358.** The tin compound **352** and the *N*-acyliminium ion **353** are presumed to be intermediates in this process (*129*). The formation of **354–358** from **351** occurs with remarkably high regio- and stereoselectivity. From **350** (R = CH_3), **354a** was prepared as the sole product in 50% yield by using ethyl vinyl ether as nucleophile and ethanolytic workup

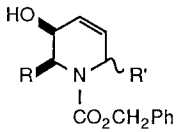
(*130*). Compound **358a** was obtained in a similar fashion (*131*) in 66% yield as the sole product from **350** (R = $C_5H_{11}C{\equiv}C$). 1-(Trimethylsilyl)oxybuta-1,3-diene was used as nucleophile to convert **350** (R = C_2H_3) into **355a** and **355b** as a 66 : 34 mixture in 58% yield (*132*), and to convert **350** (R = CH_3) into **356a** and **356b** as a 79 : 21 mixture in 43% yield (*133*). With α-(trimethylsilyl)oxystyrene as nucleophile **350** (R = $CH_3C{\equiv}C$) was transformed into a 84 : 16 mixture of **357a** and **357b** in 64% yield (*131*).



354 R = CH_3 ; R' = $CH_2CH(OEt)_2$
355 R = $CH{=}CH_2$; R' = $CH_2CH{=}CHCHO$
356 R = CH_3 ; R' = $CH_2CH{=}CHCHO$
357 R = $C{\equiv}CCH_3$; R' = CH_2COPh
358 R = $C{\equiv}Cn\text{-}C_5H_{11}$; R' = $CH_2CH(OEt)_2$

Compounds **354–358** proved to be excellent starting materials for the synthesis of a wide variety of piperidine alkaloids. Most of these syntheses begin with the highly selective inversion of the hydroxyl function to **359** by way of simple oxidation and reduction. Carpamic (**360**) and azimic acid (**361**) were synthesized from **354a** in eight steps, including elaboration of the side chain using a Wittig reaction (*130*). Pseudocarpamic acid (**362**) was synthesized in a similar fashion from **356b** (*133*), and prosafrinine (**363**) from **356a** (*133*). Compound **355a** was the starting material for the synthesis of prosophylline (**364**). This synthesis involved Wittig chemistry for the construction of the C_{12}-substituent and sequential use of $KMnO_4$, $NaIO_4$, and $NaBH_4$ for conversion of the vinyl substituent into a hydroxymethyl group (*132*).

The *Sedum* alkaloids sedacryptine (**365**) and sedinine (**366**) were synthesized from **357a** (*131, 134*). Crucial steps in both syntheses were the mercuric sulfate-induced regioselective hydration of the triple bond, assisted by a *cis*-oriented hydroxyl function (see **359**). For the synthesis of sedinine, the double bond in



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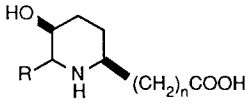
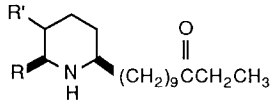


360 R = ▬ CH_3; n = 7 carpamic acid

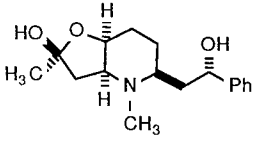
361 R = ▬ CH_3; n = 5 azimic acid

362 R = ≡ CH_3 ; n = 7 pseudocarpamic acid

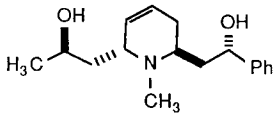


363 R = CH_3 ; R' = ▬ OH prosafrinine

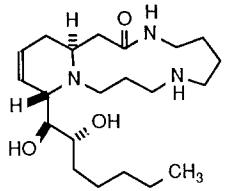
364 R = CH_2OH ; R' = ≡ OH prosophylline



365 sedacryptine



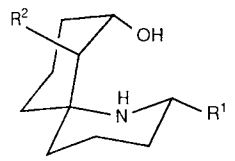
366 sedinine



367 cannabisativine

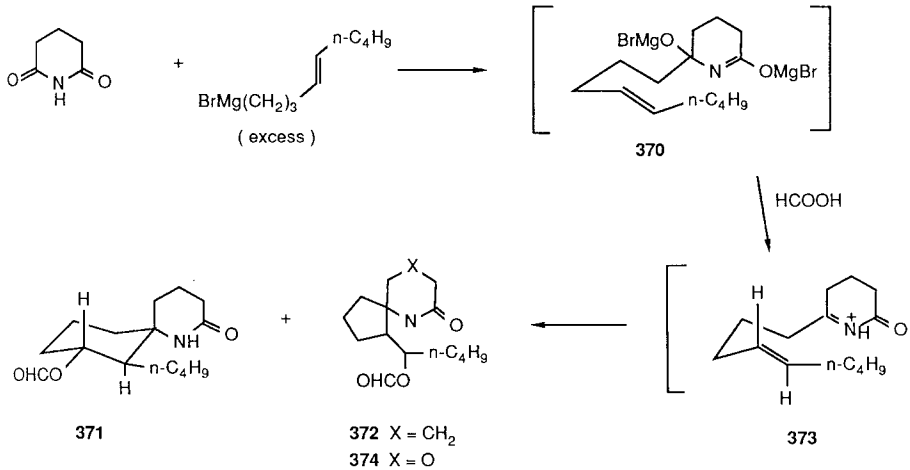
359 was hydrogenated and the required double bond was introduced by base-promoted elimination of a mesylate (*134*). The total synthesis of cannabistivine (**367**) proceeded along similar lines from **358a** (*135*).

Histrionicotoxin (**368**) and related alkaloids (*136*) have enjoyed considerable synthetic attention due to their important neuropharmacological activity. Perhydrohistrionicotoxin (**369**), the fully hydrogenated analogue of **368,** has been synthesized several times (*137*). The most direct approach, developed by the groups of Speckamp and Evans, employs *N*-acyliminium ion chemistry. Reaction of glutarimide with excess (*E*)-4-nonenylmagnesium bromide in THF for 18 hr at ambient temperature, furnished a solution of **370.** After evaporation of THF, the residue was dissolved in formic acid and stirred for 14 days at 42°C. This protocol provided the desired azaspirane **371** in 30% yield from glutarimide (*138, 139*), along with a small amount of five-membered ring isomer **372.** The six-membered ring of **371** was formed with complete stereoselectivity through the intermediacy of *N*-acyliminium ion **373,** and with the incipient ring in a chair-like transition state conformation. Evans and co-workers used refluxing dichloromethane as medium for the Grignard reaction to **370,** and stirred the crude product after hydrolytic workup for 48 hr in formic acid at 25°C. In this



368 $R^1 = CH_2CH{=}CHC{\equiv}CH$; $R^2 = CH{=}CHC{\equiv}CH$
histrionicotoxin

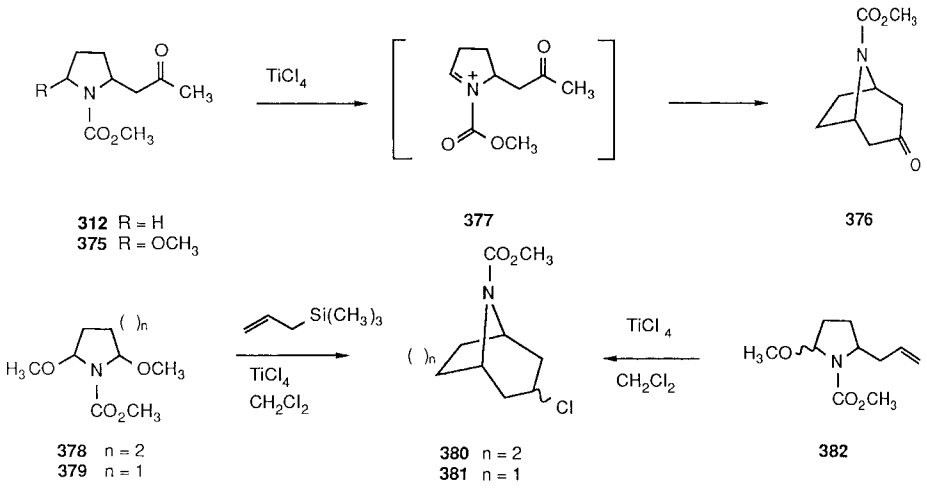
369 $R^1 = n\text{-}C_5H_{11}$; $R^2 = n\text{-}C_4H_9$
perhydrohistrionicotoxin



way, **371** was obtained in a slightly improved yield of 33%, but contaminated with about 25% of **372** (*140, 141*). Surprisingly, 6,5-azaspirane **374** was the sole product when the sequence of reactions was performed starting with morpholine-3,5-dione (*139, 142*). Recently, Gessner *et al.* have prepared various analogues of histrionicotoxin by using similar methodology (*143*). In a related reaction sequence, Tanis and Dixon used the furan ring as π-nucleophile (*143a*). From **371,** perhydrohistrionicotoxin (**369**) was synthesized in a few steps, including reductive pentylation of the lactam via the corresponding thiolactam (*141, 144, 145*).

C. Tropane and Related Alkaloids

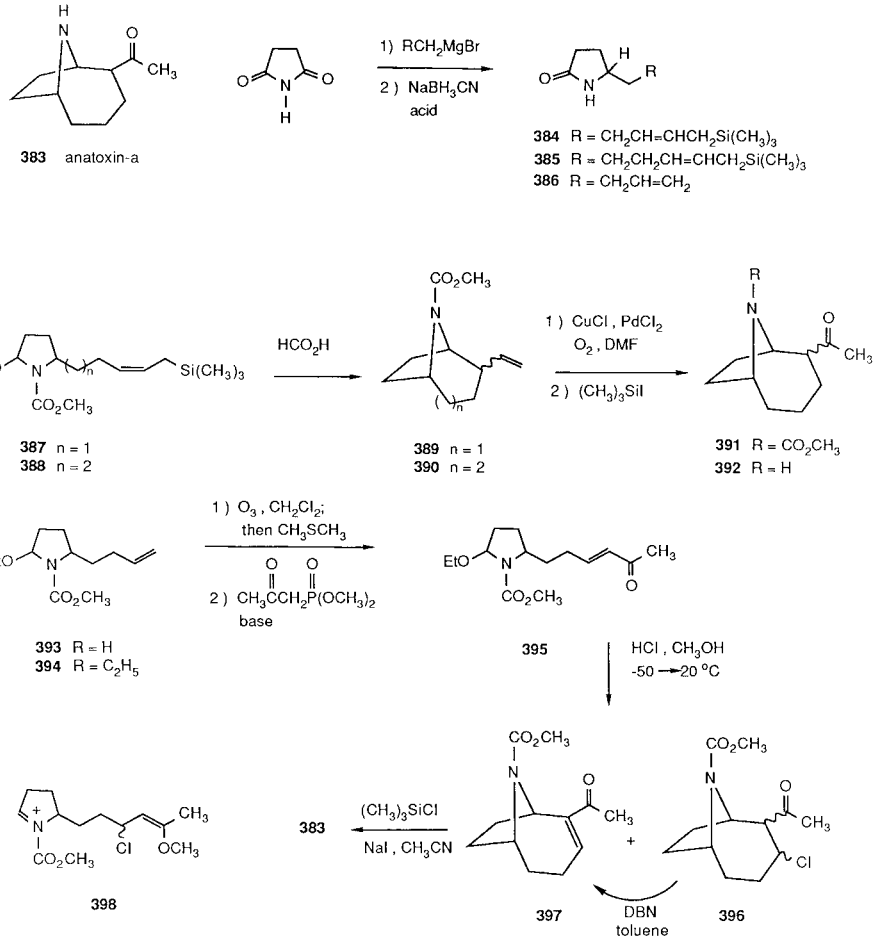
The synthesis of the skeleton of the tropane alkaloids and related bridged heterocycles can be conveniently executed by employing the *N*-acyliminium ion as intermediate. Shono *et al.* converted carbamate **312,** discussed in Section IVA, into methoxy derivative **375** by anodic oxidation in methanol in 52% yield. Treatment of **375** with titanium tetrachloride in dichloromethane provided bicyclic **376** in 50% yield, most likely through the intermediacy of *N*-acyliminium ion **377** (*118*). Double anodic methoxylation of *N*-methoxycarbonylpiperidine



and *N*-pyrrolidine furnished good yields of **378** and **379.** Reaction of these dimethoxy compounds with allyltrimethylsilane in dichloromethane, promoted by titanium tetrachloride, afforded **380** and **381** in yields of 85 and 41%, respectively (*127*). These [3 + 3]-type annulation reactions are characterized by two consecutive *N*-acyliminium ion reactions. In accord with this mechanistic rationale, cyclization of **382** under the same conditions furnished **381** in 89% yield (*127*).

Recently, our research group (*146, 147*) accomplished two related synthetic routes to the unique homotropane alkaloid anatoxin-a (**383**). Reaction of succinimide with excess Grignard reagent (about 3 equiv) and immediate reduction of the product hydroxy lactams with sodium cyanoborohydride in acidic medium furnished the substituted pyrrolidinones **384–386** in yields of about 60% from succinimide. *N*-carbomethoxylation of these products was best achieved by treating their lithium salts with methyl cyanoformate. Chemoselective reduction of the ring carbonyl group at −20°C using the pH-controlled $NaBH_4$ method furnished the *N*-acyliminium precursors **387, 388,** and **393.** Cyclization of **387** and **388** readily occurred in high yield on dissolution in formic acid to afford bicyclic carbamates **389** and **390** (*146*). The latter product was subjected to a Wacker oxidation to give acetyl derivative **391.** Deprotection of nitrogen by using *in situ*-generated iodotrimethylsilane, furnished dihydroanatoxin-a **392,** which has been transformed into **383** by Koskinen and Rapoport (*148*).

The second synthesis of anatoxin-a (**383**) continued with ethanolysis of **393** to **394.** Ozonolysis and reductive workup of **394,** followed by olefination of the resulting aldehyde, furnished the *N*-acyliminium precursor **395.** When **395** was stirred in methanol, which had been saturated with HCl at −50°C, for 18 hr



383 anatoxin-a

384 R = $CH_2CH{=}CHCH_2Si(CH_3)_3$
385 R = $CH_2CH_2CH{=}CHCH_2Si(CH_3)_3$
386 R = $CH_2CH{=}CH_2$

387 n = 1
388 n = 2

389 n = 1
390 n = 2

391 R = CO_2CH_3
392 R = H

393 R = H
394 R = C_2H_5

395

396

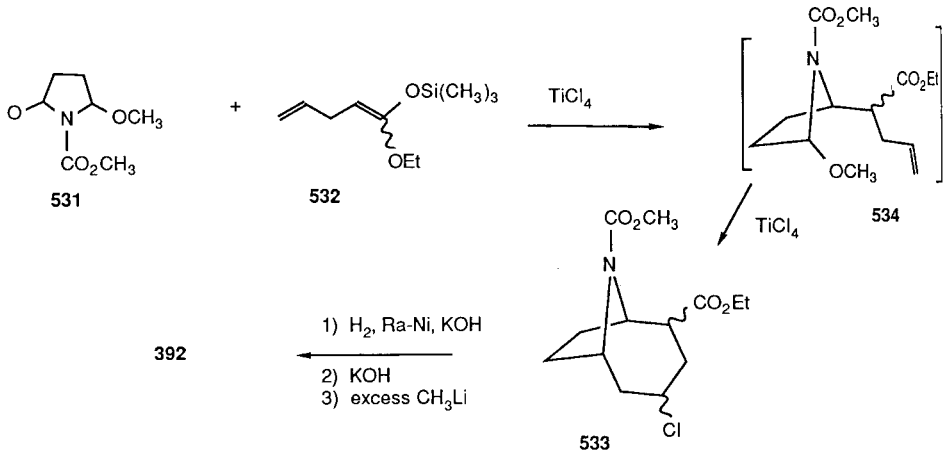
397

398

while the temperature was allowed to slowly rise to room temperature, cyclization took place to a 81 : 19 mixture of chloride **396** and enone **397** in 58% yield (*147*). The former product was readily dehydrochlorinated into the latter by treatment with 1,5-diazabicyclo[4.3.0]non-5-ene in refluxing toluene. Deprotection of nitrogen completed this synthesis of anatoxin-a (*147*). The *N*-acyliminium ion **398** is presumed to be the crucial intermediate in the key cyclization reaction. The side chain functionality most likely arises via conjugate HCl addition to **395,** followed by methyl enol ether formation.

Very recently, Shono and co-workers (*148a*) published a short synthesis of anatoxin-a (**383**) from dimethoxycarbamate **531** and skipped dienol ether **532.** Lewis acid promoted coupling of these reactants furnished the homotropane

skeleton **533** in one step in 60% yield. This interesting process most probably follows a stepwise mechanism which involves two N-acyliminium ion reactions with **534** as an intermediate. In three steps, **533** was transformed into dihydroanatoxin-a (**392**), which completed a formal synthesis of the natural product (*148*).

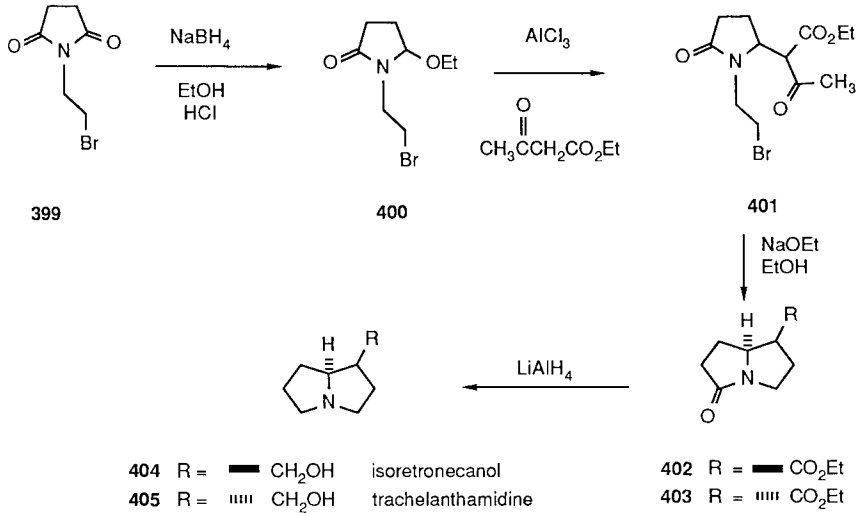


V. Izidine Alkaloids

A. Pyrrolizidine Alkaloids

The pyrrolizidine alkaloids (*149, 150*) comprise a very large group of natural products, which are characterized by the presence of the 1-azabicyclo[3.3.0]octane system, usually called the necine base. *N*-acyliminium chemistry has been shown to be quite useful for the synthesis of necine bases. Three different methodologies involving *N*-acyliminium intermediates can be distinguished: a) use of stabilized carbanions as nucleophiles, b) use of π-nucleophiles, and c) syntheses via aza-Cope rearrangements.

a) **Stabilized carbanions as nucleophiles** Ethoxy lactam **400** is the precursor to an *N*-acyliminium intermediate in the synthesis of isoretronecanol (**404**) and its epimer trachelanthamidine (**405**) by Kraus and Neuenschwander (*151*). Compound **400** was readily synthesized by means of acid-promoted reduction of imide **399.** Reaction of **400** with ethyl acetoacetate, induced by aluminum chloride in dichloromethane, produced in 54% yield the amidoalkylation product **401.** When treated with sodium ethoxide at room temperature, **401** underwent ring closure and deacetylation to a 1 : 4 mixture of **402** and **403** in 84% yield.

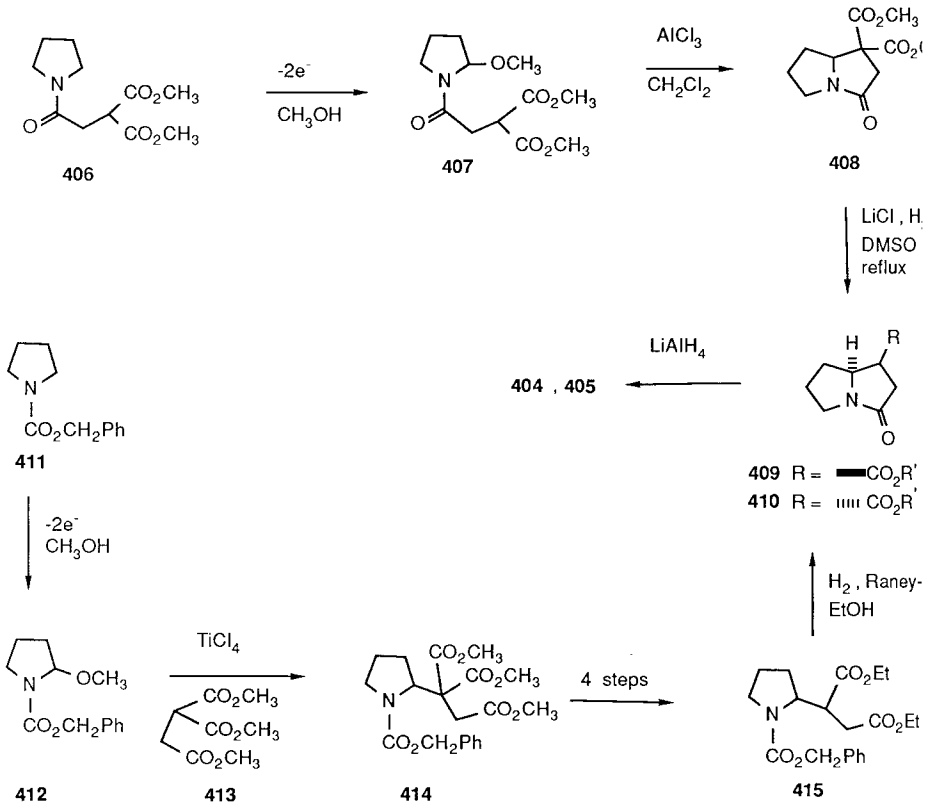


These isomers were separated and reduced with $LiAlH_4$ providing the natural products **404** and **405** in 90% yield (*151*).

The simple necine bases **404** and **405** have been synthesized from pyrrolidine by Wistrand *et al.* by using electrochemistry. Anodic oxidation of **406** in methanol produced methoxy amide **407** in 90% yield. *N*-acyliminium ring closure occurred after treatment with aluminum chloride in dichloromethane to give **408** in 90% yield. Demethoxycarbonylation under Krapcho conditions gave a thermodynamic 4 : 1 mixture of **409** and **410** (85% yield), which was separated chromatographically. Reduction of the individual isomers furnished the desired natural products **404** and **405** (*152*).

Shono *et al.* (*153*) synthesized a mixture of ethyl esters **409** and **410,** by starting with the electrochemical oxidation of carbamate **411,** resulting in **412** in 67% yield. Amidoalkylation with triester **413** in the presence of titanium tetrachloride gave **414** (57%), which, in a number of steps, was transformed into **415** (44% overall). Hydrogenolysis of the benzyl ester was spontaneously followed by lactam formation giving a 62 : 38 mixture of **409** and **410** in 79% yield (*153*).

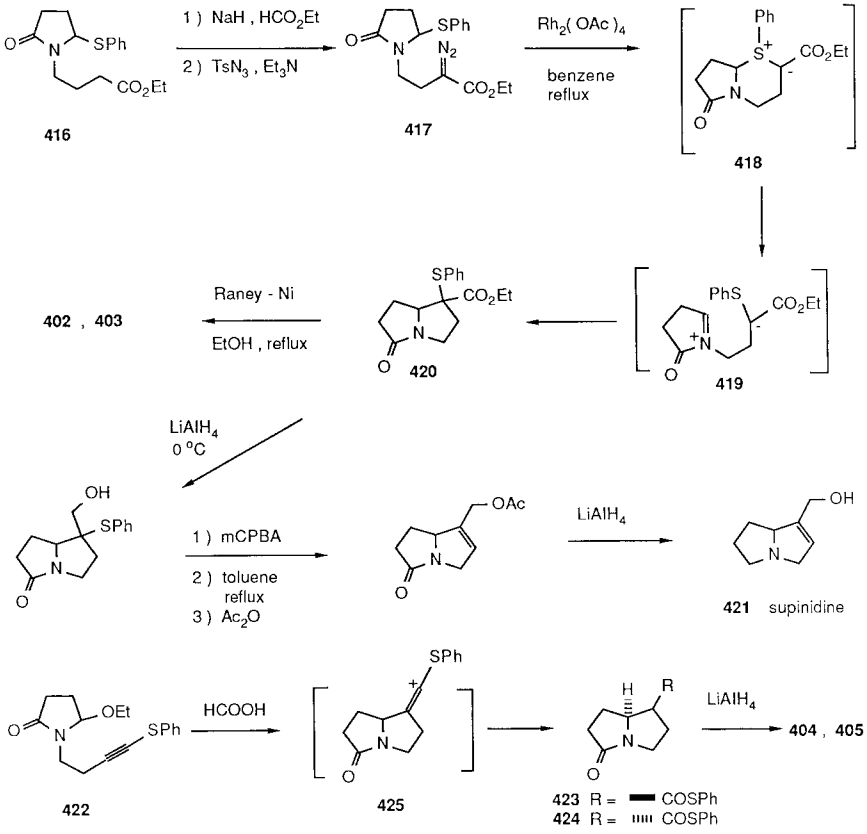
Very recently, Kametani *et al.* (*154*) reported another synthesis of the mixture of esters **402** and **403.** Compound **416,** readily prepared from succinimide, was converted into α-diazoester **417** in two steps in 66% overall yield. The crucial step in this synthesis was the intramolecular carbenoid displacement reaction, which occurred when **417** was refluxed in benzene in the presence of a catalytic amount of rhodium acetate. Through the intermediates **418** and **419,** bicycle **420** was obtained in 55% yield. Raney-nickel reduction yielded a 1 : 1 mixture of **402**



and **403.** Alternatively, reduction and sulfoxide elimination led to supininidine (**421**) (*154*).

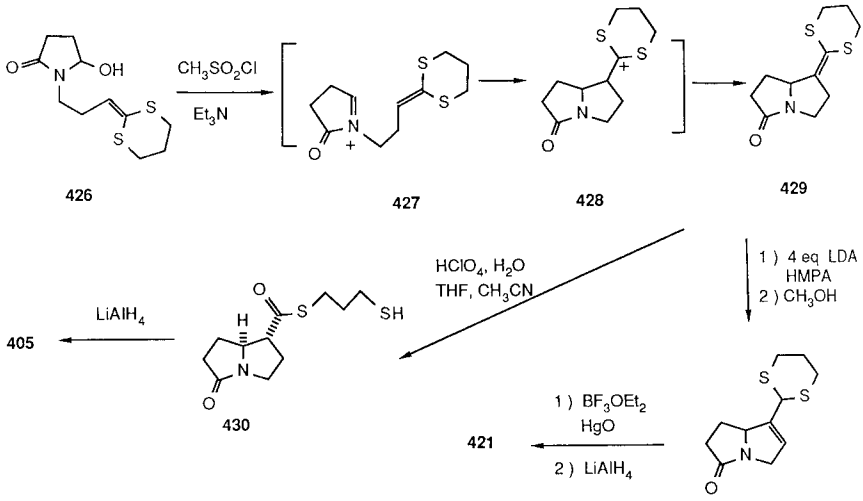
b) **π-Nucleophiles** The phenylthioacetylene moiety was used by Nossin and Speckamp as π-nucleophile in an *N*-acyliminium cyclization reaction in order to prepare isoretronecanol (**404**) and trachelanthamidine (**405**). Thus, stirring of thioether **422,** readily prepared from succinimide, in formic acid for 144 hr furnished, with complete regioselectivity, a 1 : 4 mixture of thioesters **423** and **424** in 80% yield. Apparently, the sulfur substituent stabilizes the linear vinyl cation **425** to such an extent that none of the six-membered ring product is formed. After chromatographic separation, **423** and **424** were reduced to the natural products **404** and **405** (*155*).

Chamberlin and Chung have investigated the ketene dithioacetal group as π-nucleophile in *N*-acyliminium ion cyclizations (*156*). Hydroxy lactam **426,** obtained from the corresponding imide through $NaBH_4$ reduction in methanol (*156, 157*), was converted into *N*-acyliminium intermediate **427** by way of a nonacidic

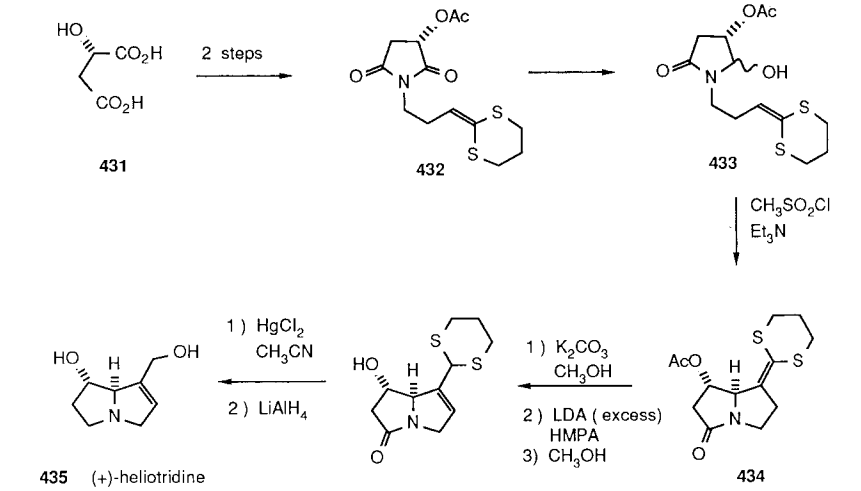


method, in view of the acid lability of the ketene dithioacetal functionality. This method involved formation of a methanesulfonate, which, *in situ,* suffered cyclization to ketene dithioacetal **429** in 86% yield. Later (*157*), it was found that this special method was not necessary, since **426** could be transformed into **427** in 89% yield by using trifluoroacetic acid in dichloromethane. The cyclization occurred with complete regioselectivity due to the cation-stabilizing influence of the sulfur atoms in **428.** From **429,** supinidine (**421**) was obtained in 45% overall yield by means of base-induced migration of the double bond, dithioacetal hydrolysis, and reduction (*156, 157*). Hydrolysis of **429** with perchloric acid furnished the thermodynamically more stable thioester isomer **430** in 82% yield. Reduction with $LiAlH_4$ then yielded trachelanthamidine (**405**). Isoretronecanol (**404**) could not be synthesized through this route (*157*).

The ketene dithioacetal methodology was applied to the synthesis of enantiomerically pure (+)-heliotridine (**435**) by starting from readily available (*S*)-malic acid (**431**) (*158*). The reduction of imide **432,** readily obtainable from **431**

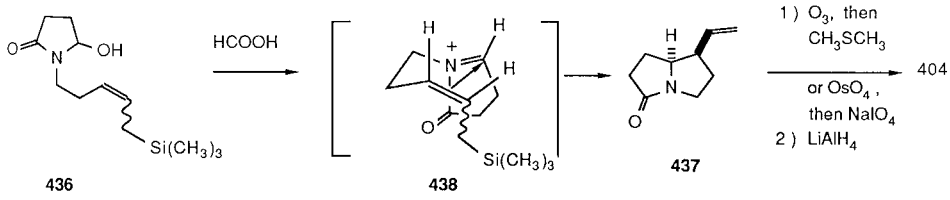


in two steps (36% yield), proceeded with complete regioselectivity by using $NaBH_4$ in methanol at $-4°C$, to give a 10 : 1 mixture of stereoisomers **433** in 85% yield. The crucial *N*-acyliminium ion cyclization was effected through treatment with methanesulfonyl chloride as described before to give pyrrolizidine **434** in 68% yield. Very little stereoisomeric material was obtained, which indicates that the acetoxy group effectively blocks the α-face of the *N*-acyliminium ion. The remaining steps of the synthesis included deprotection of the hydroxyl function, isomerization of the double bond, hydrolysis of the dithiane, and



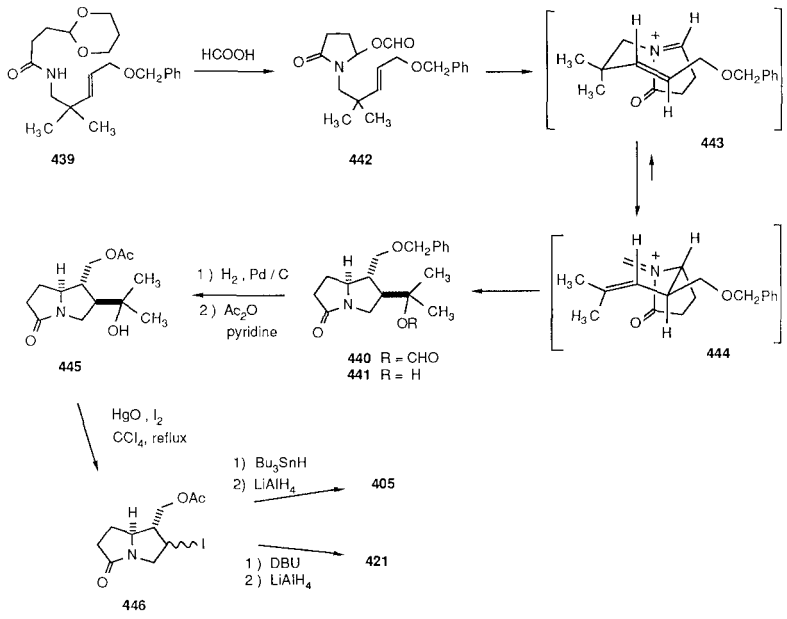
reduction to give natural heliotridine (**435**) in 33% yield from **434** (*158*). Recently, the preparation of various other enantiomerically pure pyrrolizidine diols was reported from cyclization product **434** (*159*).

Another π-nucleophile which has shown excellent behavior in *N*-acyliminium ion cyclizations is the allylsilane moiety (*160, 161*). Cyclization of either (*E*)- or (*Z*)-allylsilane **436** by means of simple dissolution in formic acid, furnished pyrrolizidine **437** as a single compound in 80% yield. The regioselectivity of this reaction must be ascribed to the β-effect of silicon, directing the ring closure to solely five-membered ring formation. The stereoselectivity is a result of the preference for the chair-like transition state emanating from π-complex **438** (*161*), regardless of double-bond geometry. A similar result was obtained by using the corresponding allylstannane as π-nucleophile (*162*). From **437,** isoretronecanol (**404**) was synthesized in two steps by way of oxidative cleavage of the double bond and reduction.

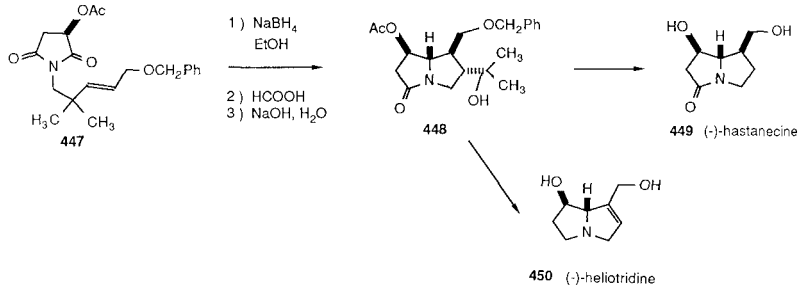


c) **Aza-Cope rearrangements** The research groups of Hart and Speckamp have independently discovered that *N*-acyliminium ions of the 2-aza-1,5-hexadienyl type are susceptible to Cope rearrangement processes (*163–168*). This aspect of *N*-acyliminium chemistry has been elaborated by Hart and Yang (*166–168*) for the synthesis of some pyrrolizidine alkaloids. When the secondary amide **439** was stirred in formic acid for 2 hr, a mixture of products was obtained, containing **440** and **441** in 80% combined yield. The mechanism of this reaction consists of the initial formation of lactam **442,** followed by heterolysis to *N*-acyliminium ion **443.** Rather than cyclizing to a six-membered ring, species **443** changes into **444** in a Cope-type sigmatropic rearrangement. Cyclization of **444** leads to pyrrolizidine system **440** via a tertiary carbenium ion, which is trapped by formic acid. The stereochemical consequences can be deduced from π-complex **444,** which prefers a chair-like conformation, thus accounting for the high stereoselectivity of the process. In order to prepare some natural pyrrolizidines, changes in protecting groups were necessary, producing **445** from **441** in 94% yield. Degradation of the side chain produced **446** in 89% yield. From **446,** both trachelanthamidine (**405,** 78%) and supinidine (**421,** 93%) were obtained after two more synthetic steps (*166*).

This methodology was then adapted for the synthesis of enantiomerically pure



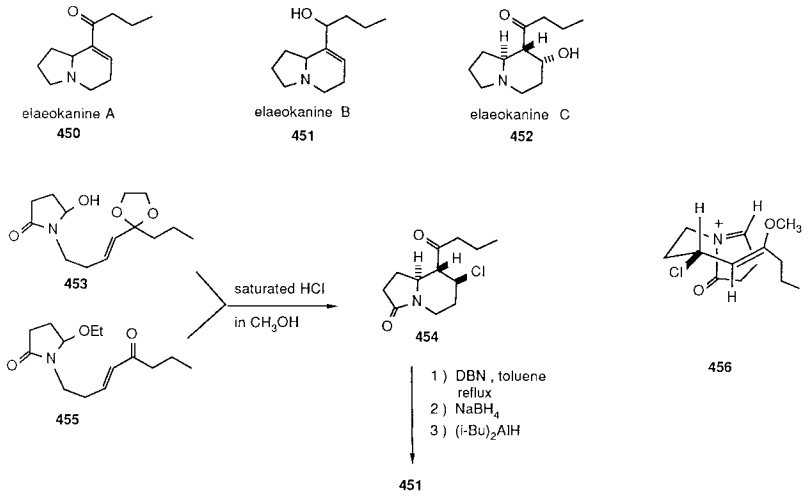
(−)-hastanecine (**449**) and (−)-heliotridine (**435**) by starting from (*R*)-malic acid (*167, 168*). Acetoxyimide **447** was synthesized from (*R*)-malic acid, and regioselectively reduced to the corresponding hydroxy lactam (83% yield). After stirring this hydroxy lactam in formic acid for 24 hr and the subsequent saponification of the product, pyrrolizidine system **448** was obtained in 77% yield. In the same manner as described before, **448** was transformed into the natural products **449** and **435.**



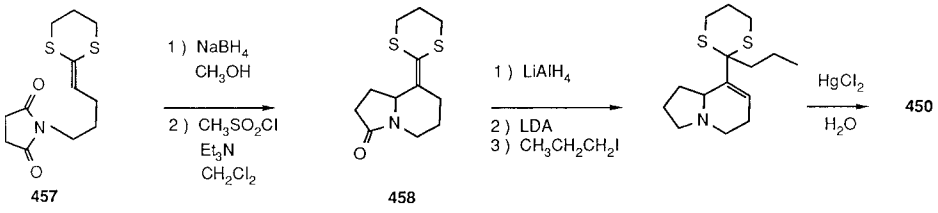
B. Indolizidine Alkaloids

Of the various types of alkaloids belonging to this group, the Elaeocarpus alkaloids (for example **450–452**) (*169*) have received the most attention from

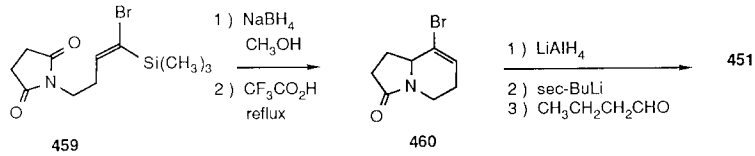
research groups engaged in *N*-acyliminium chemistry. Wijnberg and Speckamp investigated the ring closure of unsaturated acetal **453.** They obtained a quantitative yield of chloride **454** as a single isomer following treatment of **453** with saturated HCl in methanol (*170*). Later (*171*), it was found that enone **455** also cyclized quantitatively under the same conditions. Surprisingly, formic acid was unsuitable as medium for cyclization. The detailed mechanism of formation of **454** is unknown, but *N*-acyliminium ion **456** might be the crucial intermediary π-complex, which is the result of conjugate HCl addition and enol ether formation (*147*). Chloride **454** was converted into elaeokanine B (**451**) in three steps in 80% overall yield.



Chamberlin *et al.* reported the synthesis of elaeokanine-A (**450**) by using the ketene dithioacetal functionality as π-nucleophile (*157*). Reduction of imide **457,** followed by cyclization under neutral conditions, produced indolizidine **458** in 70% yield. Amide reduction, followed by alkylation and dithiane hydrolysis, furnished elaeokanine-A (**450**) in 29% overall yield.

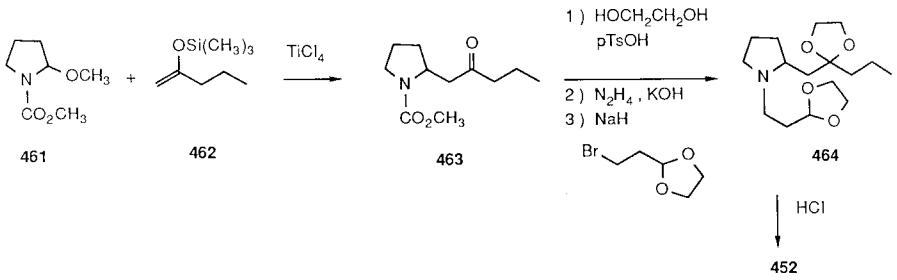


Overman *et al.* have utilized the vinylsilane moiety as π-nucleophile for the synthesis of elaeokanine-B (**451**) (*172*). Reduction of imide **459,** followed by cyclization in refluxing trifluoroacetic acid, furnished vinyl bromide **460** in 63% yield. Reduction of the amide, bromide–lithium exchange, and reaction with butanal led to the natural product **451** in 58% overall yield.



459 460

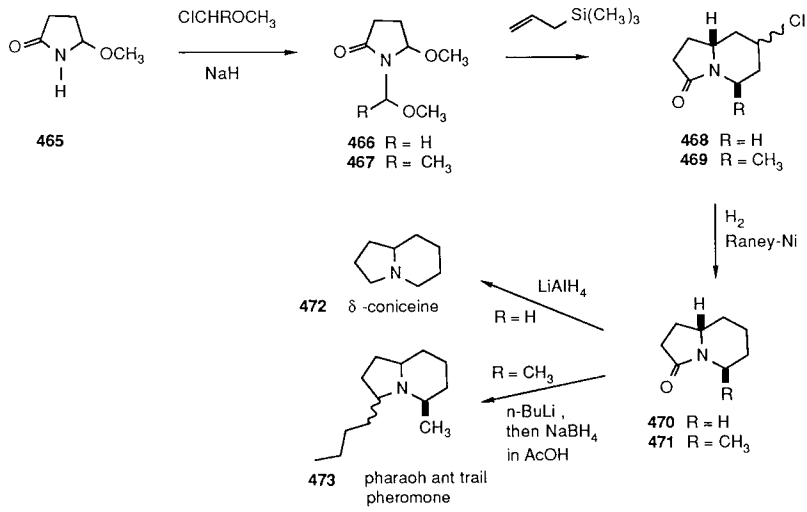
An intermolecular *N*-acyliminium ion reaction was utilized by Shono *et al.* (*153*) in a preparation of elaeokanine-C (**452**). Methoxy carbamate **461,** obtained through electrochemical oxidation, was treated with silyl enol ether **462** in the presence of titanium tetrachloride, resulting in ketone **463** in 81% yield. Protection of the ketone, deprotection of nitrogen, and attachment of a three-carbon chain furnished diacetal **464** in 61% overall yield. Aldol cyclization completed the synthesis of **452** in 45% yield.



461 462 463 464 452

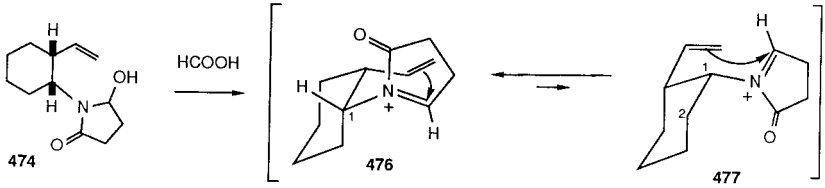
Two *N*-acyliminium ion reactions are involved in a novel [3 + 3]-type annulation process recently described by Shono *et al.* (*127*), and applied to the synthesis of δ-coniceine **472** and the pharaoh ant trail pheromone **473.** 2-Pyrrolidone was methoxylated electrochemically to methoxy lactam **465** in 67% yield. Subsequent methoxyalkylation on nitrogen produced **466** (71%) or **467** (83%). The crucial [3 + 3]-type annulation reaction occurred following treatment of **466** and **467** with allylsilane in the presence of titanium tetrachloride, giving **468** (77%) and **469** (70%), respectively. Removal of the chlorine atom provided **470** (85%) and **471** (74%) as single isomers. Finally, reduction of amide **470** with $LiAlH_4$ furnished δ-coniceine (**472**), while addition of butyllithium to **471,** followed by $NaBH_4$ reduction, gave pheromone **473** as a 2 : 1 mixture of isomers (34% yield).

An important stereochemical aspect of *N*-acyliminium cyclization reactions

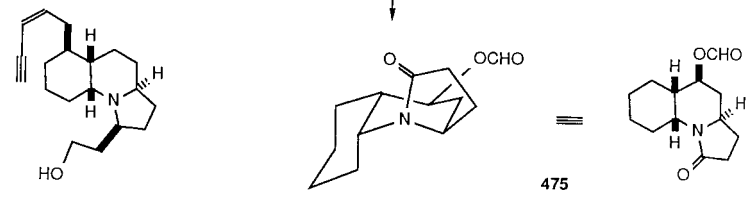


was reported by Hart in 1980 (*173*). He investigated the cyclization of hydroxy lactam **474** in formic acid and obtained tricycle **475** in 85% yield. The high stereoselectivity of this process was explained by considering the relative stability of the two possible intermediary π-complex conformations **476** and **477.** In **476** the C1–H bond is coplanar with the *N*-acyliminium part, while in **477** this is the case with the C1–C2 bond. Due to the unfavorable A(1,3) strain between C2 and the carbonyl group, **477** is less stable than **476.** Cyclization of **476** leads to the observed product **475** (*173*). This stereochemical result was applied in the total synthesis of gephyrotoxin (**478**) (*136*) by Hart and Kanai (*174, 175*). The Diels–Alder adduct of butadiene with cyclohexenone (**479**) was converted in five steps (22% overall) into hydroxy lactam **480,** having both vinyl groups in an axial orientation. *N*-acyliminium cyclization in formic acid led to a single formate **481** in 79% yield. The formate moiety was then replaced by hydrogen via radical reduction of a xanthate to give **482** in 66% overall yield. The synthesis of gephyrotoxin (**478**) was completed through a quite efficient conversion of the carbonyl group into a 2-hydroxyethyl moiety, and of the vinyl group into a *cis*-enyne function (*175*).

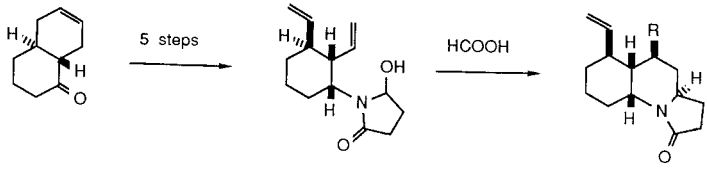
Very recently, the first total synthesis of a Daphniphyllum alkaloid, methyl homodaphniphyllate (**483**), was reported by Heathcock *et al.* (*176*). Early in this synthesis, *N*-acyliminium chemistry was utilized to construct tricyclic system **485.** Heating of amide **484** in toluene in the presence of *p*-toluenesulfonic acid furnished **485** in 83% yield, presumably via intermediates **486** and **487.** The remaining steps included the annulation of the fourth ring, giving **488,** and, the most crucial step, the intramolecular, acid-induced Michael addition to the desired alkaloid skeleton **489** (*176*).



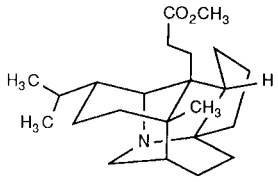
474 476 477



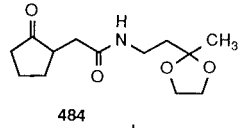
478 gephyrotoxin 475



479 480 481 R = OCHO 482 R = H



483 methyl homodaphniphyllate



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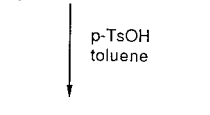

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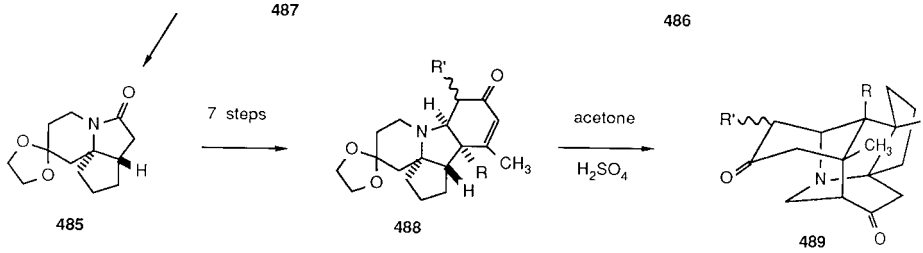
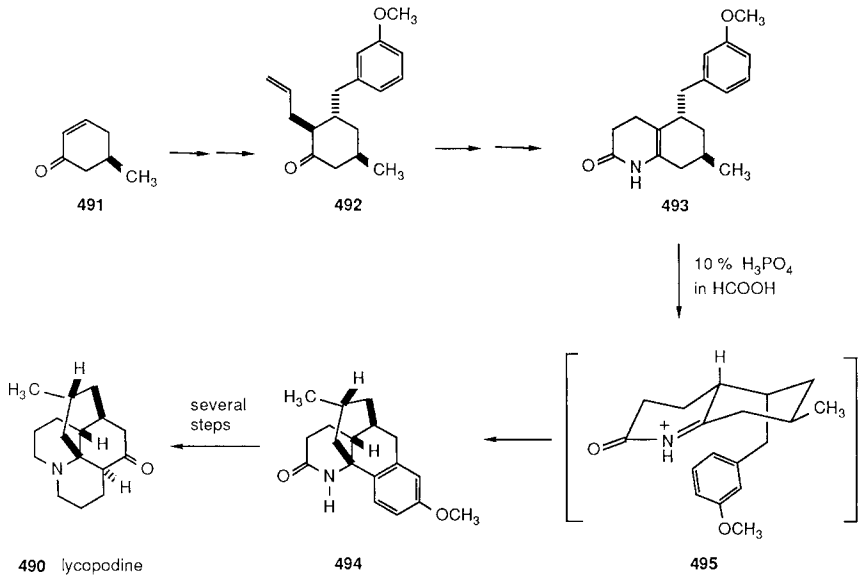


485 488 489

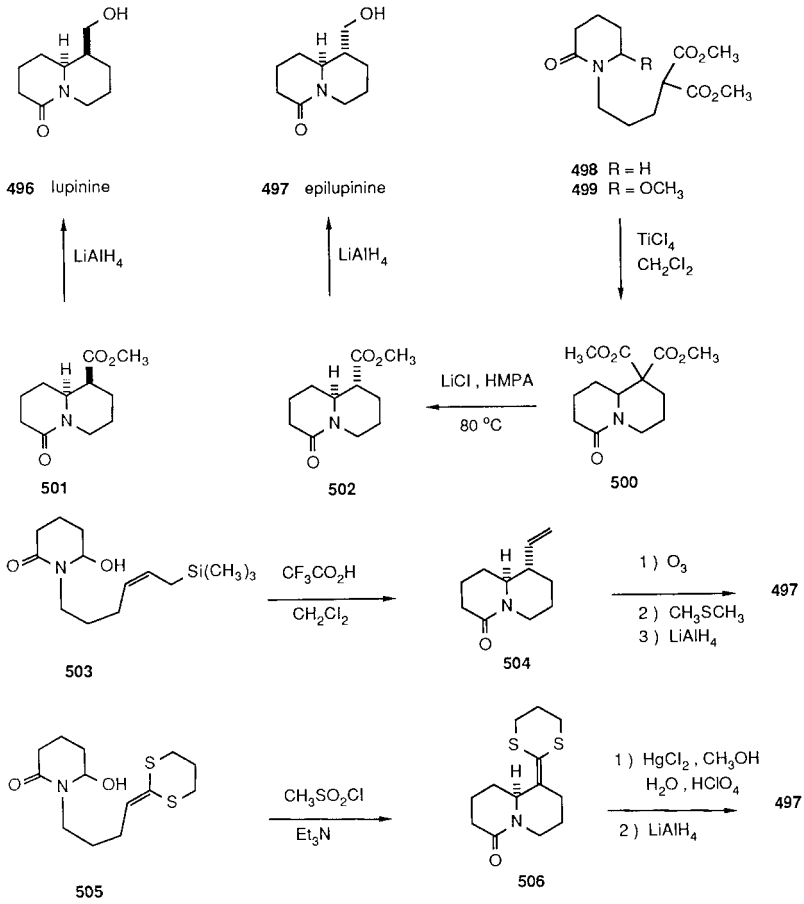
C. Quinolizidine Alkaloids

An early and beautiful illustration of the great potential of *N*-acyliminium ion cyclizations in alkaloid synthesis is found in the total synthesis of lycopodine (**490**) by Stork and co-workers, published in 1968 (*177, 178*). Conjugate addition of Grignard reagent to enone **491,** followed by allylation of the magnesium enolate, gave **492,** which in a few steps was converted into enamide **493,** the crucial precursor to the *N*-acyliminium intermediate. Treatment of **493** with a 10% solution of phosphoric acid in formic acid for 20 hr produced 55% of para-substitution product **494,** along with 29% of the corresponding ortho product. Most likely, **495** served as intermediate in this reaction, which established the quaternary center of **490** in the correct stereochemical sense. The remaining steps of the total synthesis included $LiAlH_4$ reduction of the amide carbonyl, Birch reduction of the aromatic ring, regioselective oxidative cleavage of this ring, and recyclization on nitrogen (*177, 178*).

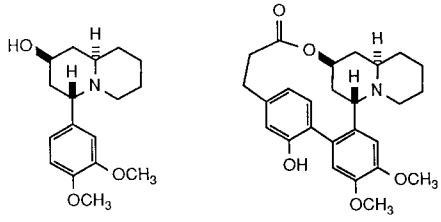


Lupinine (**496**) and epilupinine (**497**) constitute much simpler target molecules. Okita *et al.* synthesized these lupine alkaloids by using as the crucial step the cyclization of methoxy lactam **499,** obtained through electrochemical oxidation of **498** (*179*). After treatment of **499** with titanium tetrachloride, ring closure occurred to **500** in 77% yield. Demethoxycarbonylation was effected using lithium chloride in HMPA to give a mixture of esters **501** and **502** in yields of 19% and 49%, respectively. Reduction with $LiAlH_4$ completed the synthesis of

496 (55%) and **497** (93%). Our research group reported a stereoselective synthesis of racemic epilupinine (**497**) using the silicon-directed cyclization of hydroxy lactam **503,** obtained by imide reduction (*161*). Treatment of **503** with trifluoroacetic acid produced a single vinyl compound **504** in 74% based on the imide. The high stereoselectivity was explained as being the result of a chair-like transition state with an equatorially oriented allylsilane moiety. Ozonolysis of **504,** followed by reduction, produced **497** in 95% overall from **501.** Similarly, Chamberlin *et al.* synthesized epilupinine (**497**) by using the ketene dithioacetal as π-nucleophile. Cyclization of **505** via its methanesulfonate gave bicycle **506** in 79% yield, based on the imide. Hydrolysis of the acetal, followed by reduction, resulted in **497** in 75% yield (*157*).

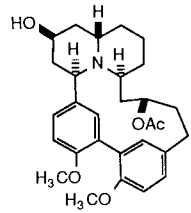


Three aryl-substituted quinolizidine alkaloids (**507–509**) have been synthesized in a highly stereoselective manner by taking advantage of the A (1,3) strain effect discovered by Hart (*173*). Racemic lasubine I (**507**) was prepared by Speckamp *et al.* from glutarimide and homoallyl alcohol **510** (*180*). Mitsunobu coupling, followed by reduction, gave hydroxy lactam **511** in 26% yield. After dissolving **511** in formic acid, ring closure occurred with high selectivity. Subsequent formate hydrolysis and carbonyl reduction created lasubine I (**507**) in 68% overall yield from **511.**

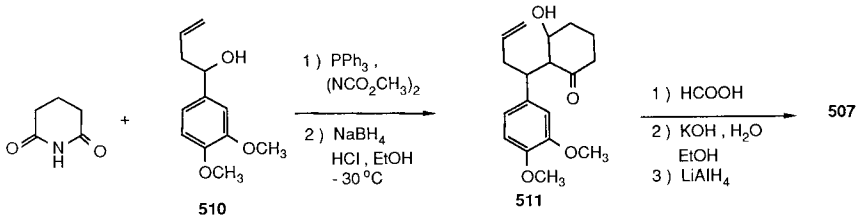


lasubine I
507

vertaline
508

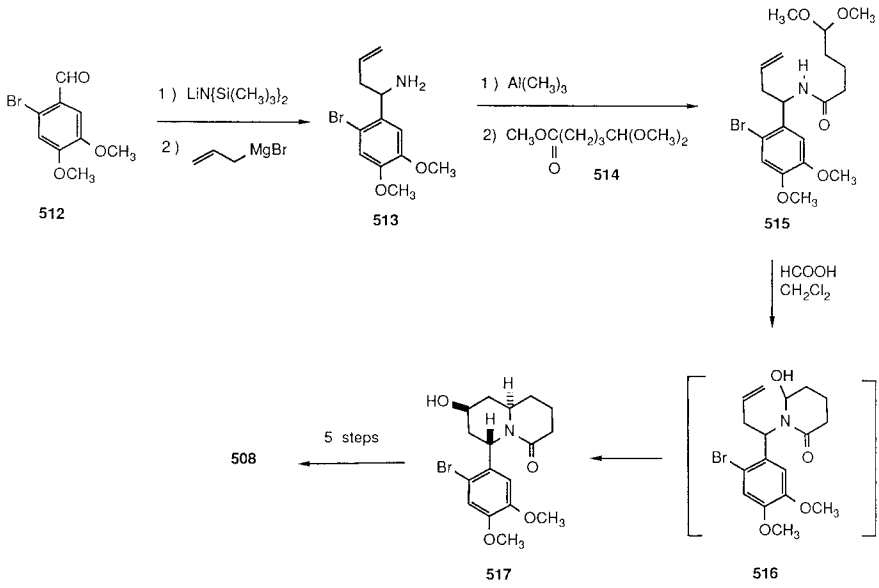


lythrancepine II
509

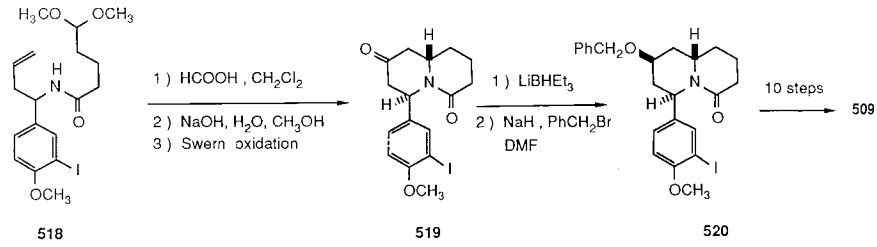


The synthesis of vertaline (**508**) by Hart and Kanai (*181*), followed a similar strategy, although the benzylic carbon–nitrogen bond could not be formed by using a Mitsunobu reaction. An interesting sequence of reactions was then developed in order to arrive at the precursor **515** for the crucial *N*-acyliminium cyclization. Aldehyde **512** was treated with lithium hexamethyl disilazide, followed by allylmagnesium bromide, to produce homoallylamine **513** in 97% yield. The *N*-trimethylsilylimine probably served as intermediate in this sequence. Amide formation using ester **514** gave **515** in 88% yield. When **515** was stirred with formic acid in dichloromethane, double cyclization occurred via **516** to furnish the desired stereoisomer **517** in 60% yield. In another five steps, **517** was transformed into vertaline (**508**) (*181*).

In the synthesis of lythrancepine II (**509**) by Hart and Hong (*182*), amide **518** was prepared in the same manner as **515.** Treatment of **518** with formic acid in

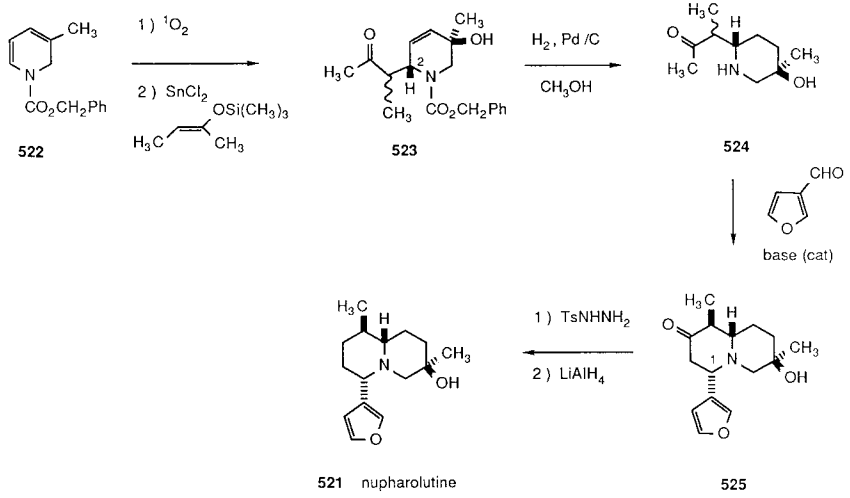


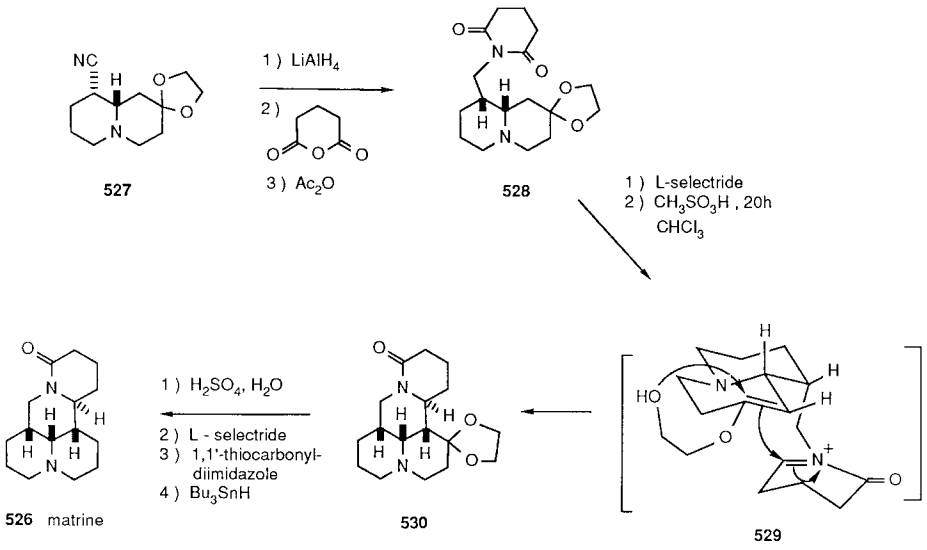
dichloromethane, followed by formate hydrolysis and Swern oxidation of the resulting alcohol, furnished **519** in 74% overall yield. Ketone reduction with lithium triethyl borohydride, established the correct stereochemistry of the hydroxyl function, which was protected as benzyl ether **520** (66% from **519**). After another 10 steps, racemic lythrancepine II (**509**) was obtained (*182*).



The Nuphar alkaloid nupharolutine (**521**) was synthesized by Natsume and Ogawa by using a completely different approach from the conceptual point of view, compared with the above syntheses (*183*). Dihydropyridine **522,** readily obtainable from pyridine, was treated with singlet oxygen. The resulting endoperoxide was not isolated, but directly subjected to reaction with the thermodynamic silyl enol ether derived from butanone, in the presence of stannous

chloride. In this *N*-acyliminium-type reaction, **523** was formed in 35% yield with high selectivity, since only 4% of the C2 epimer was produced. One-pot hydrogenation and hydrogenolysis of **523** furnished the secondary amine **524** in good yield. The base-catalyzed condensation of **524** with 3-furylaldehyde was a poor step, producing **525** in 17% yield in addition to 15% of its C1 epimer. Removal of the carbonyl function completed the synthesis of nupharolutine (**521**) (*183*).





Recently, a total synthesis of matrine (**526**) was reported by Chen *et al.* (*184*). Bicyclic nitrile **527,** prepared in a few steps from 3-cyanopyridine, was reduced to the primary amine with $LiAlH_4$. Treatment of this amine with glutaric anhydride, followed by dehydration with acetic anhydride, gave glutarimide **528** in 56% overall yield. Reduction of **528** with L-selectride gave the corresponding hydroxy lactam, which was subjected to treatment with methanesulfonic acid in chloroform. *N*-acyliminium ion cyclization then occurred, presumably through the intermediacy of enol ether **529,** to give tetracycle **530.** Replacement of the acetal function by hydrogen took another four steps, furnishing matrine (**526**) in 11% overall yield from **528** (*184*).

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CHAPTER 5

QUINOLINE ALKALOIDS RELATED TO ANTHRANILIC ACID

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I. Introduction

The quinoline alkaloids derived from anthranilic acid were last reviewed in Volume 17 of this series. In the intervening 10 years more than 80 new quinoline alkaloids have been isolated, a new group, the dimeric quinolinones, has been discovered, and considerable advances have been made in the synthesis of the alkaloids. The main features of the biosynthetic pathways to hemiterpenoid quinoline alkaloids were determined in the period 1966–1975; the plan adopted for the previous article in Volume 17 and for this review is based on the biosynthetic sequence as summarized in Scheme 1. Derivatives of 4-hydroxy-2-quinolinone (**5**) are discussed first and then 3-prenyl-2-quinolinones, *cf.* (**6**), derived by *C*-allylation of intermediate **5.** The 3-prenyl-2-quinolinones occupy a central position in the sequence; oxidative cyclization leads to dihydropyranoquinolines or dihydrofuroquinolines exemplified by isobalfourodine (**7**) and platydesmine (**9**), respectively; dehydration of the latter compounds may be responsible for example, for the formation of terminal olefins (**12**). On the other hand, oxidation of 3-prenyl-2-quinolinones to a diene **4** provides the opportunity for intramolecular cyclization to the large group of dimethylpyranoquinolinones, *cf.* **2,** and intermolecular addition to give dimeric quinolinone alkaloids. The widely distributed furoquinoline alkaloids, for example choisyine (**13**), are known to be formed from dihydrohydroxyisopropylfuroquinolines, *cf.* **9,** through the simplest member, dictamnine (**10**). The 1,1-dimethylallyl and 1,2-dimethylallyl alkaloids, *cf.* **11,** and their geranyl counterparts are discussed with the 4-prenyloxy-2-quinolinones, for example (**8**), and 4-geranyloxy-2-quinoli-

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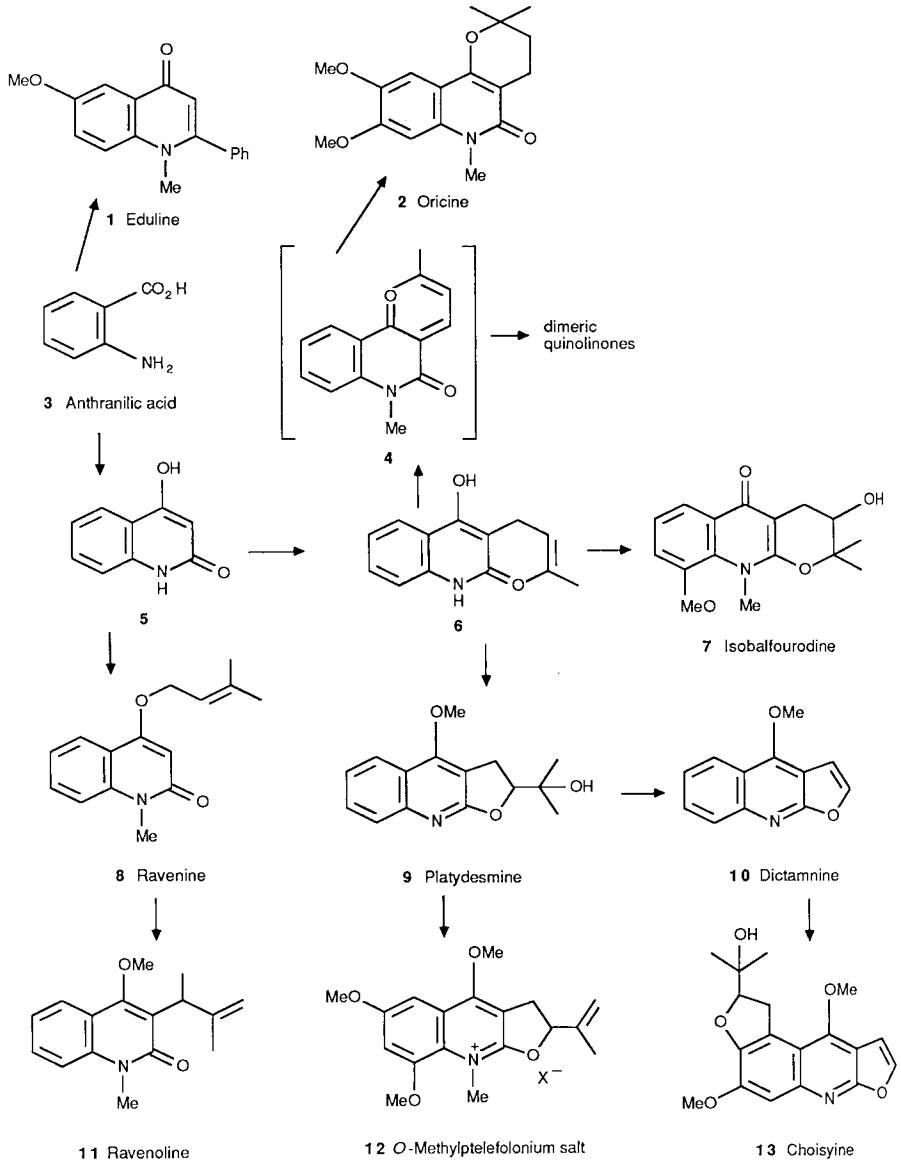
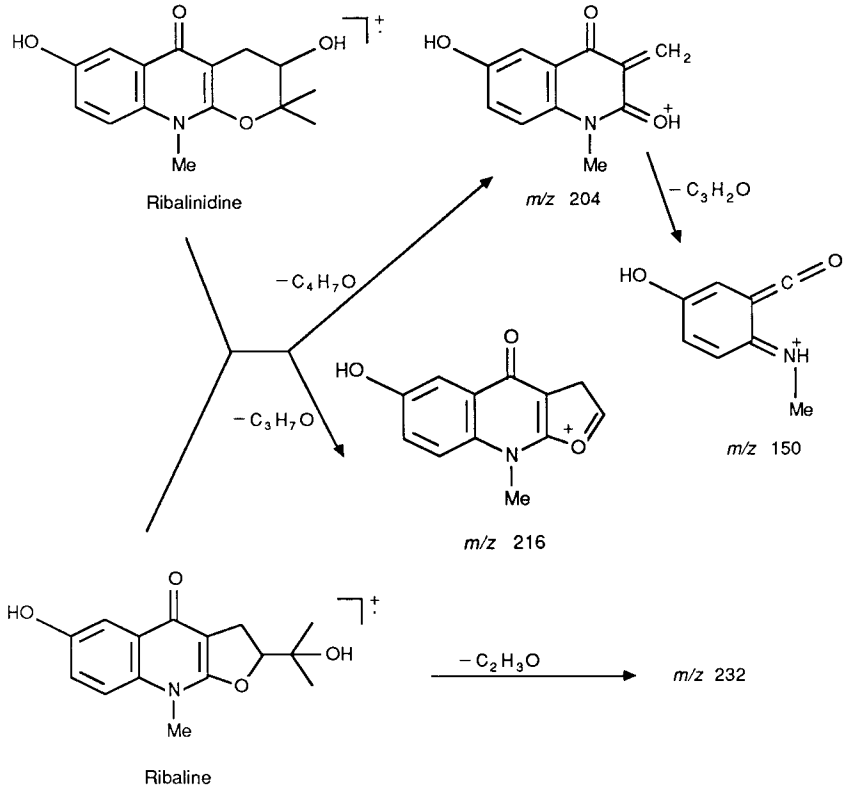


SCHEME 1. Origin of quinoline alkaloids.

nones from which they appear to be derived, and the review ends with an account of the nonterpenoid 2-alkyl-, and 2-aryl-quinolinones, *cf.* **1.**

Spectroscopic methods continue to be crucial in the elucidation of the structures of quinoline alkaloids and data will only be given when of special significance. ^{1}H-NMR data for a selection of quinoline alkaloids were given in the article in Volume 17 (Schemes 1 and 22), but reviews on the application of mass spectrometry and ^{13}C NMR spectroscopy to quinoline alkaloids have appeared more recently and will be discussed here.

A survey of the mass spectra of 16 dihydrofuro- and dihydro-pyrano-4-quinolinone alkaloids was carried out by Hammerum and co-workers (*1*). The mass spectra of furoquinolines not containing a hydroxy function in the acyclic portion closely resemble the spectra of their pyrano-isomer and when a hydroxy group is present, as in ribaline and ribalinidine, the fragmentation patterns are again surprisingly similar (Scheme 2); for example, both compounds produce



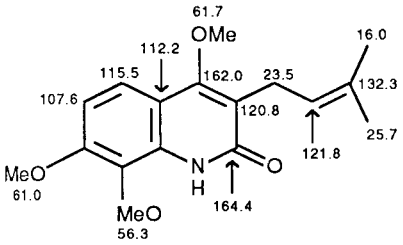
SCHEME 2. Mass spectra of ribaline and ribalidine.

abundant ion peaks at *m/z* 204 and 150. The peak at *m/z* 216, which is formed by loss of C_3H_7O from the molecular ions of both compounds, is four times as abundant in the spectrum of the furo-isomer. The formation of an abundant [M-C_2H_3O]$^+$ fragment is a characteristic feature of the mass spectra of hydroxyisopropyldihydrofuro-4-quinolinones.

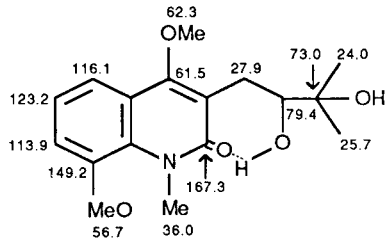
The ^{13}C-NMR spectrum of the dimethylpyranoquinolinone alkaloid zanthophylline was determined by Stermitz and Sharifi (*2*), and that of the furoquinoline alkaloid, skimmianine, by Ahond *et al.* (*3*). In 1980 a study of the spectra of 25 hemiterpenoid quinoline alkaloids and related prenylquinolines was reported (*4*). ^{13}C-NMR data for a selection of quinoline alkaloids is given in Scheme 3. The following general observations show how ^{13}C-NMR spectroscopy can help to determine structure in hemiterpenoid quinoline alkaloids. (*a*) In 2-quinolinones, the presence of a hydroxy group at C-4 produces a C-4 signal at about 157 ppm which is shifted to about 161 ppm in 4-methoxyquinolinones. (*b*) The signal for an *N*-methyl group appears at about 30 ppm when C-8 is unsubstituted, *cf.* isoplatydesmine, ribalinine and araliopsine, but at about 36 ppm when a methoxy group is present at C-8, *cf.* balfourolone. (*c*) Comparison of the ^{13}C-NMR spectra of hydroxyisopropyldihydrofuro-4-quinolinones with their pyrano isomers shows a large difference in chemical shifts between equivalent carbon atoms, for example +23.8 ppm between C-11 of isoplatydesmine and ribalinine and −12.0 ppm between C-12 in the same pair; this seems to be the most unequivocal spectroscopic method for distinguishing between such isomers. (*d*) In tricyclic compounds, an important difference between the angular compounds (2-quinolinones) such as araliopsine and the linear isomers (4-quinolones) like isoplatydesmine is that the chemical shift of the carbon of the carbonyl group of the 2-quinolinones is at higher field by approximately 10 ppm than that in the 4-quinolinones. (*e*) In the ^{13}C-NMR spectra of furoquinolines, for example skimmianine and tecleamine, the signals for furanoid α-carbons occur at about 143 ppm and β-carbons at about 104 ppm; these chemical shifts are at higher field than in compounds containing furan rings attached directly to benzenoid rings.

Review articles on quinoline alkaloids have been written by Sainsbury (*5*) and by Cordell (*6*) and annual comprehensive coverage is provided by the Royal Society of Chemistry, London, in Specialist Periodical Reports "The Alkaloids," 1976–1982 (*7*) and subsequently in "Natural Product Reports" (*8*). Mester's valuable account of the occurrence of rutaceous alkaloids up to April 1973 has been extended to 1982 with the publication of two further articles (*9, 10*). Aspects of the biosynthesis of quinoline alkaloids were discussed in 1983 (*11*).

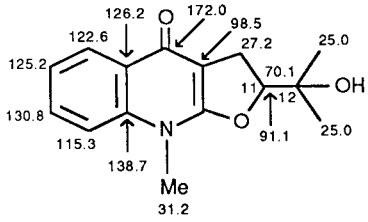
This review includes work published during the period mid-1976 to the end of 1986.



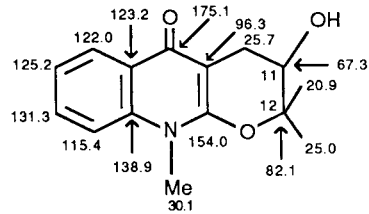
Preskimmianine (*4*)



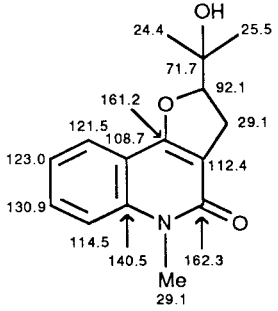
Balfourolone (*4*)



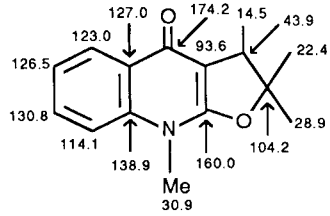
Isoplatydesmine (*4*) (in DMSO - d_6)



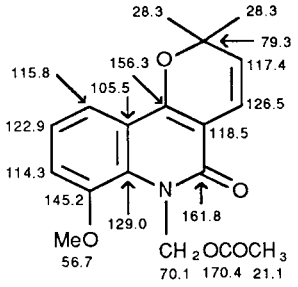
Ribalinine (*4*)



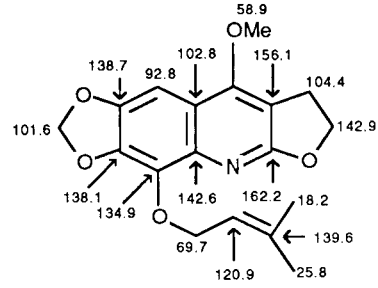
Araliopsine (*4*)



Lemobiline (*4*)



Zanthophylline (*2*)



Tecleamine (*12*)

SCHEME 3. ^{13}C-NMR data for typical quinoline alkaloids. In $CDCl_3$, unless stated otherwise. Figures refer to δ values in ppm.

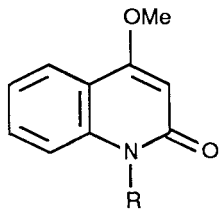
II. Simple Alkaloids Related to 4-Hydroxy-2-quinolinone

Methylation products of 4-hydroxy-2-quinolinone and related alkaloids bearing additional hydroxy or methoxy groups are listed in Table I (*13–27* and *229*). Several compounds discussed in Volume 17, including 4-methoxy-1-methyl-2-quinolinone (**15**), edulitine (**16**), and folinine (**17**) have been identified from new sources with compound (**15**) being particularly widespread. Since these compounds are polar and essentially nonbasic, it is probable that they have been missed in some extraction procedures.

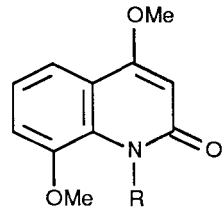
Alkaloids isolated since the last review are 4-methoxy-2-quinolinone (**14**) from *Hortia longifolia* (*13*), the 7-hydroxy derivative integriquinolone (**18**), which is a constituent of *Zanthoxylum integrifoliolum* (*22*), and daurine (**20**) and folidine (**21**), obtained by Yunusov and his collaborators from *Haplophyllum dauricum* (*26*) and from *H. foliosum* (*27*), respectively. The structure of integriquinolone was determined by spectroscopy and by reaction with diazoethane to

TABLE I
SIMPLE ALKALOIDS RELATED TO 4-HYDROXY-2-QUINOLINONE

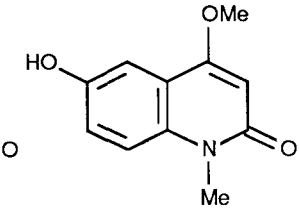
Compound	Structural number	Source	Reference
4-Methoxy-2-quinolinone	**14**	*Hortia longifolia*	*13*
4-Methoxy-1-methyl-2-quinolinone	**15**	*Afraegle paniculata*	*14*
		Euodia lanu-ankenda	*15,16*
		Haplophyllum dauricum	*17*
		Hortia longifolia	*13*
		Myrtopsis sellingii	*18*
		Sarcomelicope glauca	*229*
		Zanthoxylum ailanthoides	*19*
		Z. cuspidatum	*20*
		Z. decaryi	*21*
		Z. integrifoliolum	*22*
Edulitine	**16**	*Haplophyllum dauricum*	*17*
Folinine	**17**	*Haplophyllum dauricum*	*17*
		H. obtusifolium	*23*
		H. perforatum	*24*
Integriquinolone	**18**	*Zanthoxylum integrifoliolum*	*22*
Swietenidine-A	**25**	*Chloroxylon swietenia*	*25*
Swietenidine-B	**27**	*Chloroxylon swietenia*	*25*
Daurine	**20**	*Haplophyllum dauricum*	*26*
Folidine	**21**	*H. foliosum*	*27*



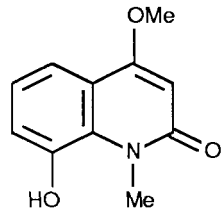
14 R = H
15 R = Me



16 R = H Edulitine
17 R = Me Folinine

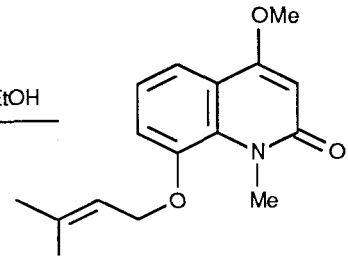


18 Integriquinolone



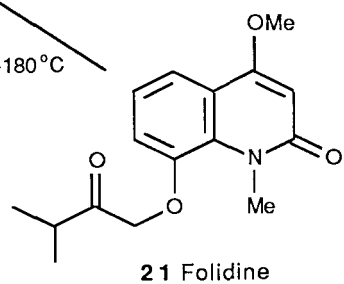
19 Folifidine

HCl, EtOH



20 Daurine

NaOH
at 160-180 °C

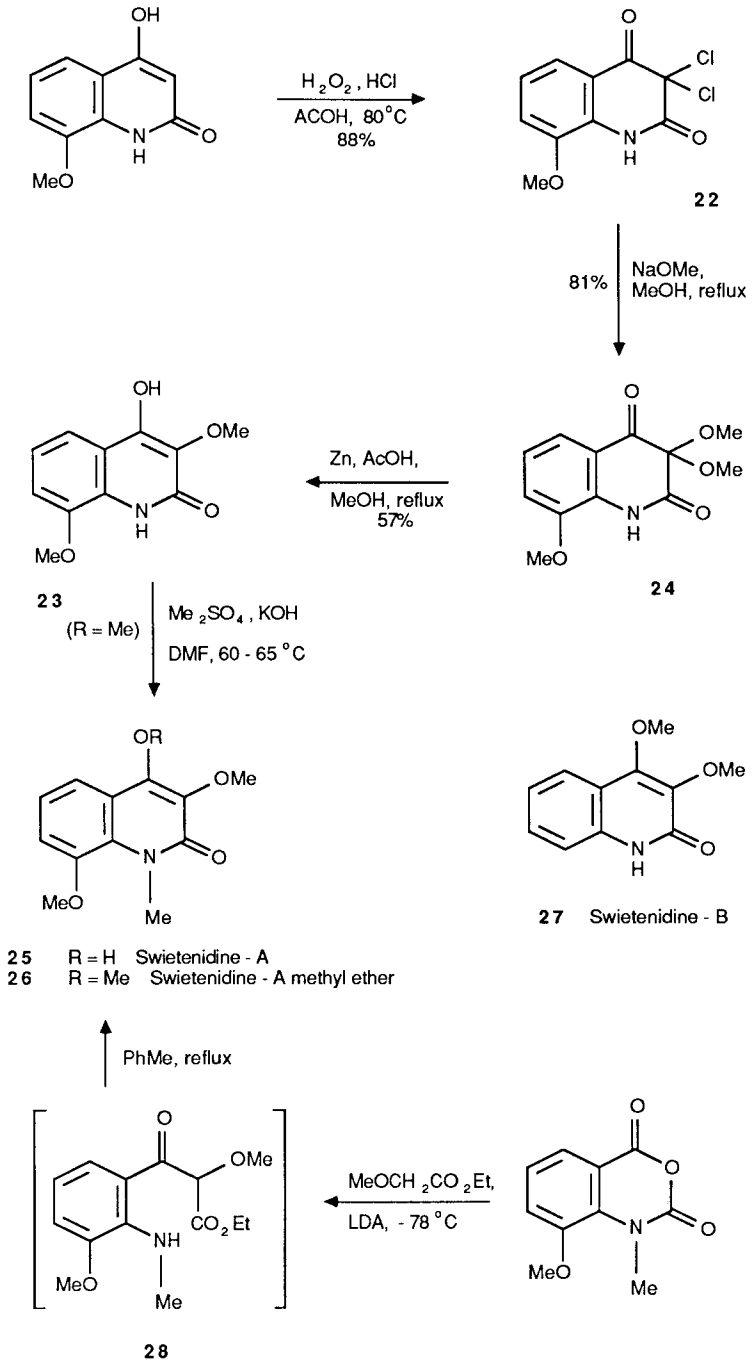


21 Folidine

give the *O*-ethyl ether; the ^{1}H-NMR spectrum of the latter compound indicated the presence of OEt, OMe, and NMe groups, three aromatic hydrogen atoms, and hydrogen at C-3 producing a singlet at δ6.02.

Daurine, mp 117–118°C, was shown by IR absorption at 1650 cm^{-1} and by the UV spectrum to be a 2-quinolinone. The ^{1}H-NMR spectrum indicated that daurine was a methoxy *N*-methylquinolinone containing an *O*-prenyl group, and structure **20** was established by hydrolysis with ethanolic hydrochloric acid to folifidine (**19**) (*26*). The structure of the related alkaloid, folidine (**21**), was also determined by spectroscopy and by its conversion into folifidine (**19**) (*27*).

The most interesting new alkaloids of the group are the 3-methoxy-2-quinol-

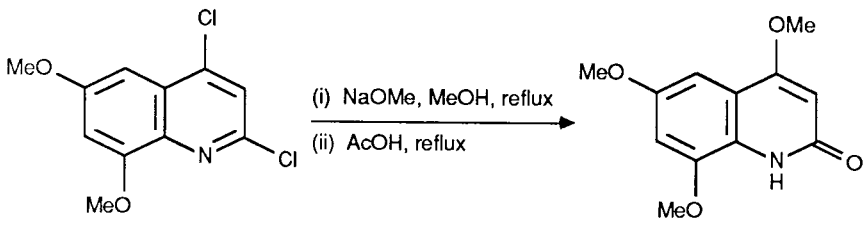


SCHEME 4. Structure and synthesis of swietenidines.

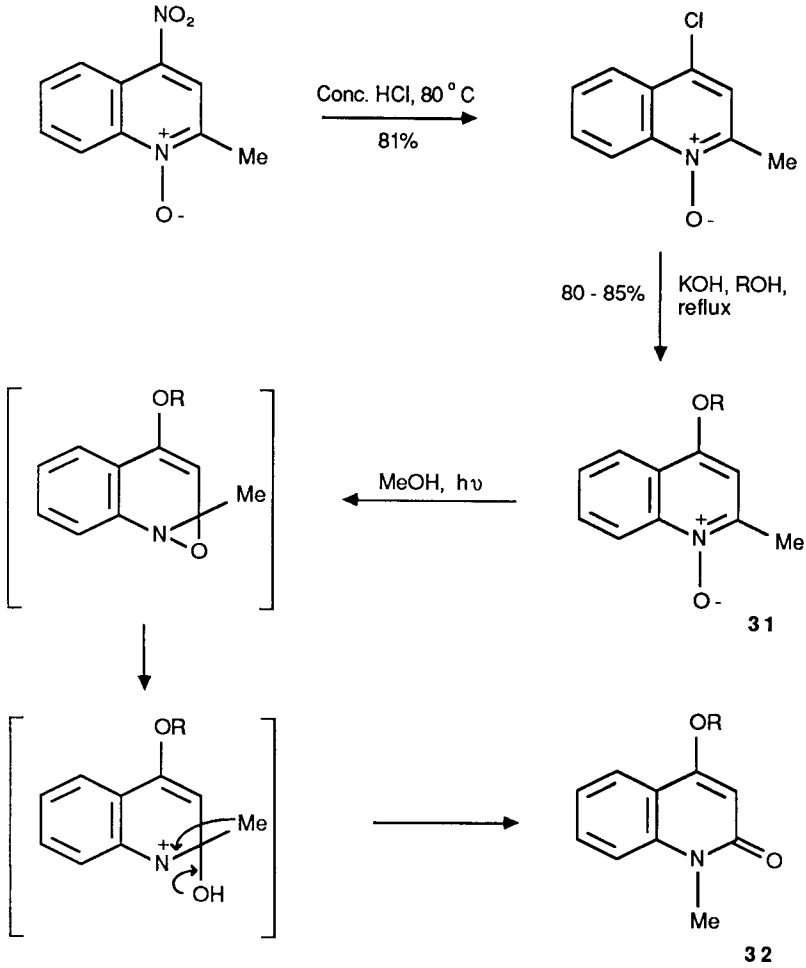
inones swietenidine-A (**25**) and swietenidine-B (**27**), isolated from the bark of *Chloroxylon swietenia* (East Indian satinwood) by Bhide and co-workers (*25*). The structures of the two alkaloids were determined by spectroscopic studies and by synthesis. Swietenidine-A, mp 158–159°C, was shown to be a 4-hydroxy-2-quinolinone derivative by its UV spectrum, by IR absorption at 3450 (OH) and 1640 cm^{-1} (–NHCO- in a 2-quinolinone), by its solubility in alkali, and by reaction with diazomethane to give a monomethyl ether (**26**). The NMR spectra of swietenidine-A and its methyl ether indicated that the alkaloid contained two methoxy groups and an *N*-methyl group (singlets at δ3.96, 3.90, and 3.86) and three adjacent aromatic protons at C-5, C-6, and C-7. The structure of swietenidine-B, mp 182°C, was apparent from its spectroscopic properties and the recognition that it had been obtained as a synthetic compound (*28*) before its isolation. The method used to prepare swietenidine-B was applied to swietenidine-A methyl ether (**26**) (*29*) (Scheme 4). Reaction of 4-hydroxy-8-methoxy-2-quinolinone with hydrogen peroxide and hydrochloric acid gave the 3,3-dichloro derivative **22** which was converted into the ketal **24;** reductive cleavage then gave the 4-hydroxy-3-methoxy-2-quinolinone **23,** which gave swietenidine-A methyl ether on dimethylation. Swietenidine-A itself was synthesized in 71% yield by reaction of an *N*-methylisatoic anhydride with the lithium enolate of ethyl methoxyacetate and cyclization of the crude product (**28**) by refluxing in toluene (Scheme 4) (*30*).

A new synthesis of halfordamine (**30**) (see Vol. 17, p. 109) reported by Mali *et al.* (*31*) involves reaction of 2,4-dimethoxyaniline with malonic acid and phosphorus oxychloride to give 2,4-dichloro-6,8-dimethoxyquinoline (**29**) which was converted into 2,4,6,8-tetramethoxyquinoline; refluxing with acetic acid then gave halfordamine.

Kaneko and co-workers (*32*) devised a new approach to the synthesis of 4-alkoxy-1-methyl-2-quinolinones by photorearrangement of 4-alkoxy-2-methylquinoline 1-oxides (Scheme 5). Irradiation of the *N*-oxide (**31;** R = Me), for example, gave 4-methoxy-1-methyl-2-quinolinone (**32;** R = Me) as the major product (55% yield), apparently by the mechanism indicated. Other 2-quinolinones have been obtained by this method (*cf.* Section VIIB).



29 **30**



SCHEME 5. Photochemical synthesis of 4-alkoxy-1-methyl-2-quinolinones.

III. 3-Prenyl-2-quinolinones and Related Alkaloids

A. INTRODUCTION

4-Hydroxy-3-prenyl-2-quinolinones were first prepared in connection with the synthesis and biosynthesis of quinoline alkaloids (Vol. 9, pp. 247, 257) but have since been isolated from natural sources (Vol. 17, p. 110 and Table I). This section will be concerned with the occurrence, structure, and synthesis of 3-

prenyl-2-quinolinones and their cyclization products; compounds derived by oxidation of 3-prenyl-2-quinolinones will be considered later (Section IV) and *O*-prenyl and *O*-geranyl ethers will be discussed in Section VII.

B. Occurrence and Structure

New alkaloids of the group and those obtained from additional sources are listed in Table II (*33–48* and *231*).

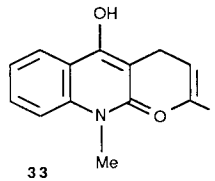
Alkaloids isolated for the first time during the last 10 years include 4-hydroxy-1-methyl-3-prenyl-2-quinolinone (**33**) from *Almeidea guyanensis* (*34*), which was obtained originally as a synthetic compound, and 8-hydroxy or 8-methoxy derivatives from *Glycosmis* species.

Thus, glycosolone (**36**) was isolated by Das and co-workers (*37*) from *Glycosmis pentaphylla* and shown to be a phenolic 2-quinolinone by its solubility in alkali, by the UV maxima at 263–298 nm unchanged on acidification, and by IR absorption at 3130 and 1635 cm^{-1}. The presence of a 3-methylbut-2-enyl

TABLE II
3-(3-Methylbut-2-enyl)-2-quinolinones and Related Alkaloids[a]

Compound	Structure number	Source	Melting point (°C)	Ref.
Atanine	**34**	*Afraegle paniculata*	—	*33*
4-Hydroxy-3-isopentenyl-1-methyl-2-quinolinone	**33**	*Almeidea guyanensis*	164	*34*
N-Methylatanine	**35**	*Almeidea guyanensis*	130	*34*
		Citrus grandis	—	*35*
		Melicope indica	130	*36*
Glycosolone	**36**	*Glycosmis pentaphylla*	159	*37*
Homoglycosolone	**36a**	*Glycomis pentaphylla*	168	*231*
Glycophylone	**42**	*Glycosmis pentaphylla*	151	*38*
Glycolone	**44**	*Glycosmis pentaphylla*	118	*39*
4,8-Dimethoxy-3-isopentenyl-1-methyl-2-quinolinone	**37**	*Glycosmis mauritiana*	—	*40*
		Glycosmis citrifolia	—	*41*
Preskimmianine	**46**	*Acronychia oligophylebia*	154–156	*42*
		Citrus grandis	—	*35*
		Dictamnus angustifolius	—	*43*
N-Methylpreskimmianine	**47**	*Vepris louisii*	88–89	*44*
Pteleprenine	**45**	*Ptelea trifoliata*	—	*48*
	48	*Haplophyllum bucharicum*	—	*45*
	49	*Esenbeckia flava*	oil	*46*
N-Methylkhaplofoline	**38**	*Almeidia guyanensis*	119–120	*34*
Ravesilone	**39**	*Ravenia spectabilis*	272	*47*

[a] This table is supplementary to Table I in Chapter 2 of *The Alkaloids* (*17*).



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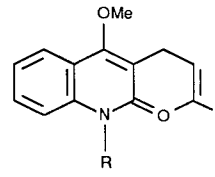
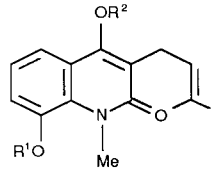
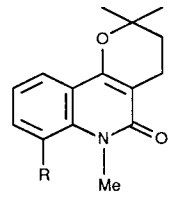


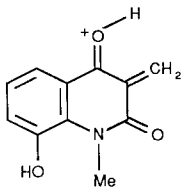
34 R = H Atanine
35 R = Me *N*-Methylatanine



36 R^1 = H, R^2 = Me Glycosolone
36a R^1 = H, R^2 = CH_2 CH_3 Homo-glycosolone
37 R^1 = Me, R^2 = Me



38 R = H *N*-Methylkhaplofoline
39 R = OH Ravesilone
40 R = OMe



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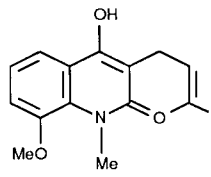
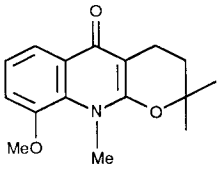


42 Glycophylone



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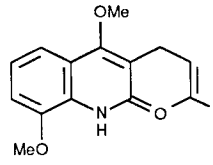
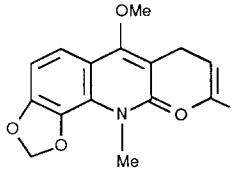
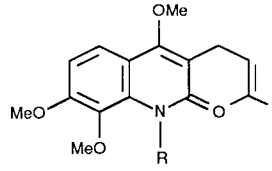


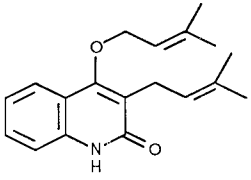
44 Glycolone



45 Pteleprenine



46 R = H Preskimmianine
47 R = Me *N*-Methylpreskimmianinne



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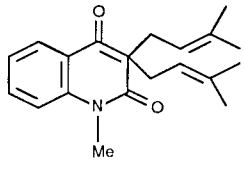

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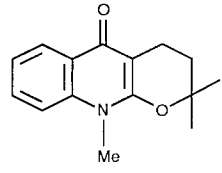


50

group in glycosolone was apparent from the ^{1}H- and ^{13}C-NMR spectra of the alkaloid. A resonance in the ^{13}C spectrum at δ35.87 is typical of an OMe group and one at 61.87 indicates that the NMe group responsible for this resonance is shielded by the OH group at C-8. In the mass spectrum, the parent ion peak appears at *m/z* 273 and a fragment peak at *m/z* 204 was attributed to ion **41.** On heating glycosolone with 6N hydrochloric acid, a compound, $C_{15}H_{17}NO_3$ (mp 274°C), was formed and was shown by spectroscopic methods and by its solubility in base to be the tricyclic derivative **39;** the reaction, which confirms the structure of glycosolone, appears to involve hydrolysis of the methoxy group at C-4 followed by cyclization with the prenyl group at C-3. The dihydropyranoquinolinone structure (**39**) was assigned subsequently to an alkaloid, ravesilone (mp 272°C), isolated from leaves of *Ravenia spectabilis* (*47*); ravesilone was later synthesized by refluxing the 8-methoxyquinolinone **40** with hydrogen bromide in acetic acid (*230*).

An alkaloid, $C_{17}H_{21}NO_3$, from *Gycosmis pentaphylla* was named homoglycosolone and assigned the 4-ethoxyquinolinone structure **36a** (*231*). The presence of an ethoxy group is not firmly established by the ^{1}H-NMR spectrum and further work on the structure of the alkaloid is needed.

The 8-methoxy-1-methyl-3-prenyl-2-quinolinone **37,** previously known as a synthetic compound, was isolated from *Glycosmis mauritiana* (*40*) and on heating with formic acid was converted into a mixture of dihydropyrano quinolinones (**40**) and (**43**) in approximately equal yields. The alkaloid was isolated subsequently from *G. citrifolia* (*41*).

The 4-hydroxy-8-methoxy-1-methyl-3-prenyl-2-quinolinone **42** (glycophylone) (*38*) and the isomeric 4,8-dimethoxy-3-prenyl-2-quinolinone (**44**) (glycolone) (*39*) are also constituents of *Glycosmis pentaphylla.* The structures of these alkaloids were assigned from their spectroscopic properties and those of the acid cyclization products, but the position of the aromatic methoxy group remained uncertain; however, the near correspondence of melting points of the alkaloids and their derived pyrano-derivatives with synthetic samples in the case of glycophylone (**42**) and glycolone (**44**) provides good support for the proposed structures.

The 4-methoxy-1-methyl-7,8-methylenedioxy-3-prenyl-2-quinolinone pteleprenine (**45**), was isolated from *Ptelea trifoliata* (*48*) and the corresponding 7,8-dimethoxy derivative, *N*-methylpreskimmianine (**47**), was obtained from *Vepris louisii* (*44*). The structures of these two alkaloids were determined by spectroscopic methods and, in the case of compound **47,** by its formation from preskimmianine (**46**) by methylation with methyl iodide and potassium carbonate in boiling acetone.

N-Methylkhaplofoline (**38**), which was first prepared by methylation of khaplofoline and later synthesized (Vol. 9, p. 255), has now been isolated by Wirasutisna and co-workers from *Almeidea guyanensis* (*34*).

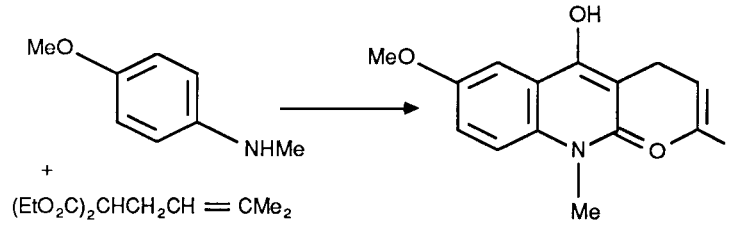
The *C*-, *O*-diprenyl alkaloid **48** of *Haplophyllum tuberculatum* (*see* Vol. 17, p. 112) is also a constituent of *H. bucharicum* (*45*); additional reactions of the compound are discussed later (Section VII, D). Another example of the preparation of an alkaloid before its isolation is the *C,C*-diprenyl derivative **49,** which was a principal product from the reaction of 4-hydroxy-1-methyl-2-quinolinone with 3,3-dimethylallyl bromide (*49*) and has now been found in the wood of *Esenbeckia flava* (*46*). When heated with perchloric acid, compound **49** was converted into the dihydropyranoquinolinone **50.**

C. Synthesis

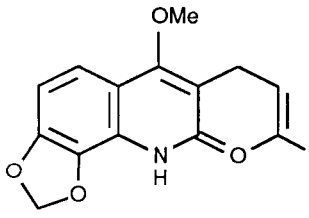
1. Synthesis from Prenylmalonates

The traditional method of preparing 3-prenyl-2-quinolinones involves refluxing aromatic amines and diethyl (3-methylbut-2-enyl) malonate (**51**) in diphenyl ether (Vol. 9, p. 247). Although yields are not high (12–37%), selective methylation increases the scope of the reaction and the method continues to be used. Thus, the 3-prenyl-2-quinolinone **52,** which is an intermediate in the synthesis of *O*-methylribaline (*cf.* Section IV, C, 1), was obtained from 4-methoxy-*N*-methylaniline (37% yield) (*50*). The alkaloids **53** (*51*) and **54** (*52*) were prepared by brief methylation of the corresponding 4-hydroxy compounds with diazomethane.

Young and co-workers (*53*) carried out an interesting study of the by-products formed in the reaction of aromatic amines with diethyl (3-methylbut-2-enyl)mal-



51 **52**



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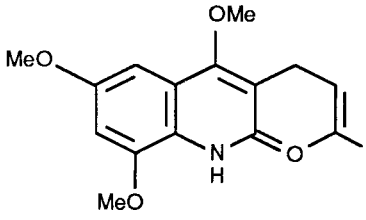
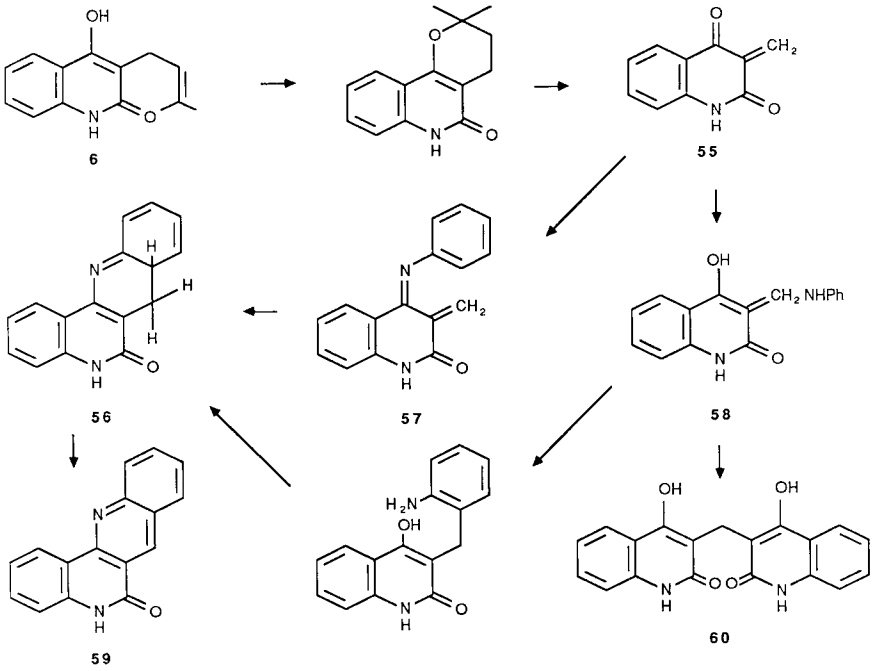


54

onate and identified dibenzo[b,h] [1, 6] naphthyridin-6(5H)-ones (*cf.* **59**) and 3,3′-methylenebis-4-hydroxy-2-quinolinones (*cf.* **60**). Since reaction of the 3-prenyl-2-quinolinones (*cf.* **6**) with the amines gave the same tetracyclic products it seemed that the quinolinones were intermediates. The mechanism proposed is given in Scheme 6 for the case of the products from aniline. The quinone methide **55,** formed by retro-Diels–Alder cleavage, may react with aniline to give intermediates **57** or **58,** which could both be transformed into tetracyclic derivative **56,** easily oxidized to the naphthyridinone **59.** In the reaction of 2,4-dimethoxyaniline with diethyl (3-methylbut-2-enyl)malonate, the isolation of a 4-hydroxy-3-methyl-2-quinolinone (*54*), which could arise from an oxidation–reduction reaction with compounds of type **56** and **55,** provides some support for the mechanism. It was also suggested that reaction of 4-hydroxy-2-quinolinone (formed, for example, by a retro-Mannich reaction of compound **58**) with the quinone methide **55** might yield the bisquinolinone **60** (*53*).

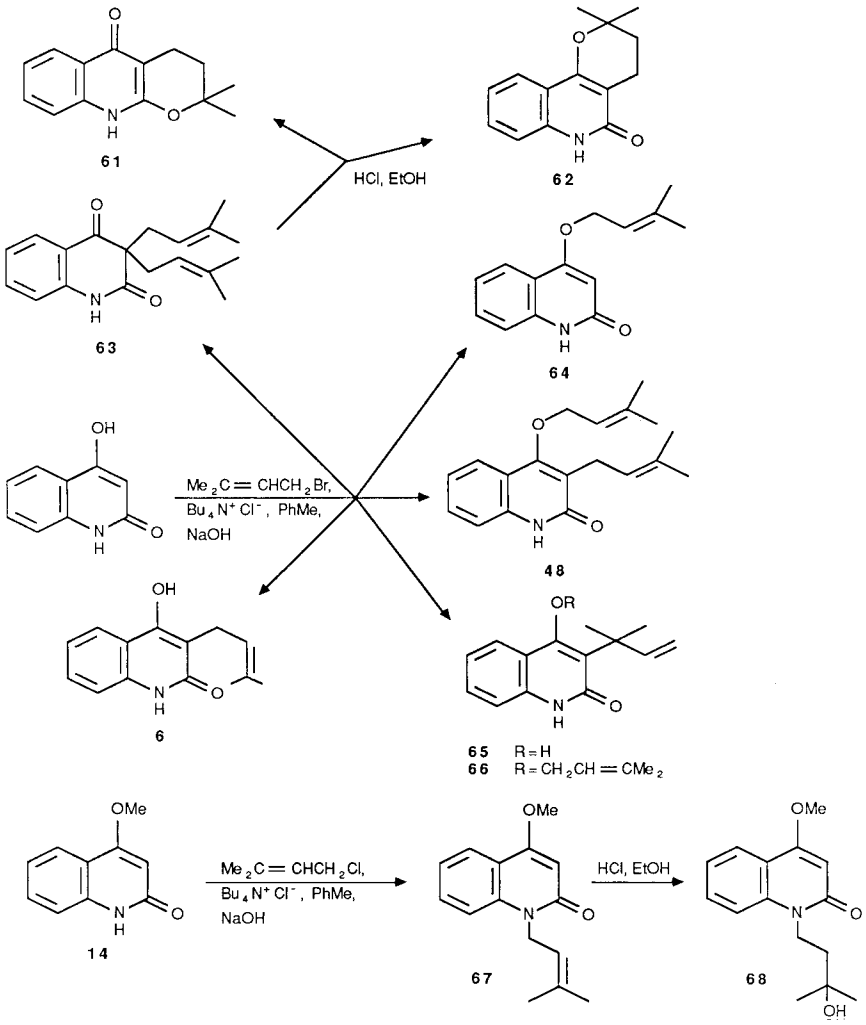
2. *Direct Allylation of Quinoline Derivatives*

Direct allylation of 4-hydroxy-2-quinolinone with 3,3-dimethylallyl bromide and base gives 3-prenyl-2-quinolinones in only 11% yield (*55*), although this is a convenient method. A similar reaction with 4-hydroxy-1-methyl-2-quinolinone

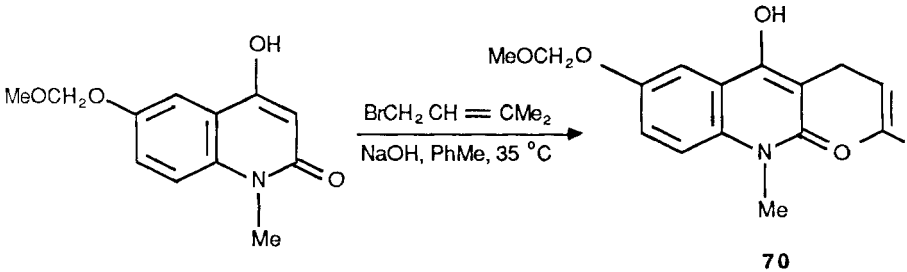


SCHEME 6. Byproducts of the reaction of aniline and diethyl prenylmalonate.

gives alkaloid **33** in low yield (*49*). A systematic study of the allylation of 4-hydroxy-2-quinolinone using a phase-transfer catalyst has been carried out by Reisch and co-workers (*56*) and yielded the bisprenyl derivative **63** (50%) and five other products **64, 48, 6, 65** and **66** (Scheme 7). Similar allylation of 4-methoxy-2-quinolinone (**14**) gave the *N*-allylquinolinone **67** which was hydrated to compound **68.** The bisprenyl derivative **63** was converted into a mixture of khaplofoline (**61**) and its angular isomer **62** on treatment with acid.



SCHEME 7. Reaction of 4-hydroxy-2-quinolinone with 3,3-dimethylallyl bromide.



69 **70**

A recent example of allylation of a 4-hydroxy-2-quinolinone is the preparation of compound **70** (59% yield) from the protected derivative **69** in one stage of the synthesis of ribaline (Section IV, C, 1) (*57*).

3. Synthesis from Isatoic Anhydrides

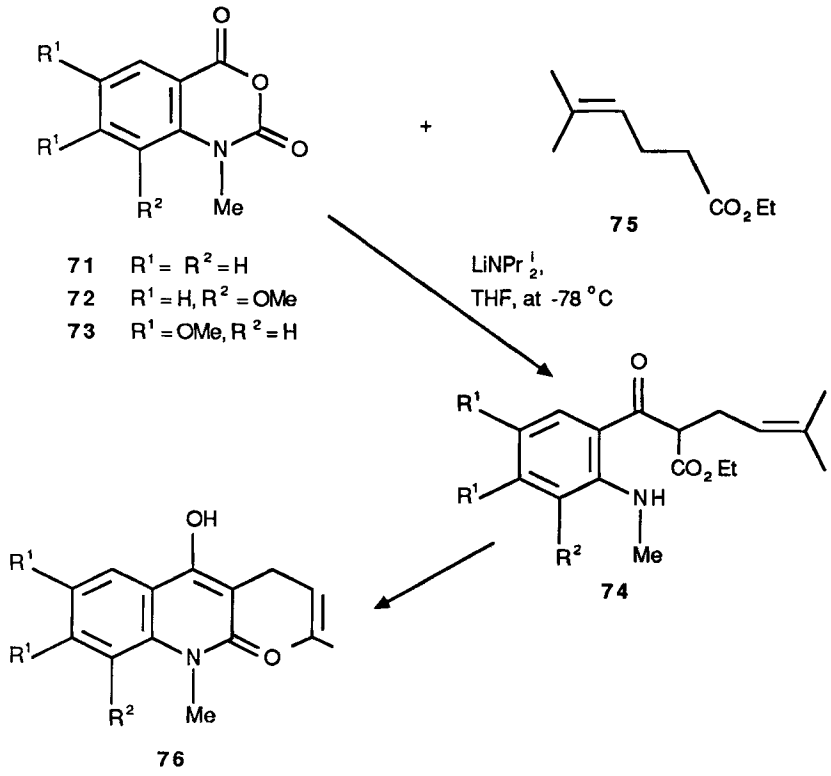
A synthesis of 3-prenyl-2-quinolinones from *N*-methylisatoic anhydride was described earlier (Vol. 17, p. 111) and a new approach from the same compounds **71–73** has now been devised by Coppola (*58*) (Scheme 8). The ester **75,** which was obtained by alkylation of lithio ethyl acetate with 3,3-dimethylallyl bromide, was converted into its lithium enolate with LDA; reaction with the isatoic anhydrides gave intermediates **74,** which were cyclized to the prenylquinolinones **76** with overall yields from the isatoic anhydrides of 45–52%.

4. Synthesis from 3-Carboxymethyl-2-quinolinones

Shanmugam and co-workers (*59, 60*) have introduced an interesting new synthesis of 3-(3-methylbut-2-enyl)-2-quinolinones and their 3-methylbut-1-enyl isomers which is illustrated in Scheme 9 with the 4,8-dimethoxy compounds. Aldol condensation of the 3-carboxymethyl-2-quinolinone **77** with 2-methylpropanal gives the lactone **78,** which is hydrolyzed to the carboxylic acid **80,** both stages occurring in high yield. Decarboxylation then furnished glycolone (**44**) (37%) and its isomer **79.** The synthesis has been applied to the 3-carboxymethyl-4-methoxy-2-quinolinone without a substituent in the aromatic ring and to 3-carboxymethyl-4-hydroxy-2-quinolinones (**81**)–(**84**). The inaccessibility of starting compounds for the synthesis of 3-prenyl-2-quinolinones containing substituents in the aromatic ring is a problem for this as for other methods of synthesis; compound **77,** for example, was prepared in six steps from 7-methoxyisatin.

5. Synthesis by Photochemical Prenylation

Momose and Kaneko (*61*) employed photochemical prenylation to prepare a 3-prenylquinolinone and its cyclization product (Scheme 10). Thus the 2 + 2 head-to-tail adduct **85** was obtained as a mixture of stereoisomers by irradiation of 4-



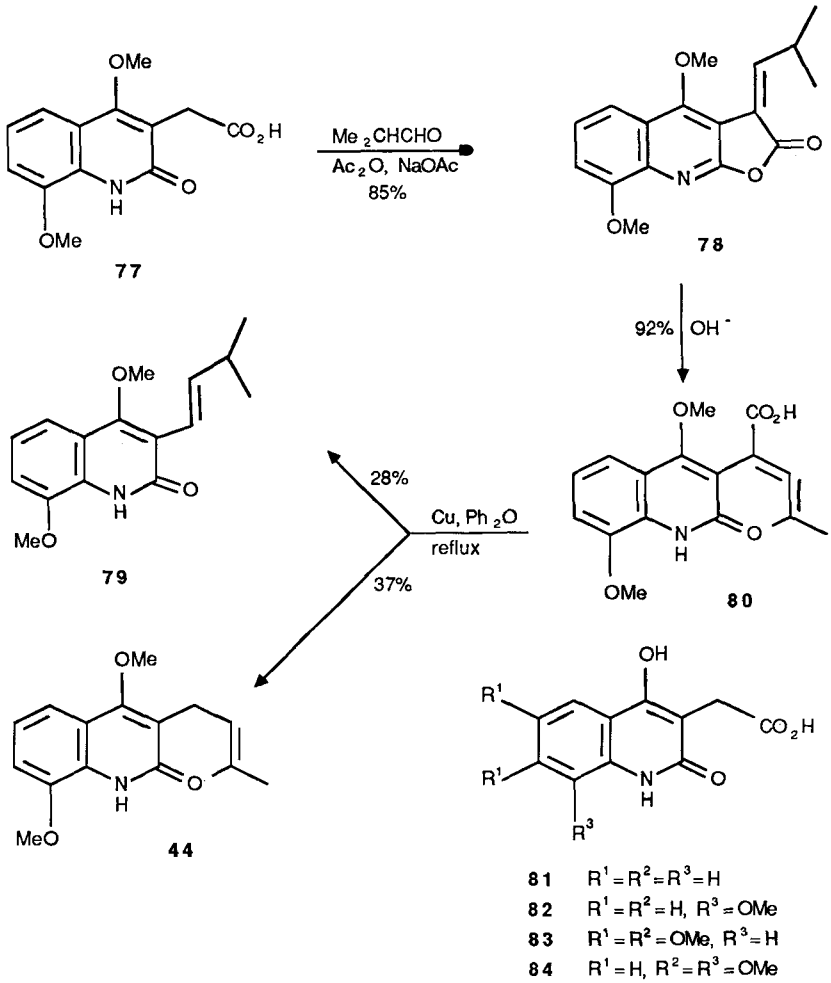
SCHEME 8. Synthesis of 3-prenyl-2-quinolines from isatoic anhydrides.

acetoxy-2-quinolinone and 2-methylbut-3-en-2-ol in methanol; acid-catalyzed cleavage then gave 4-hydroxy-3-prenyl-2-quinolinone (32% yield), and by alternative loss of acetate, the cyclobutaquinolinone **86.** 4-Methoxy-2-quinolinone reacted similarly to give the adduct **87,** which with formic acid gave khaplofoline (**61**) in 50% yield and the 4-methoxydihydropyrano-quinoline **88** as a minor product.

IV. Tricyclic Alkaloids Derived by Oxidation of 3-Prenyl-2-quinolinones and Related Compounds

A. INTRODUCTION

The occurrence, structure, and synthesis of dihydrohydroxyisopropylfuroquinolines and dihydrodimethylhydroxypyranoquinolines were discussed previously (Vol. 17, p. 109) and terminal olefins and bicyclic oxidation products of

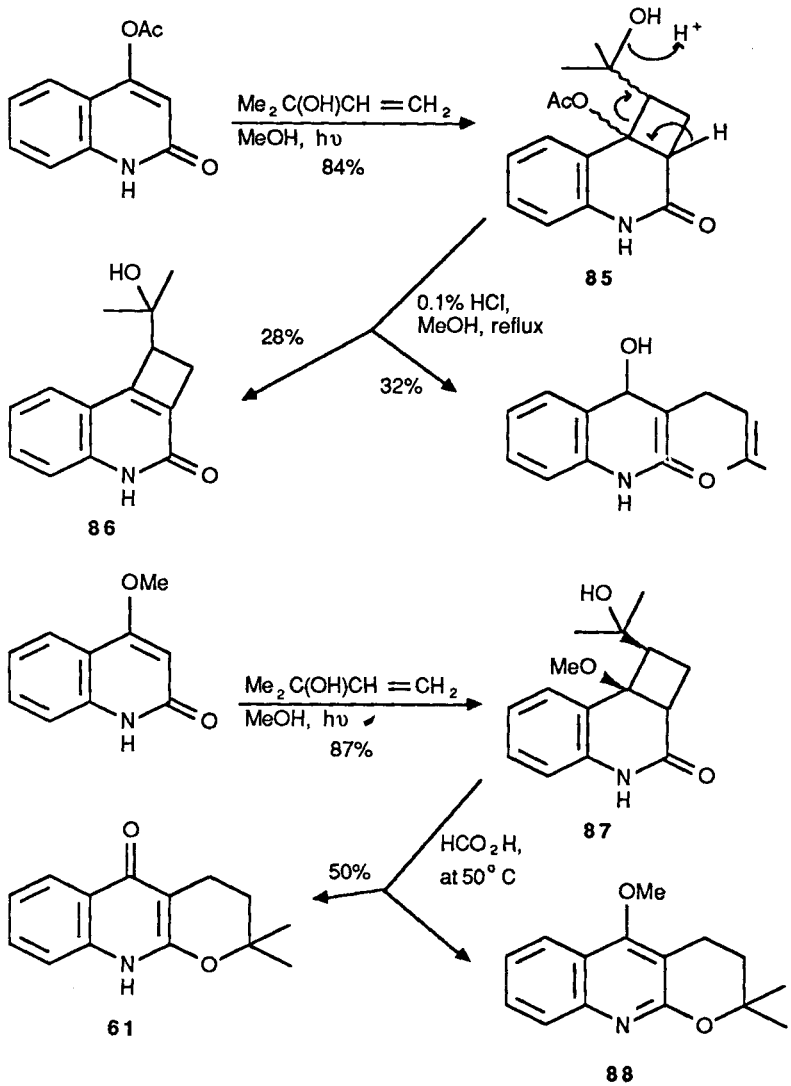


SCHEME 9. Synthesis of 3-(3-methylbut-2-enyl)-2 quinolinones and 3-(3-methylbut-1-enyl)-2 quinolinones from 3-carboxymethyl-2 quinolinones.

3-prenyl-2-quinolinones were included in the account. As well as structural work on new alkaloids, studies of the asymmetric synthesis, absolute stereochemistry, and rearrangement reactions were featured in research published in the period 1966–1976. During the last 10 years these themes have continued, but the greatest advances have occurred in the development of new methods of synthesis.

B. OCCURRENCE AND STRUCTURAL STUDIES

Data published 1976–1986 are given in Table III (*18*, *22*, *24*, *34*, *62–73*).



SCHEME 10. Photochemical prenylation of 4-acetoxy-2-quinolinone and 4-methoxy-2-quinolinone.

1. *Platydesmine, N-Methylplatydesminium Salts, Ribalinine, Geibalansine, Araliopsine, Edulinine, and Myrtopsine*

The 4-methoxyquinoline alkaloid platydesmine (**9**), which was shown to be a crucial intermediate in the biosynthesis of furoquinoline alkaloids (Vol. 17,

TABLE III

DIHYDROFUROQUINOLINES, DIHYDROPYRANOQUINOLINES, AND RELATED ALKALOIDS[a]

Compound	Structure number	Source	Melting point (°C)	$[\alpha]_D$ (Solvent)	Ref.
Unsubstituted in aromatic ring					
N-Methylplatydesminium chloride	**89**	*b*	192–196	—	*62*
N-Methylplatydesminium perchlorate		*b*	200–203	+16.2°(MeOH)	*62*
		c	180	−24.3°(MeOH)	*62*
Platydesmine	**9**	*d*	135–137	$[\alpha]_{578}$ +39°($CHCl_3$)	*63*
		e	138–139	—	*24*
		f	137–138	+43.8°(MeOH)	*22*
		g	—	0°	*64*
Ribalinine (folifine)	**90**	*h*	235–236	—	*65*
		i	238–240	0°	*66*
Geibalansine	**91**	*j*	179	−2°(EtOH)	*67*
Geibalansine acetate	**92**	*j*	amorphous	—	*67*
Edulinine	**116**	*h*	103–105	−17°($CHCl_3$)	*68*
		i	99–102	−23°	*66*
		g	—	—	*64*
Araliopsine	**113**	*i*	141–143	−4°	*66*
Almeine	**151**	*k*	amorphous	—	*34*
Dubinidine	**152**	*e*	—	—	*24*
Myrtopsine	**93**	*l*	208	−5°(MeOH)	*18*
		f	100–105	0°(MeOH)	*22*
		m	201–202	−5°(MeOH)	*69*
6-Hydroxy and 6-methoxy compounds					
Ribalinium perchlorate	**94**	*b*	205	+23.6(MeOH)	*62*
Ribaline	**109**	*n*	268–269	0°	*70*
		n	>210–211	$[\alpha]_{589}$ +72.1 $[\alpha]_{578}$ +75.5 $[\alpha]_{546}$ +86.6 $[\alpha]_{436}$ +157.3 } (MeOH)	*70*
Ribalinidine	**95**	*o*	265		*71*
		n	260–261	$[\alpha]_{589}$ +15.3 $[\alpha]_{578}$ +16.5 $[\alpha]_{540}$ +19.6 $[\alpha]_{436}$ +41.6 } (MeOH)	*70*

(*continued*)

TABLE III (*Continued*)

Compound	Structure number	Source	Melting point (°C)	$[\alpha]_D$ (Solvent)	Ref.
Riedelianine	**96**	*n*	257	$[\alpha]_{589}$ +25.3 $[\alpha]_{578}$ +26.2 $[\alpha]_{540}$ +28.9 (MeOH)	*70*
8-Methoxy compounds					
O-Methylbalfourodinium chloride	**98**	*c*	184–185	−15.3(MeOH)	*62*
O-Methylbalfourodinium perchlorate		*c*	200–204	−14.64(MeOH)	*62*
7,8-Methylenedioxy compounds					
O-Methylhydroxyluninium chloride	**99**	*p*	225	−15.6(MeOH)	*62*
O-Methylhydroxyluninium perchlorate		*p*	176–178	—	*62*
7,8-Dimethoxy compounds					
Veprisinium iodide	**101**	*q*	126–127	−12.2(MeOH)	*72*
Veprisinium perchlorate		*q*	171–172	−11.8(MeOH)	*72*
Veprisilone	**100**	*q*	135–136		*73*
O-Methylisoptelefolonium perchlorate	**150**	*p*	146–148	+9.83(MeOH)	*62*
6,8-Dimethoxy compounds					
O-Methylptelefolonium chloride	**12**	*p*	135	+15.28(MeOH)	*62*
O-Methylptelefolonium perchlorate		*p*	254–256	—	*62*

[a] This table is supplementary to Table II in Chapter 6 of *The Alkaloids*(9) and to Table II in Chapter 2 of The Alkaloids (17).

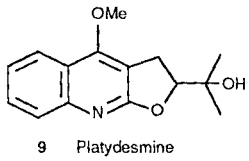
[b] *Ruta graveolens* [c] *Choisya ternata* [d] *Flindersia fournieri*
[e] *Haplophyllum perforatum* [f] *Zanthoxylum integrifoliolum* [g] *Amyris diatripa*
[h] *Fagara mayu* [i] *Zanthoxylum simulans* [j] *Geijera balansae*
[k] *Almeidea guyanensis* [l] *Myrtopsis sellingii* [m] *Haplophyllum foliosum*
[n] *Balfourodendron riedelianum* [o] *Ruta chalepensis* [p] *Ptelea trifoliata*
[q] *Vepris louisii*

p. 186) has been isolated in the form of its dextrorotatory enantiomer from *Flindersia fournieri* (*63*) and *Zanthoxylum integrifoliolum* (*22*). Platydesmine has also been isolated as a racemate from a *Haplophyllum* species for the first time (*24*) and from *Amyris diatripa* (*64*).

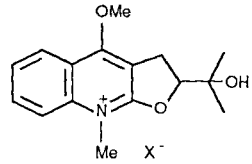
(+)-*N*-Methylplatydesminium salt (**89**) is present in *Araliopsis*, *Ruta*, and

Skimmia species (Vol. 17, Table II), and the (−)-enantiomer has been isolated from *Choisya ternata* as its perchlorate salt (*62*).

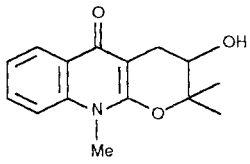
The dihydrohydroxypyrano-4-quinolinone ribalinine (folifine) (**90**) has been obtained from two new sources, *Zanthoxylum simulans* as a racemate (*66*) and *Fagara mayu* as a sample of unspecified optical rotation (*65*). The new alkaloid (−)-geibalansine was isolated from *Geijera balansae* and shown by spectroscopic studies to be the hydroxy-4-methoxydihydropyranoquinoline (**91**) iso-



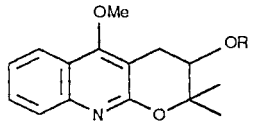
9 Platydesmine



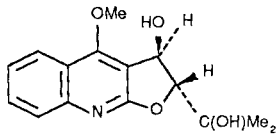
89 *N*-Methylplatydesminium



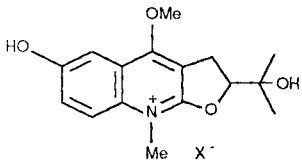
90 Ribalinine (Folifine)



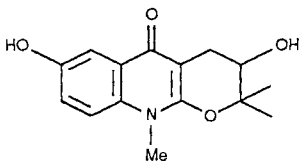
91 R = H Geibalansine
92 R = Ac *O*-Acetylgeibalansine



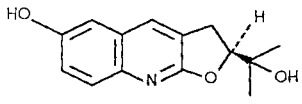
93 Myrtopsine



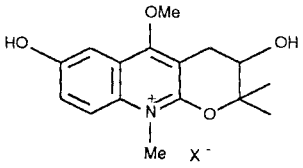
94 Ribalinium salt



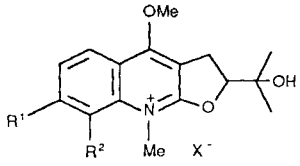
95 Ribalinidine



96 (+)- Riedelianine



97 Rutalinium salt



98 R^1 = H, R^2 = OMe *O*-Methylbalfourodinium salt
99 R^1, R^2 = OCH_2O *O*-Methylhydroxyluninium salt

meric with ribalinine (**90**); (±)-geibalansine had been synthesized previously. *O*-Acetylgeibalansine (**92**) was obtained from *G. balansae* as an amorphous solid (*67*).

Araliopsine (**113**), the only known dihydrohydroxyisopropylfuroquinoline alkaloid with angular annulation, was first obtained from *Araliopsis soyauxii* as the (+)-enantiomer (Vol. 17, p. 120) and (−)-araliopsine has since been isolated from *Zanthoxylum simulans* (*66*).

The diol edulinine (**116**), in the form of one of the enantiomers or as a racemate, is widely distributed and may be an artifact formed by hydrolysis of the quaternary salt *N*-methylplatydesminium (**89**). (−)-Edulinine has been obtained more recently from *Fagara mayu* (*68*) and *Zanthoxylum simulans* (*66*); the alkaloid was also isolated from *Amyris diatripa* (*64*), but in this case the optical rotation was not recorded.

(−)-Myrtopsine, which was first isolated from *Myrtopsis sellingii*, was shown to be a hydroxy-platydesmine (**93**) (*18*). The presence of a secondary and a tertiary hydroxy group was apparent from the formation of a monoacetate and, under more vigorous conditions, a diacetate; the gross structure of the alkaloid was determined from its ^{1}H-NMR spectrum and that of the monoacetate, but not the stereochemistry. (−)-Myrtopsine has since been found in *Haplophyllum foliosum* (*69*) and as the racemate in *Zanthophylum integrifoliolum* (*22*). Akhmedzhanova and Bessonova (*69*) obtained well-resolved ^{1}H-NMR spectra of myrtopsine and its monoacetate and noted that the only significant difference in the spectrum of the alkaloid from that of platydesmine (**9**) was the presence of a one-proton doublet at δ5.64 (J = 3 Hz, C*H*-OH); the shift in this resonance in the acetate to 6.74 (J = 1.5 Hz, C*H*-OAc) is that expected for the acetylation of a secondary alcohol. Theoretical values for the vicinal coupling constants for the α and β protons of the dihydrofuran ring are 10 and 3.8 for the *cis*- and *trans*-arrangements, respectively. These values indicate that in myrtopsine (J = 3 Hz) the substituent OH and C(OH)Me$_2$ have a *trans*-configuration; this accords with the proposal that myrtopsine or one of its derivatives is an intermediate in the *in vivo* conversion of platydesmine into the furoquinoline alkaloid dictamnine (see Vol. 17, p. 186).

2. *Ribalinium and Rutalinium Salts, Ribaline, Ribalinidine, and Riedelianine*

The 6-hydroxy alkaloids ribalinium salt (**94**), (+)-ribaline (**109**), and (−)-ribalinidine (**95**) were discovered by Corral and Orazi in the bark of Argentinian *Balfourodendron riedelianum;* the isolation of (±)-ribaline indicated that some racemization had occurred during isolation of the alkaloids or that, more likely, *in vivo* formation was not fully stereospecific (*cf.* p. 21). In a recent study of the heartwood of *B. riedelianum*, Jurd and Wong (*70*) again isolated (+)-ribaline as principal alkaloid, and as minor constituents, (±)-ribaline and (+)-ribalinidine; the presence of the latter compound, instead of the (−)-enantiomer found in the

bark, is unexpected. (+)-Riedelianine (**96**), which is also present in the heartwood of *B. riedelianum* is unusual in containing no oxygen function at C-4; its structure was determined by single-crystal X-ray diffraction.

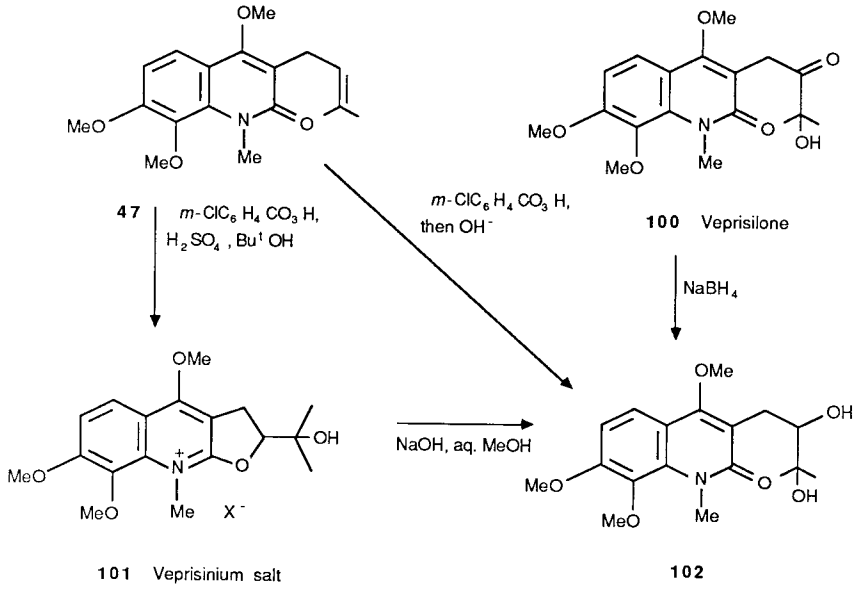
The quinolinium salts ribalinium (**94**) and rutalinium (**97**), which were isolated from *Ruta graveolens* (Vol. 17, Sect. IIIC.1 and C.2), also occur in the subspecies *hortensis* (*74*).

3. O-*Methylbalfourodinium and* O-*Methylhydroxyluninium Salts*

(+)-*O*-Methylbalfourodinium salt (**98**) is a constituent of *Balfourodendron riedelianum* and *Orixa japonica,* but the (−)-enantiomer has been isolated as its chloride and perchlorate from *Choisya ternata* (*62*). The 7,8-methylenedioxy derivative (−)-*O*-methylhydroxyluninium salt (**99**) was first obtained from *Ptelea trifoliata,* and the same enantiomer with similar specific rotation has been isolated from a different subspecies of this plant (*62*).

4. *Veprisinium Salts and Veprisilone*

Vepris louisii is a rich source of 7,8-dimethoxy quinoline alkaloids; two alkaloids of this type isolated from the stem bark by Ayafor, Sondengam, and Ngadjui (*72*), (*73*) are veprisinium salt (**101**) and veprisilone (**100**). The structures of the two alkaloids were determined by spectroscopic and chemical methods (Scheme 11). Thus, the nature of the side chain present in veprisilone was



SCHEME 11. Structures of veprisilone and veprisinium salt.

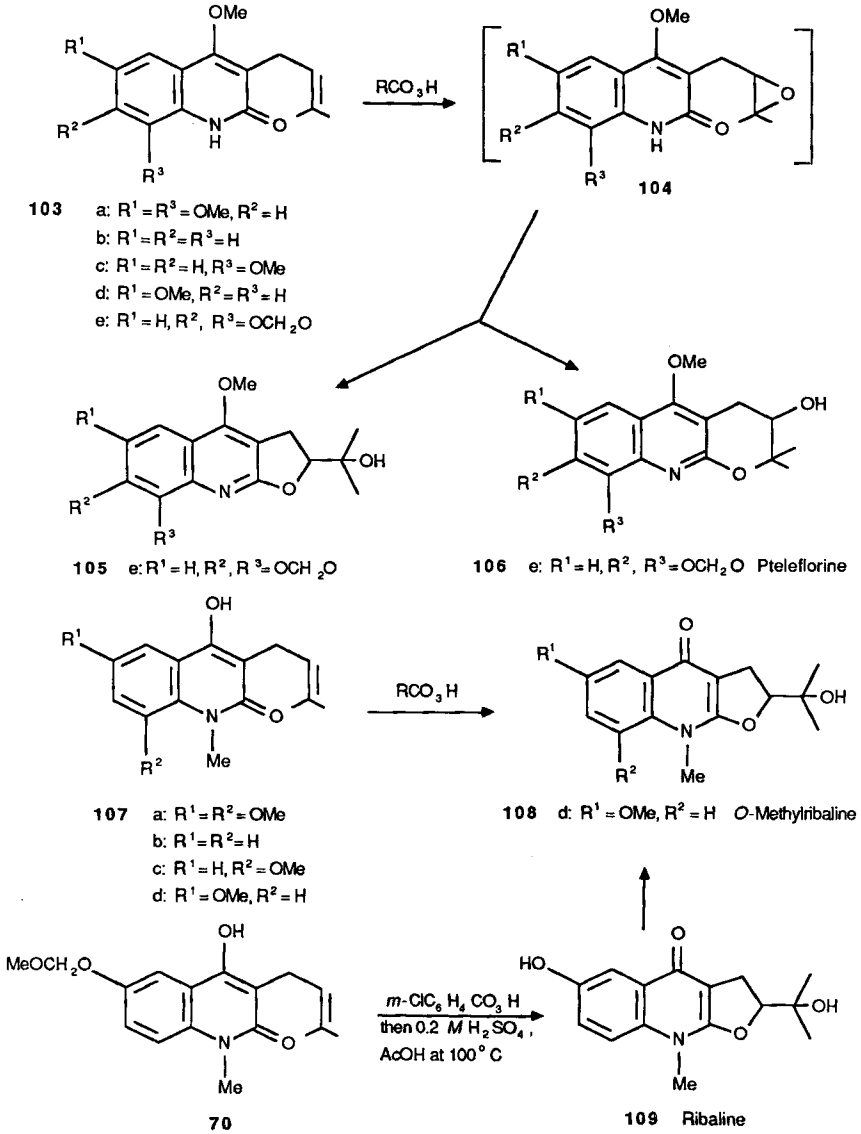
revealed by IR absorption at 3490 (OH), 1710 (ketonic C = 0), and 1640 cm^{-1} (quinolinone C = 0), by ^{1}H-NMR resonances at δ3.93 (2H, s, CH_2) and 1.50 (6H, s, $C(OH)Me_2$), and by the fragmentation peak at M^+-$C_4H_7O_2$, arising from benzylic cleavage. Reduction of veprisilone with sodium borohydride gave the diol **102,** which was obtained from *N*-methylpreskimmianine (**47**) by reaction with a peroxy acid and then base (*73*). When treatment with base was omitted from the latter reaction, veprisinium salt (**101**) was formed, and then with aqueous sodium hydroxide afforded the diol **102.** The structure of veprisinium salt was confirmed by the ^{1}H-NMR spectrum which showed resonances for *ortho*-coupled aromatic protons at δ7.98 and 7.60 (J = 4.9 Hz) and for the group $\geq CHCH_2$- at 5.20 (1H, t) and 3.90 (2H,d). Veprisinium salt is responsible for the significant antibacterial activity of the bark of *Vepris louisii* (*72*).

C. Synthesis of Hydroxyisopropyldihydrofuroquinolines and Hydroxydimethyldihydropyranoquinolines

1. Oxidative Cyclization of 3-Prenyl-2-quinolinones

The first and most widely used synthesis of dihydrofuroquinoline and dihydropyranoquinoline alkaloids involves reaction of 3-prenyl-2-quinolinones with peracids and a discussion of the stereoelectronic factors involved in the ring closure of the intermediate epoxides has been published (*50*) (Scheme 12). Treatment of 4-methoxy-3-prenyl-2-quinolones (**103**) with peracids under non-basic conditions gives a mixture of dihydrofuro- (**105**) and dihydropyranoquinolines (**106**) (Vol. 9, p. 249 and Vol. 17, p. 128), ring closure of epoxide **104** through the nucleophilic oxygen of the 2-quinolinone group apparently occurring by a 5-*exo*- or 6-*endo*-process; *exo*-modes are generally preferred on stereochemical grounds (*75*), but lack of discrimination in this case may be due to an electronic effect favoring ring closure at the tertiary carbon atom of the epoxides. A recent example involves oxidation of the 7,8-methylenedioxy-3-prenyl-2-quinolinone (**103e**) to give the dihydrofuroquinoline **105e** and the alkaloid pteleflorine (**106e**) (*51*). *N*-Methyl-4-hydroxy-3-prenyl-2-quinolinones (**107**) behave differently, and on reaction with peracids give dihydrofuroquinolinones (**108**) as the only products in 50–95% yield, even in the presence of strong acids (*cf.* preparation of veprisinium salts in Scheme 11). The reluctance to form pyrano-derivatives may be due to nonbonded interactions between the *N*-methyl group and the terminal $=CMe_2$ group in the transition state.

Reaction of the 3-prenyl-2-quinolinone **107d** with *m*-chloroperbenzoic acid furnished (±)-*O*-methylribaline (**108d**); since this compound was obtained from natural (±)-ribaline (**109**) and was correlated with ribalinidine (**95**) and ribalinium cation (**94**), the preparation provided synthetic confirmation of the structures of the three alkaloids. The six-stage preparation of ribaline (**109**) carried out by Corral, Orazi, and Autino (*57*) is the first example of the synthesis



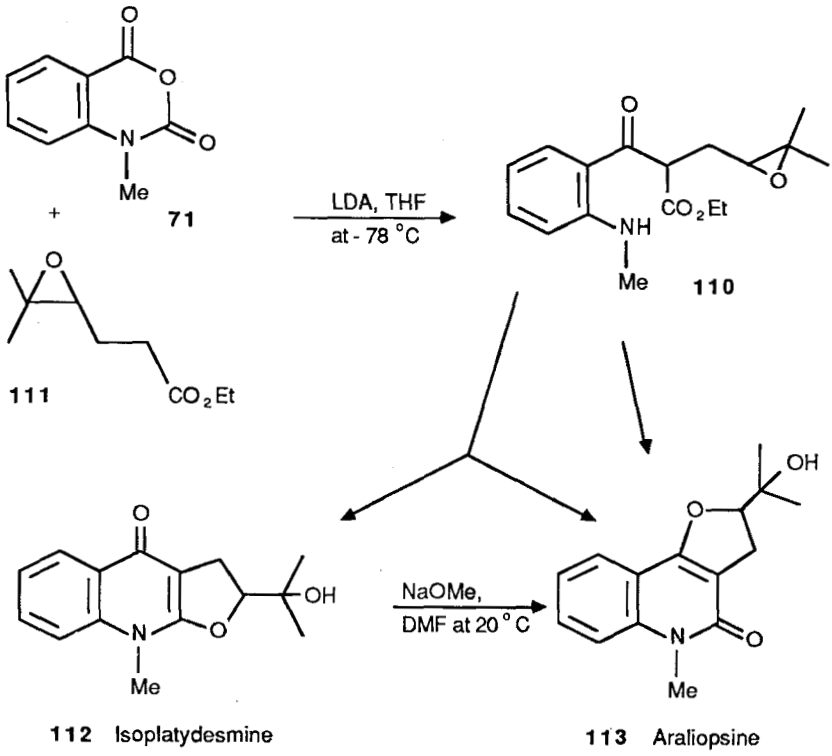
SCHEME 12. Synthesis of dihydrofuroquinilones and dihydropyranoquinolines by oxidative cyclization of prenylquinolinones.

of a hydroxyisopropyldihydrofuroquinolinone alkaloid containing a hydroxy function in the homocyclic ring. The protected prenylquinolinone **70** (*cf.* p. 357) reacted with a peracid to give a furoquinolinone which on removal of the methoxymethyl group with acid gave ribaline.

Oxidation of 4-methoxy-3-prenyl-2-quinolinone (**103b**) with a peracid gives a mixture of furo- and pyrano-quinolines (Scheme 12), but a more selective synthesis of geibalansine (**91**) (68% yield) was achieved by reaction of the 2-quinolinone **103b** with iodine and silver acetate, followed by acid hydrolysis (*76*).

2. *Synthesis from Isatoic Anhydride*

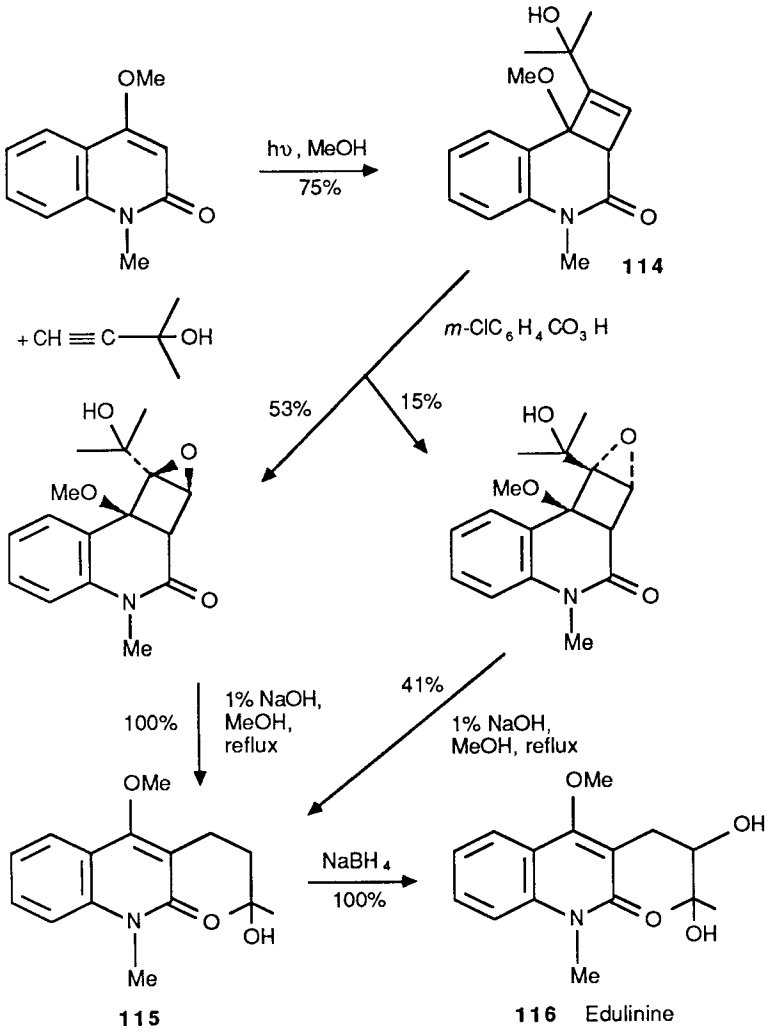
Alternative syntheses of the alkaloids araliopsine (**113**) and isoplatydesmine (**112**) have been described by Coppola (*77*) (Scheme 13). Epoxide (**111**), derived from the ester **75** (Scheme 8), reacted with isatoic anhydride **71** and LDA to give a good yield of intermediate **110,** which in a neutral medium furnished araliopsine (**113**) (33%), and in the presence of a peroxy-acid yielded a mixture of araliopsine (25%) and isoplatydesmine (11%). The first synthesis of araliopsine (82% yield) was carried out by rearrangement of isoplatydesmine with sodium methoxide in DMF (*78*) (*cf.* Section IV, D).



SCHEME 13. Synthesis of araliopsine.

3. *Photochemical Synthesis of Edulinine*

A three-step photochemical synthesis of the diol edulinine (**116**), was carried out by Naito and Kaneko (*79*) in an overall yield of 40% (Scheme 14). Irradiation of 4-methoxy-1-methyl-2-quinolinone and 2-methyl-3-butyn-2-ol in methanol resulted in 2 + 2 cyclo-addition to give the cyclobutene derivative **114.** Reaction of this compound with a peracid furnished a mixture of stereoisomeric epoxides



SCHEME 14. Photochemical synthesis of edulinine.

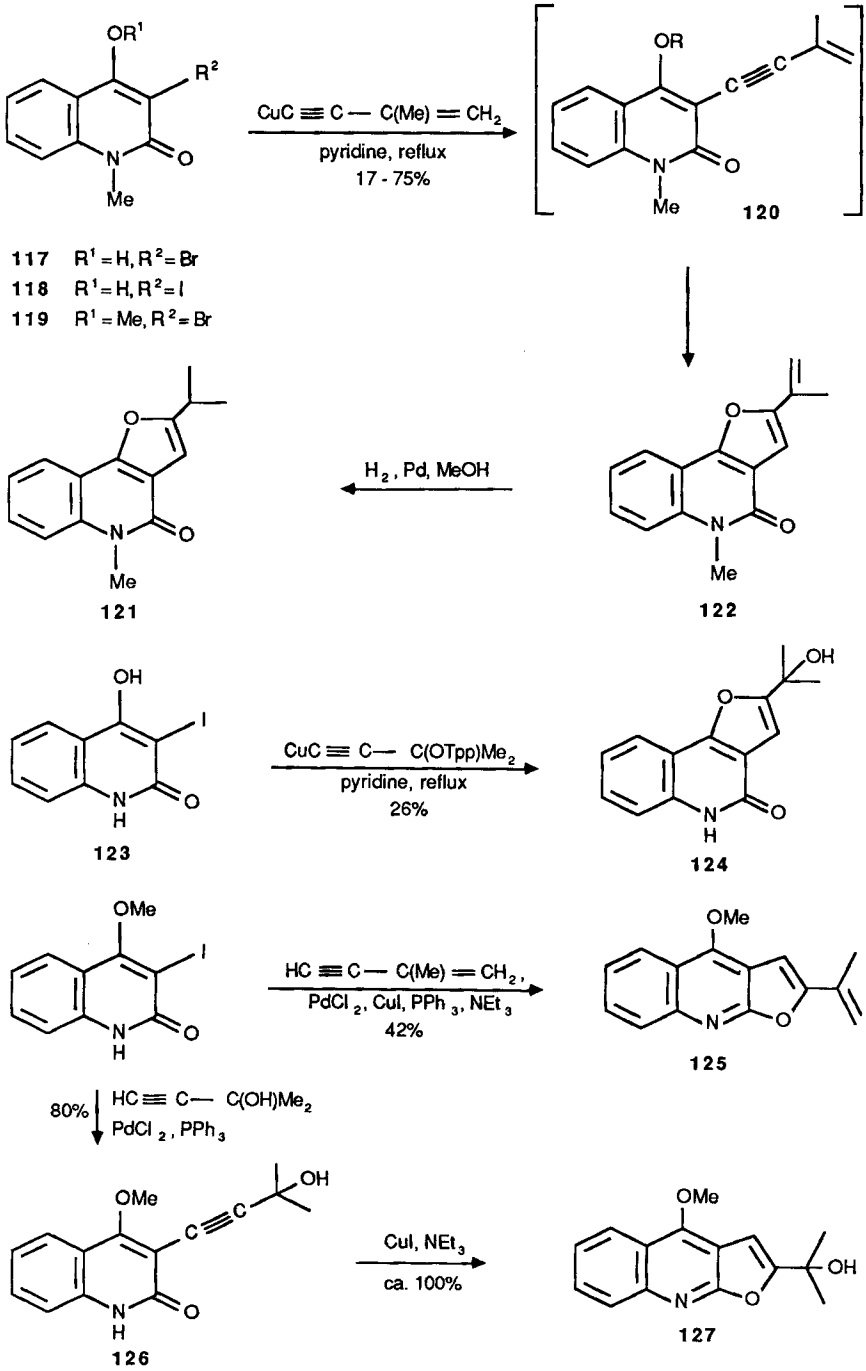
which were separately treated with base to give the α-hydroxyketone **115** (*cf.* the alkaloid veprisilone **100**), which was reduced with borohydride to edulinine.

4. Syntheses from Copper(I) Acetylides

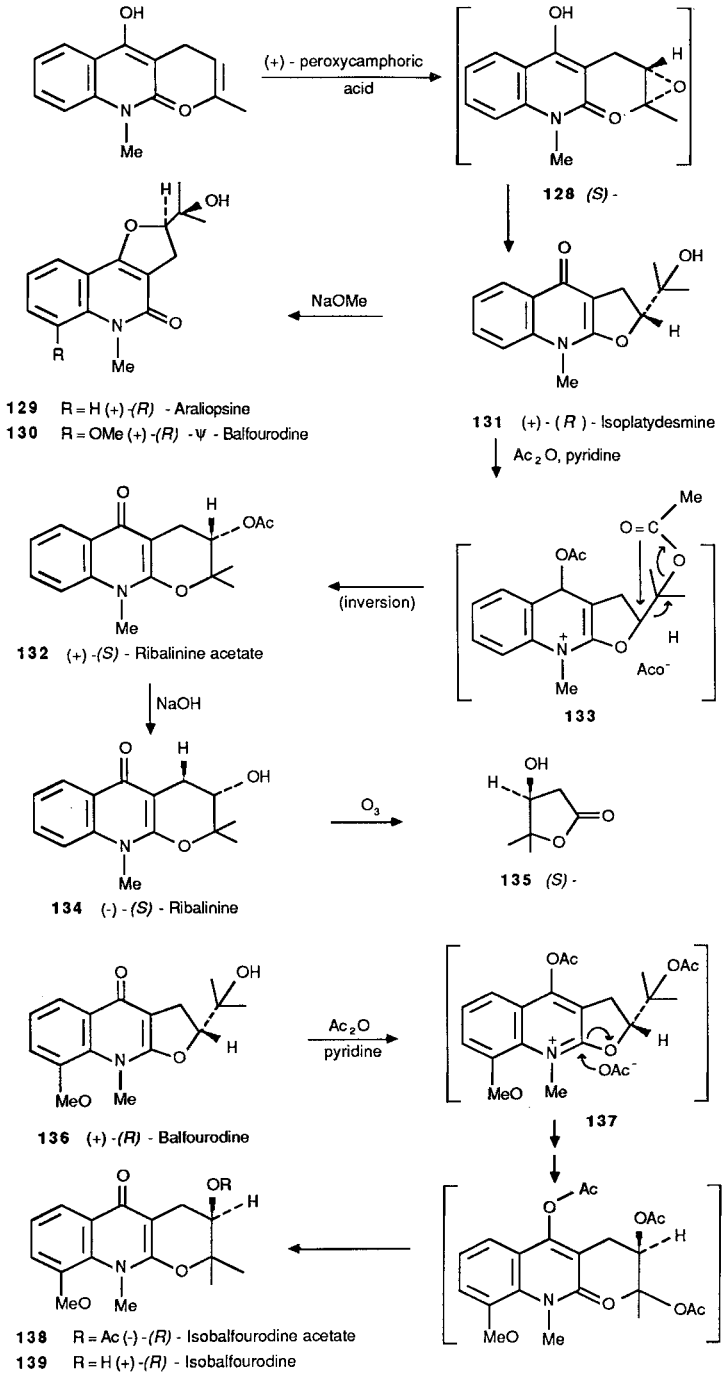
The synthesis of furoquinolines from copper acetylides has been carried out independently by Gaston *et al.* (*80*) and by Bathe and Reisch (*81*) (Scheme 15). Reaction of 3-halogeno-4-hydroxy-2-quinolinones (**117**) or (**118**) with copper(I) isopropenylacetylide in refluxing pyridine gave the isopropenylfuroquinolinone **122,** presumably via intermediate (**120;** R=H); the same product was obtained from the 4-methoxy derivative **119,** the solvent apparently effecting demethylation of the 4-methoxy group. Catalytic reduction of the isopropenylquinolinone **122** gave the same isopropylfuroquinolinone **121** obtained by dehydration of araliopsine (**113**) with concentrated sulphuric acid. When copper(I) (2-tetrahydropyranyloxyisopropyl)-acetylide was heated with the iodoquinolinone **123** in pyridine followed by removal of the protecting group with acid, the hydroxyisopropylfuroquinolone **124** was formed. The isopropenylmethoxyfuroquinoline **125** with linear annelation was obtained in a similar way (*80*), but a more convenient procedure resulting in a higher yield involved the *in situ* formation of the copper-acetylide using a palladium chloride catalyst (*81*) (Scheme 15); a stepwise method with 2-hydroxy-2-methylbut-3-yne gave compound **126** and then the linear hydroxyisopropylfuroquinoline **127.**

D. Asymmetric Synthesis and Absolute Stereochemistry of Dihydrofuroquinoline and Dihydropyranoquinoline Alkaloids

Reaction of 3-prenyl-2-quinolinones with chiral peroxy acids gave balfourodine (**136**), isobalfourodine (**7**), the diol balfourolone, and *O*-methylbalfourodinium salt (**98**) with 5–10% optical induction; the absolute configurations were determined by interconversions and by ozonolysis procedures (Vol. 17, p. 130). In a further study (*78*), the major product from the reaction of 4-hydroxy-1-methyl-3-prenyl-2-quinolinone with (+)-(*S*)-peroxycamphoric acid was isoplatydesmine containing an excess of the (+)-enantiomer; this then has the (*R*)-configuration **131** on the assumption that its formation from an intermediate (*S*)-epoxide (**128**) involves inversion at the chiral center (Scheme 16). This conclusion is confirmed by the formation of (+)-isoplatydesmine from (+)-(*R*)-*N*-methylplatydesminium iodide, *cf.* **89,** in a reaction not affecting the chiral center. (+)-Araliopsine (**129**) was obtained in 82% yield by treatment of (+)-isoplatydesmine with sodium methoxide in dimethyl formamide at ambient temperature; by analogy with the base-catalyzed rearrangement of the 8-methoxy derivative balfourodine (**136**), which was shown to occur with two inversions of configuration (*82*), (+)-araliopsine has the (*R*)-configuration. Rearrangement of



SCHEME 15. Synthesis of isopropylfuroquinolines from copper acetylides.



SCHEME 16. Asymmetric synthesis of isoplatydesmine, araliopsine, and ribalinine. Rearrangement of furoquinolinones with acetic anhydride.

(+)-isoplatydesmine, $[\alpha]_D$ +35° ($CHCl_3$) (43% optical purity) gave (+)-ribalinine acetate (**132**), which was converted by basic hydrolysis into the dihydropyranquinolinone alkaloid, (−)-ribalinine (**134**). The latter compound was shown to have an (*S*)-configuration by ozonolysis to the (−)-(*S*)-hydroxylactone **135** of established stereochemistry.

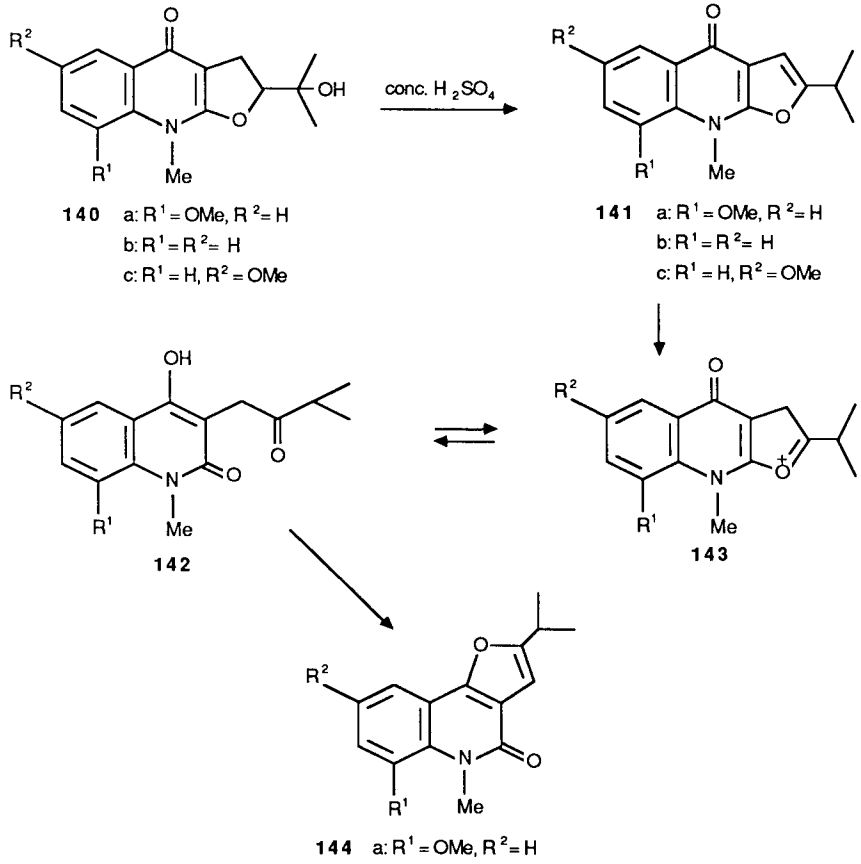
An (*S*)-configuration was assigned to (−)-veprisinium salt (**101**) on the basis of comparison of its c.d. spectrum with that of (−)-(*S*)-*O*-methylhydroxyluninium chloride (**99**) (*72*).

At least in some cases it appears that hemiterpenoid quinoline alkaloids have not been obtained as pure enantiomers. Thus, some alkaloids have been isolated as racemates—for example, ribaline (Table III) and lunacrine (Vol. 9, p. 238); furthermore, the hydroxylactone, *cf.* **135,** obtained by ozonolysis of (+)-(*R*)-*N*-methylplatydesminium salt had a specific optical rotation only 65% of the same lactone formed similarly from a natural coumarin. The lower optical purity is more likely to be due to a lack of specificity during *in vivo* formation of the alkaloids, *cf.* Section E, rather than partial racemization during isolation.

E. Rearrangement Reactions of Dihydrofuroquinoline and Dihydropyranoquinoline Alkaloids

Since the reaction of (*R*)-isoplatydesmine (**131**) with acetic anhydride and pyridine gives (*S*)-ribalinine acetate (**132**) and then by hydrolysis (*S*)-ribalinine (**134**). it is clear that the rearrangement occurs by inversion of configuration at the chiral center (Scheme 16). A mechanism that accounts for this stereochemical result is given in Scheme 16, *cf.* **133.** On the other hand, a similar rearrangement of (+)-(*R*)-balfourodine (**136**), differing from isoplatydesmine only in possessing a methoxy group at C-8, yields (−)-(*R*)-isobalfourodine acetate (**138**), which was hydrolyzed to (+)-(*R*)-isobalfourodine (**139**); a mechanism resulting in retention of configuration, *cf.* **137,** is shown in Scheme 16. The rearrangements of both alkaloids is accompanied by considerable reduction in optical purity, and this has been attributed to the existence of competing reactions with different stereochemical consequences, one predominating with isoplatydesmine and the other being favored in the 8-methoxy series (*78*). If the biosynthesis of hydroxydimethyldihydropyranoquinolinone alkaloids occurs by rearrangement of hydroxyisopropyldihydrofuroquinolinone isomers as proposed earlier, a fine balance between the two rearrangement reactions might explain the presence of (+)-(*R*)-isoplatydesmine and (−)-(*S*)-ribalinine in *Araliopsine soyauxii* and (+)-(*R*)-isoplatydesmine and (+)-(*R*)-ribalinine in *A. tabouensis;* the variation in specific optical rotation of ribalinine from various sources (0°, +8°, −10°, +14°) (Table III and Vol. 17, p. 114) is consistent with this suggestion.

Dehydration of balfourodine (**140a**) (Scheme 17) with concentrated sulfuric



SCHEME 17. Rearrangement of hydroxyisopropyl dihydrofuroquinolinones with concentrated sulfuric acid.

acid gave the isopropylfuroquinolinone **141a** in only 60% yield (Vol. 9, p. 246) and a reinvestigation showed that a second product was the 2-quinolinone **144a** with angular annelation (*54*). Isoplatydesmine (**140b**) and *O*-methylribaline (**140c**) behaved similarly to give a mixture of isopropylfuroquinolinones **141** and **144.** The linear furoquinolines **141** are probably formed first in the dehydration of the hydroxyisopropylfuroquinolinones, since they are converted into angular compounds **144** on treatment with concentrated sulphuric acid. A possible mechanism for the rearrangement involves formation of the protonated enol ether **143,** and then cleavage to ketone **142** which can cyclize to the angular furoquinolinone **144;** a similar sequence was proposed for the acid-catalyzed rearrangement of the furoquinoline dictamnine (Vol. 17, p. 167).

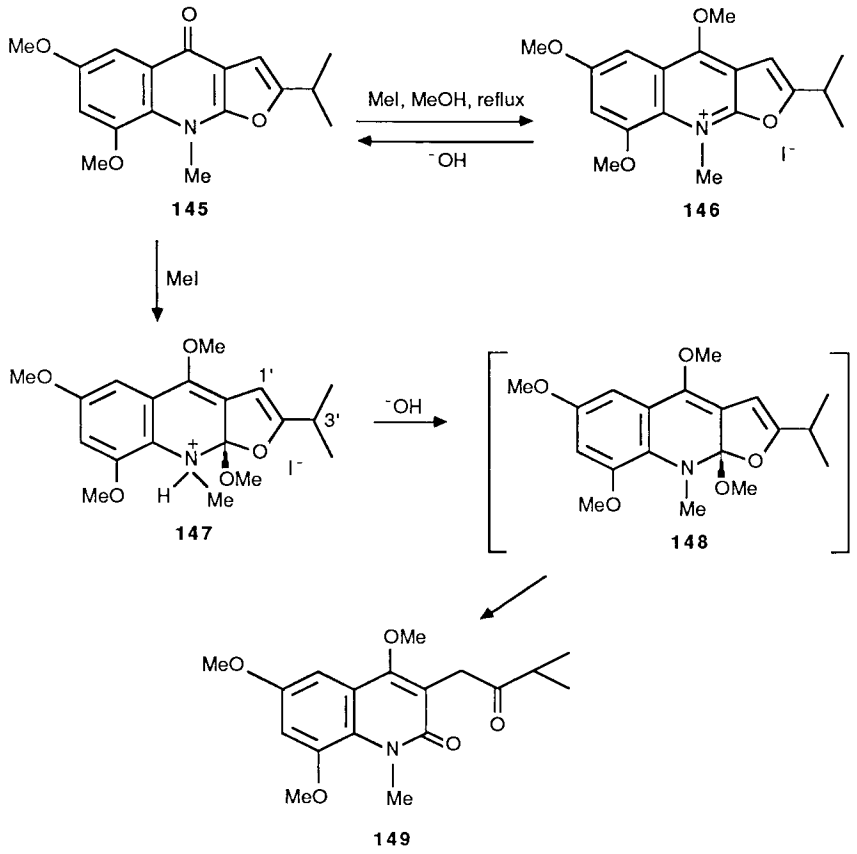
A study of Gaston and Grundon (*54*) of the reactions of isopropylfuro-

quinolinones **141a, 141b, 141c,** and the 6,8-dimethoxy derivative **145** with methyl iodide will be illustrated with the latter compound (Scheme 18). The methiodide **146,** obtained by refluxing the 4-quinolinone with methyl iodide in methanol, was not cleaved by base at C-2 to give a ketone **149,** as expected, but instead lost the methyl group of the 4-methoxy substituent to give the 4-quinolinone **145.** However, prolonged reaction of furoquinolinone **145** with methyl iodide alone gave a new salt, which reacted with sodium hydroxide to give the ketone **149** (60% yield) and the 4-quinolinone **145** (25% yield). The new salt is probably the hydriodide salt of a pseudobase with structure **147.** In the ^{1}H-NMR spectrum, three-proton singlets at δ4.74, 4.09 and 3.96 were assigned to methoxy groups at C-4, C-8 and C-6, respectively; the $\overset{+}{N}$HMe group produces a three-proton singlet at 4.67, and a high-field resonance at δ3.59, which is absent from the spectrum of methiodide **146,** is due to the methoxy group attached to the sp^3 carbon atom at C-2. In the ^{13}C-NMR spectrum, the resonance at δ102.1 ppm (C-2) shows long-range coupling to protons at C-1′ and at C-3′ and is comparable in chemical shift to that observed for the quaternary carbon atom of ortho esters when allowance is made for reduced deshielding by a nitrogen atom. The formation of ketone **149** with base apparently occurs by loss of hydrogen iodide to give the pseudo-base **148** (Scheme 18).

F. Terminal Olefins and Related Alkaloids

1. Occurrence and Structural Studies

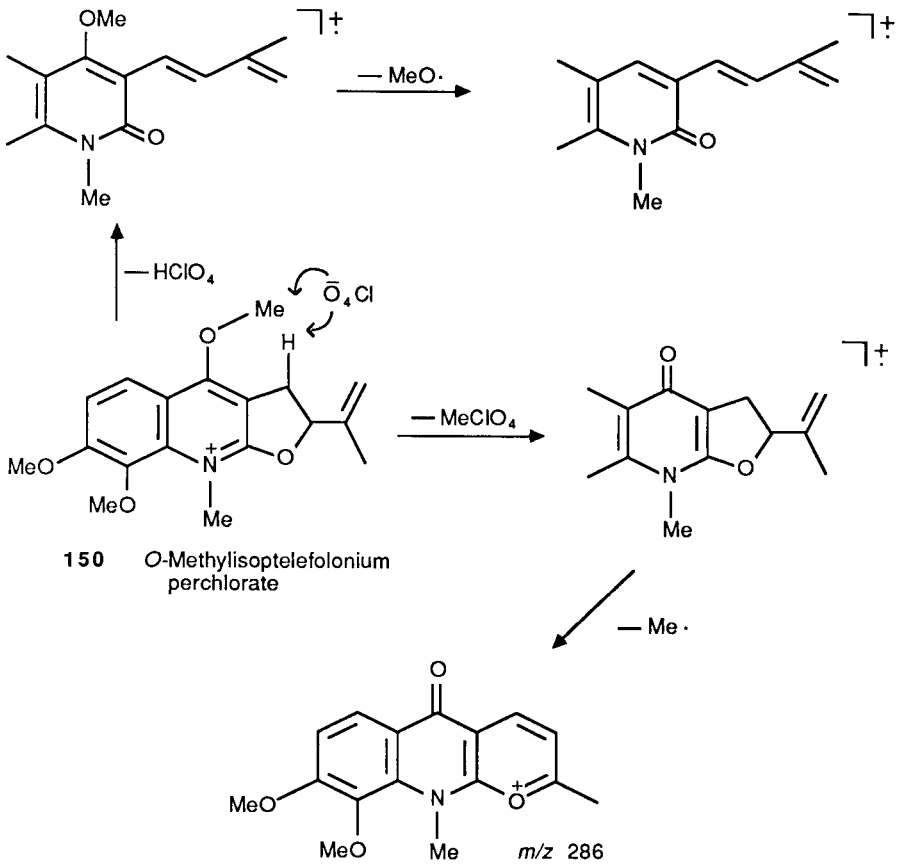
Reisch and co-workers obtained nine hemiterpenoid quinolines containing a terminal double bond from *Ptelea trifoliata,* including the quaternary quinolinium salt *O*-methylptelefolonium chloride (**12**) (Vol. 17, pp. 124–129); a second quaternary salt containing a 7,8-methylenedioxy group was thought to be present, but was not isolated. In a subsequent study of the quaternary alkaloids of *Ptelea trifoliata* by Rideau *et al.* (*62*) (+)-*O*-methylptelefolonium was isolated as its chloride and perchlorate and a new 7,8-dimethoxy alkaloid, (+)-*O*-methylisoptelefolonium salt (**150**), was obtained (Table III). The structure of the latter compound was determined by spectroscopy. In the ^{1}H-NMR spectrum the nature of the dihydrofuran ring was indicated by a one-proton triplet at δ5.80 ($CH_2CH<$) and two one-proton singlets at 5.11 and 5.26 ($=CH_2$). The behavior of the perchlorate salts of quaternary terminal olefins on electron impact differs from that of hydroxyisopropyl derivatives such as *O*-methylbalfourodinium salts (**98**). In the mass spectrum of *O*-methylisoptelefolonium perchlorate (**150**) (Scheme 19), loss of perchloric acid and then the radical MeO· gives a diene fragment ion, but the base peak at M^+-129 is due to loss of methyl perchlorate to give a radical ion and then loss of a methyl radical with rearrangement to give the stable ion at *m/z* 286 (*62*).



SCHEME 18. Conversion of isopropylfuroquinolinones into 3-(3-methylbutan-2-onyl) quinolinones via pseudobases.

Almeine (**151**), the first example of a terminal olefin in the quinoline group to be found in a species other than *P. trifoliata*, was isolated from *Almeidea guyanensis* as an amorphous solid by Moulis and co-workers (*34*). The presence of a 2-quinolinone function in almeine was indicated by infrared absorption at 1640 cm^{-1} and by the lack of a shift in the UV spectrum on addition of acid. The presence of a group $-C(Me)=CH_2$ attached to a dihydrofuran ring was apparent from the ^1H-NMR spectrum, which showed resonances at δ5.11 and 4.96 (2H, two singlets, $=CH_2$), 5.41 (1H, t, $>CHCH_2$-) and 1.78 (3H, s, Me), and from the mass spectrum which showed fragment ion peaks at *m/z* 226 (M^+-Me, 100%) and 200 [M^+-$C(Me)=CH_2$].

Dubinidine (**152**), which can be regarded as a hydroxylated derivative of a terminal olefin has been isolated from several *Haplophyllum* species and most recently from *H. perforatum* (*24*).

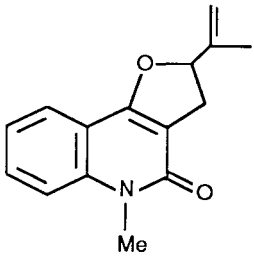


150 *O*-Methylisoptelefolonium perchlorate

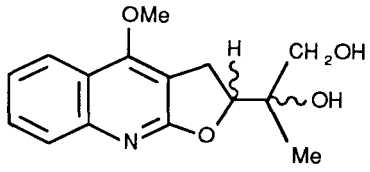
SCHEME 19. Mass spectrum of *O*-methylisoptelefolonium perchlorate.

2. *Synthesis*

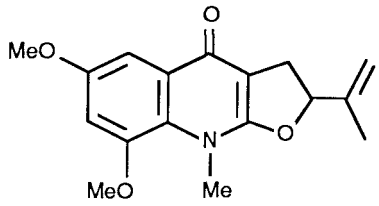
Full accounts have now been published of the synthesis of *O*-methylptelefolonium salt (**12**) (*52*), ptelefolone (**153**) (*50*), and dubinidine (**152**) (*52*) (summarized in Vol. 17, p. 128) and Grundon *et al.* (*83*) have reported a high-yield synthesis of ptelefoline (**158**) and ptelefoline methyl ether (**160**) which are also terminal olefins isolated from *Ptelea trifoliata* (Scheme 20). Rearrangement of isoplatydesmine (**131**) into ribalinine acetate (**132**) was effected with acetic anhydride in refluxing pyridine (*cf.* Section IV, E), but when hydrochloric acid was added the diacetate **156** was isolated almost quantitatively. The structure of this compound was indicated by IR absorption at 1770 (4-AcO), 1737 (2′-AcO), and 1655 cm^{-1} (2-quinolinone C=O) and by the ^{1}H-NMR spectrum which



151 Almeine



152 Dubinidine

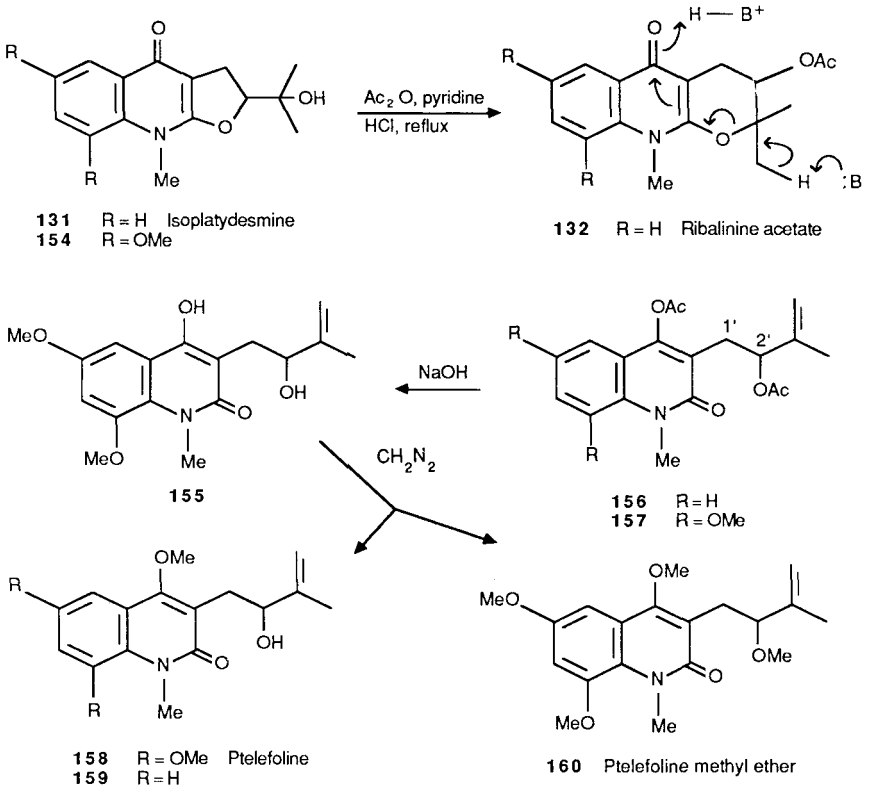


153 Ptelefolone

showed resonances at δ5.55 (C*H*OAc), 4.96, and 4.90 (=CH_2) and 1.87 [–C(Me)=]. Since compound **156** was obtained in the same way from ribalinine acetate (**132**), the latter is probably an intermediate. A mechanism was suggested, *cf.* (**132**), in which protonation of the 4-carbonyl group by the acid catalyst promotes ring fission through a β-elimination reaction. Application of the reaction to the 6,8-dimethoxy-4-quinolinone **154** gave the diacetate **157** (75%). Treatment of the diacetate **157** with aqueous sodium hydroxide gave the allylic alcohol **155,** which with diazomethane furnished ptelefoline (**158**) (72%) and ptelefoline methyl ether (**160**) (25%). Since the 4-methoxy allylic alcohol **159** is unaffected by diazomethane it appears that diazomethane, after protonation by the acidic 4-hydroxy group of compound **155,** reacts at either oxygen center; intramolecular hydrogen bonding of the 2-quinolinone carbonyl group to the allylic hydroxy substituent may render the side chain hydroxy group of compound **155** sufficiently nucleophilic to compete with the 4-oxy anion in the methylation reaction.

V. 2,2-Dimethylpyranoquinolinone Alkaloids of the Flindersine Group and Related Compounds

By 1976 flindersine (**161**) and *N*-methylflindersine (**162**) had been isolated from a number of rutaceous plants, two other alkaloids of the group, haplamine (**163**) and oricine (**164**) had been discovered, and a number of methods of



SCHEME 20. Synthesis of ptelefoline and ptelefoline methyl ether.

synthesis of the ring system had been elaborated. Since that date a considerable number of new alkaloids of the flindersine group have been obtained.

A. OCCURRENCE AND STRUCTURAL STUDIES

Sources of flindersine, *N*-methylflindersine, and oricine discovered since 1976 and the occurrence of new alkaloids of the group are given in Table IV (*2, 18, 34, 44, 84–97, 229, 238*).

Flindersine (**161**) has been isolated from six new sources. *N*-Methylflindersine (**162**) has been recognized as a constituent of six additional species and the antifeedant activity of three of these species, *Fagara chalybea, F. hostii,* and *Xylocarpus granatum,* was shown by Nakanishi and co-workers (*91*) to be due to the presence of the quinoline alkaloid.

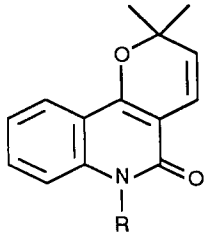
The methoxyflindersine **166** is a constituent of *Myrtopsis macrocarpa* (*18*) and its *N*-methyl derivative, zanthobungeanine (**167**), was isolated from *Zathoxylum bungeanum* (*93*) and from *Z. simulans* (*94*). *Oricia renieri* contains the methoxy

TABLE IV
2,6-DIHYDRO-2,2-DIMETHYL-5H-PYRANO[3,2-c]QUINOLIN-5-ONE ALKALOIDS OF THE FLINDERSINE GROUP

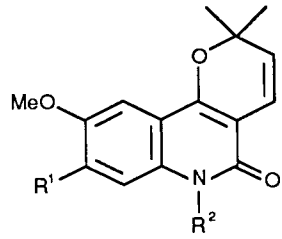
Compound	Structure number	Source	Melting point (°C)	Ref.
Flindersine	**161**	*Atalantia roxburghiana*		*84*
		Fagara heitzii		*85*
		Geijera balansae		*86*
		Haplophyllum saveolens	185	*87*
		H. glabrinum		*238*
		Micromelum minutum		*88*
		Zanthoxylum coco		*89*
N-Methylflindersine	**162**	*Almeidea guyanensis*		*34*
		Euxylophora paraensis	82	*90*
		Fagara chalybea		*91*
		F. holstii		*91*
		Myrtopsis novae-caledoniae		*18*
		Sarcomelicope glauca		*229*
		Xylocarpus granatum		*91*
—	**176**	*Haplophyllum buxbaumii*	amorphous	*92*
Haplophylline	**177**	*H. suaveolens*	amorphous	*87*
—	**166**	*Myrtopsis macrocarpa*		*18*
Zanthobungeanine	**167**	*Geijera balansae*		*86*
		Zanthoxylum bungeanum	—	*93*
		Z. simulans		*94*
—	**165**	*Oricia renieri*	oil	*95*
—	**174**	*Zanthoxylum monophyllum*	—	*2*
Zanthophylline	**175**	*Z. monophyllum*	126–127	*2*
—	**171**	*Vepris stolzii*	oil	*96*
Oricine	**164**	*Oricia renieri*	—	*95*
Veprisine	**168**	*O. renieri*	87–89	*95*
		Vepris louisii	89–90	*44*
		V. stolzii	—	*96*
—	**169**	*Vepris stolzii*	oil	*96*
—	**170**	*V. stolzii*	oil	*96*
—	**178**	*Geijera balansae*	208	*86*
—	**179**	*Euxylophora paraensis*	159–160	*90*
—	**182**	*Geijera balansae*	234–235	*86*
—	**183**	*Zanthoxylum simulans*	—	*97*
—	**184**	*Z. simulans*	—	*97*

N-methylflindersine **165** (*95*). The structures of the three alkaloids were established by spectroscopy; alkaloid **166** was synthesized (Vol. 17, p. 143) before its isolation.

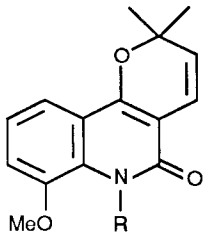
The dimethoxy-*N*-methylflindersine veprisine (**168**) was first isolated by Ayafor and co-workers (*44*) from *Vepris louisii* and then by Khalid and Water-



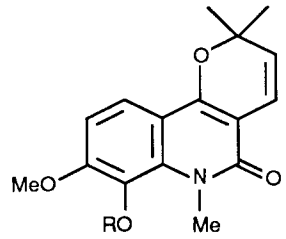
161 R = H Flindersine
162 R = Me *N*- Methylflindersine



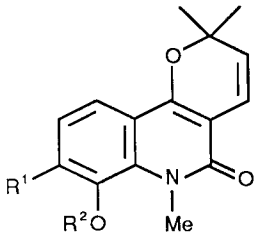
163 $R^1 = R^2 = H$ Haplamine
164 $R^1 = OMe, R^2 = Me$ Oricine
165 $R^1 = H, R^2 = Me$



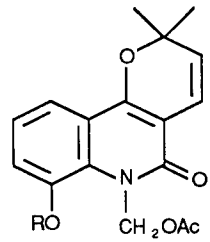
166 R = H
167 R = Me Zanthobungeanine



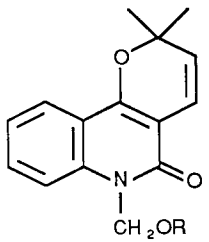
168 R = Me Veprisine
169 $R = CH_2CH{=}CMe_2$
170 $R = CH_2CH{-}CMe_2$ (O)



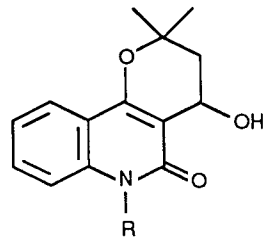
171 $R^1 = H, R^2 = CH_2CH{=}CMe_2$
172 $R^1 = R^2 = H$
173 $R^1 = OMe, R^2 = H$



174 R = H
175 R = Me Zanthophylline



176 R = H
177 R = COCH═ CMe_2 Haplophylline



178 R = H
179 R = Me

man from *V. stolzii* (*96*) and from *Oricia renieri* (*95*). The structure of the alkaloid was determined by spectroscopic methods and by synthesis (*cf.* Section B).

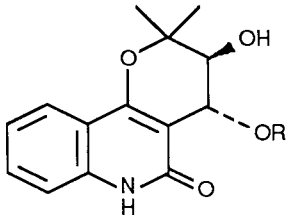
Khalid and Waterman (*96*) isolated three prenyloxy derivatives of *N*-methylflindersine, (**171**), (**169**), and (**170**) from *Vepris stolzii*. The three alkaloids were shown to be 2,2-dimethylpyranoquinolinones by resonances in the ^{1}H-NMR spectra at δ5.50 and 6.75 (AB quartet for protons at C-1′ and C-2′). In the mass spectra of alkaloids, **169** and **171** cleavage of the prenyloxy groups produced fragmentation peaks at M^+-68 (C_5H_8) and in alkaloid **170** corresponding C—O cleavage resulted in a peak at M^+-84 (C_5H_8O). The positions of the sidechains in the three alkaloids were indicated by acid hydrolysis to derivatives **172** or **173** that were shown by positive Gibbs tests to contain aromatic protons *para* to phenolic hydroxy groups.

A number of *N*-functionalized derivatives of *N*-methylflindersine have been isolated. Stermitz and Sharifi (*2*) obtained the *N*-acetoxymethyl derivative zanthophylline (**175**) from *Zanthoxylum monophyllum* and determined its structure by ^{1}H- and ^{13}C-NMR spectroscopy and by hydrolysis to the methoxy flindersine **166** and formaldehyde (indentified as its 2,4-dinitrophenylhydrazone derivative). When aqueous acid was used in the isolation procedure, only the methoxy compound **166** was obtained and appears to be an artifact formed by hydrolysis of zanthophylline. A minor constituent of *Z. monophyllum* that was obtained in too small a yield for full characterization is believed to be the desmethylzanthophylline **174,** based on its mass spectrum and IR absorption at 3500 (OH) and 1750 cm^{-1} (MeCOO).

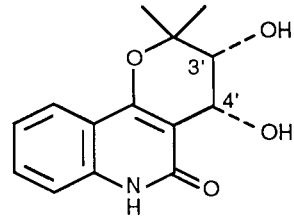
Ulubelen has recently identified two new *N*-functionalized alkaloids of the flindersine group from *Haplophyllum* species. The structure of haplophylline (**177**) from *H. suavelens* was apparent from the ^{1}H-NMR and mass spectra and from IR absorption at 1720 cm^{-1} (*87*). An alkaloid from *H. buxbaumii* was shown to be *N*-hydroxymethylflindersine (**176**) by spectroscopic studies and by its formation by alkaline hydrolysis of haplophylline (*92*).

The optically-active hydroxydimethyltetrahydropyranoquinolinone (**179**), isolated by Jurd and Wong (*90*) from *Euxylophora paraensis,* can be regarded as the hydrate of *N*-methylflindersine which occurs in the same plant; the structure was determined by spectroscopic studies. Venturella and Bellino (*98*) synthesized the alkaloid by methylation of the known quinolinone (**178**) (*see* Vol. 17, p. 232) with methyl iodide and potassium carbonate in acetone. Quinolinone **178,** $[\alpha]_D$-6° ($CHCl_3$), was subsequently obtained from *Geijera balansae* (*86*).

G. balansea also contains the *cis*-diol (**182**), $[\alpha]_D$-2°($CHCl_3$) (*86*). Although the mass spectrum of the alkaloid did not show a molecular ion peak, the presence of a hydroxy group was indicated by IR absorption at 3400–3200 cm^{-1}, and that of a 2-quinolinone group at 1645 cm^{-1}. That the alkaloid was a dihydroxydihydroflindersine was apparent from the ^{1}H-NMR spectrum which, after addition of D_2O, showed one-proton doublets at δ4.74 (H-4′) and 3.65



180 R = H
181 R = Ac



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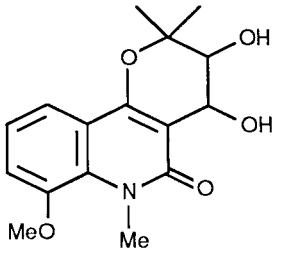

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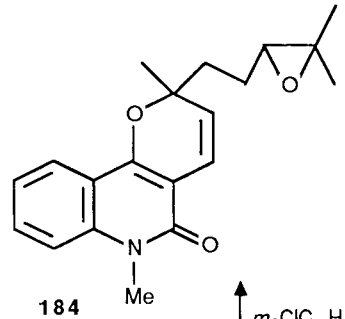

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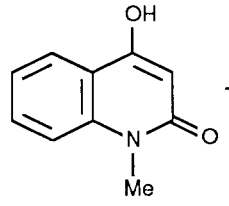
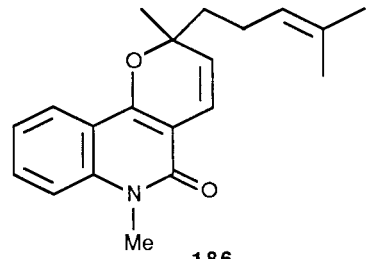
m-$ClC_6H_4CO_3H$



pyridine
reflux



186

\+

$Me_2C{=}CHCH_2CH_2C(Me){=}CHCHO$

185

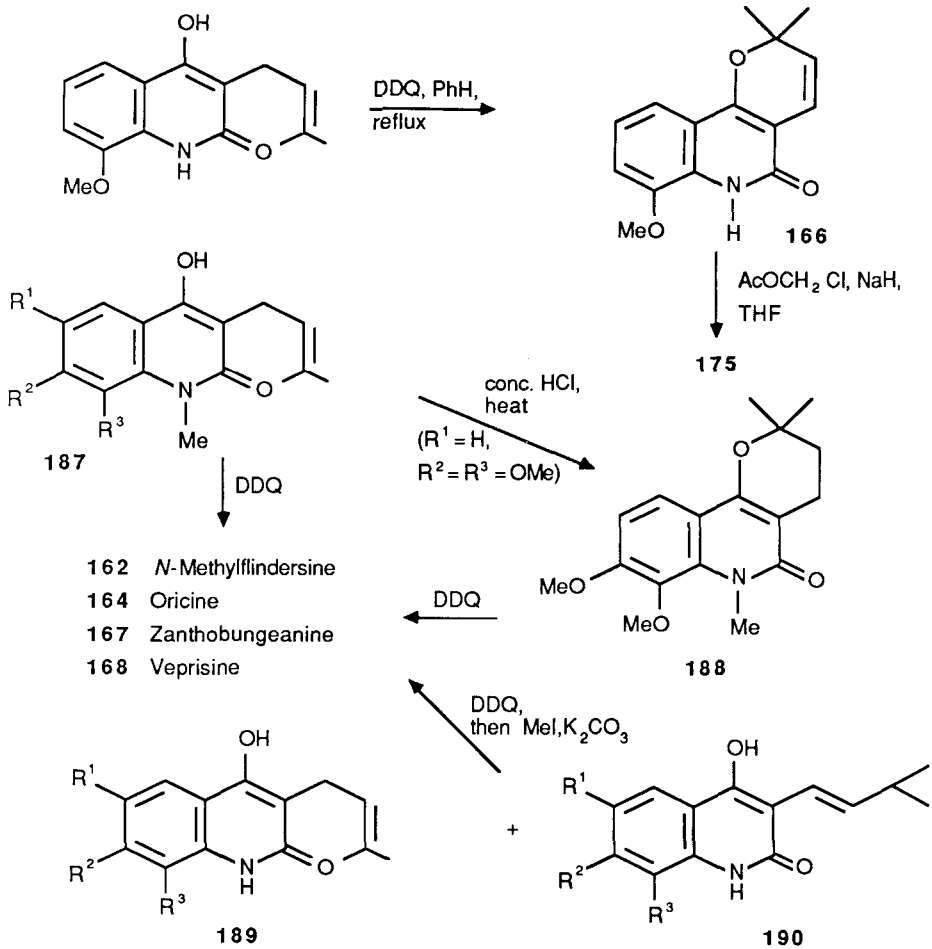
(H-3′); the coupling constant (J = 6 Hz) excluded a *trans*-diaxial arrangement for the OH groups, but did not distinguish between *trans*-diequatorial and *cis* configurations. This problem was solved by chemical correlation. Thus, reaction of flindersine with chromic acid in acetic acid gave the *trans*-acetoxyhydroxydihydroflindersine **181,** hydrolyzed with sodium methoxide in methanol to the *trans*-diol **180;** the coupling constants $J_{3'\text{-}4'}$ = 5 Hz for the diol and $J_{3'\text{-}4'}$ = 3 Hz for its acetate indicate that the substituents at C-3′ and C-4′ are in an equatorial configuration. Oxidation of flindersine with osmium tetraoxide gave the (±)-*cis*-diol **182** with spectroscopic properties identical to the natural diol. A related diol (**183**) is reported to be a constituent of *Zanthoxylum simulans* (*97*).

Another alkaloid isolated by Gray and O'Sullivan (*97*) from *Z. simulans* was

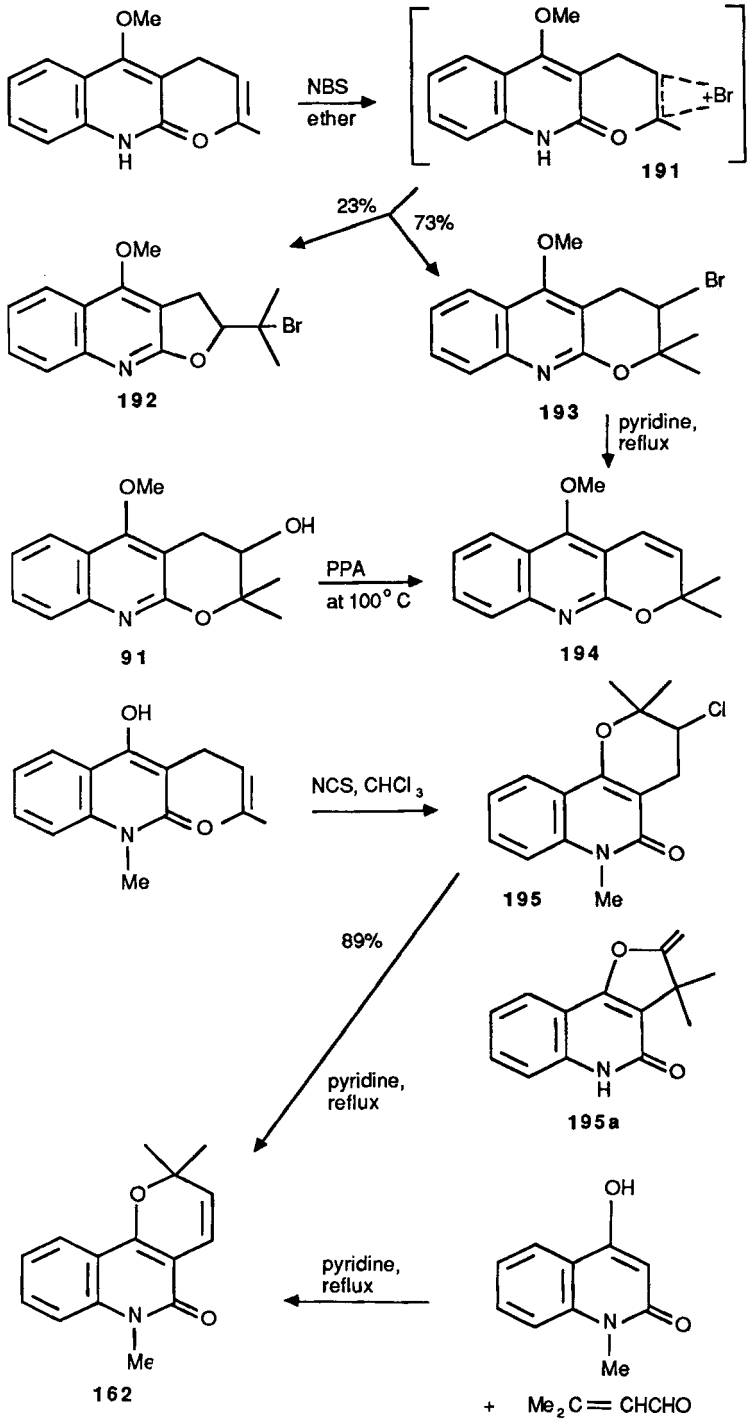
shown to be the flindersine derivative **184,** apparently formed from a *C*-geranylquinolinone. Reaction of 4-hydroxy-1-methyl-2-quinolinone with the aldehyde **185** in refluxing pyridine afforded compound **186;** epoxidation then furnished a mixture of diastereoisomers corresponding to alkaloid **184** (*99*).

B. Synthesis

Of the synthetic methods discussed previously (Vol. 17, p. 142), that involving reaction of 4-hydroxy-3-prenyl-2-quinolinones with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) has proved to be the most popular (Scheme 21). Venturella *et al.* (*100*) prepared the methoxyflindersine **166** in this way and



Scheme 21. Synthesis of 2,2-dimethyl pyranoquinolinones by reaction of prenylquinolinones with DDQ.



SCHEME 22. Synthesis of 5-methoxy-2,2-dimethyl-2H-pyrano (2,3-*b*) quinoline and *N*-methyl flindersine.

converted it into zanthophylline (**175**). 4-Hydroxy-*N*-methyl-3-prenyl-2-quinolinones (**187**) behave in a similar way to give *N*-methylflindersine (**162**) (*101*) (*58*) and oricine (**164**) (*58*); in a modification of this route, acid-catalyzed cyclization of 7,8-dimethoxy-4-hydroxy-1-methyl-3-prenyl-2-quinolinone (**187;** R^1=H, R^2=R^3=OMe) furnished compound **188** which on reaction with DDQ was converted into veprisine (**168**) (*44*). The mixture of (3-methylbut-2-enyl)- (**189**) and (3-methylbut-1-enyl)-2-quinolinones (**190**), prepared by Shanmugam and collaborators (*102*) (*cf.* Section III,C,4), was converted by the usual oxidative cyclization with DDQ followed by methylation into *N*-methylflindersine (**162**), zanthobungeanine (**167**), oricine (**164**), and veprisine (**168**).

The synthesis of flindersine from 4-hydroxy-2-quinolinone, 3-methylbut-2-enal, and boiling pyridine (Vol. 17, p. 143) has now been applied to 4-hydroxy-*N*-methyl-2-quinolinone to give *N*-methyl-flindersine (Scheme 22) (*101*).

A study of the reactions of 3-prenyl-2-quinolinones with *N*-halosuccinimides by Grundon *et al.* (*101*) resulted in new syntheses of dimethylpyranoquinolines (Scheme 22). Treatment of 4-methoxy-3-prenyl-2-quinolinone (atanine) with *N*-bromosuccinimide in ether or carbon tetrachloride with or without a radical initiator did not result in allylic substitution, but gave a mixture of bromofuroquinoline **192** and bromopyranoquinoline **193,** possibly via a bromonium ion **191.** The bromopyranoquinoline, which was the major product, reacted with boiling pyridine to give 5-methoxy-2,2-dimethyl-2H-pyrano[2,3-b]quinoline (**194**); this was apparently the first example of a 2,2-dimethylpyranoquinoline with linear annelation, but the compound was prepared independently by Shanmugam and co-workers (*76*) by heating the hydroxypyranoquinoline geibalansine (**91**) with polyphosphoric acid (Scheme 22). Reaction of 4-hydroxy-1-methyl-3-prenyl-2-quinolinone with *N*-chlorosuccinimide gave a single product that was shown by spectroscopy to be the chloropyranoquinolinone with angular annelation (**195**) (Scheme 22). When this product was submitted to an elimination reaction with pyridine, *N*-methylflindersine was obtained in 89% yield (*101*).

Flindersine and its 8-methoxy derivative were prepared previously (Vol. 17, p. 143) from thallous salts of 4-hydroxy-2-quinolinone and 3-chloro-3-methylprop-1-yne and Reisch *et al.* (*232*) have carried out a new study of this reaction using a phase-transfer catalyst to give flindersine (**161**) in good yield and the by-product **195a** (12%).

VI. Dimeric Quinolinone Alkaloids

The first dimeric quinolinone alkaloid was discovered by Reisch in 1978 and since that time 14 more dimers have been isolated (Table V) (*86, 90, 103–107*). As indicated in the Introduction, alkaloids of this group may all be derived by

TABLE V
DIMERIC QUINOLINONE ALKALOIDS

Compound	Structure number	Source	Melting point (°C)	Ref.
Pteledimerine	**197**	*Ptelea trifoliata*	319–321	*103*
Pteledimeridine	**198**	*P. trifoliata*	340–342	*104*
Paraensidimerin D	**205**	*Euxylophora paraensis*	259	*90*
Paraensidimerin B	**206**	*E. paraensis*	286–287	*107*
Vepridimerine A	**212**	*Vepris louisii*	343–345	*105*
Vepridimerine B	**214**	*Oricia renieri*	278–279	*105*
		V. loisii		*105*
Vepridimerine C	**218**	*O. renieri*	272	*105*
		V. loisii		*105*
Vepridimerine D	**219**	*O. renieri*	—	*105*
Paraensidimerin A	**213**	*E. paraensis*	311–312	*106*
Paraensidimerin C	**215**	*E. paraensis*	210 (as hydrate)	*106*
Paraensidimerin E	**216**	*E. paraensis*	289–290	*107*
Paraensidimerin F	**217**	*E. paraensis*	310	*107*
Paraensidimerin G	**210**	*E. paraensis*	280–281	*107*
Geijedimerine	**220**	*Geijera balansae*	205–207	*86*
Pteledimericine[a]		*P. trifoliata*	275–277	*104*

[a] $C_{30}H_{30}N_2O_5$, unknown structure.

dimerization of a diene formed by dehydrogenation of 4-hydroxy-1-methyl-3-prenyl-2-quinolinone; in the following account, the three known structural types will be discussed separately.

A. PTELEDIMERINE AND PTELEDIMERIDINE

Pteledimerine (**197**), which was isolated from the root bark of *Ptelea trifoliata* by Reisch and co-workers (*103*), is a high melting crystalline compound. The molecular formula, $C_{30}H_{30}N_2O_4$, was apparent from the mass spectrum, and IR absorption at 1645 and 1630 cm^{-1} indicated the presence of 2- and 4-quinolinone carbonyl groups. Structure **197** was established mainly by ^{1}H-NMR spectroscopy which showed a resonance at δ8.32 typical of a proton at C-5 deshielded by the carbonyl group at C-4; other important features of the spectrum include a two-proton singlet at 3.44 (3-CH_2), two-proton doublets at 2.16 and 2.08 attributed to methylene groups at C-10′ and C-11 each coupled to the methine proton at C-9′ (3.21 ppm), and a three-proton singlet at 1.85 (methyl at C-10). In accord with the proposed structure, a fragment ion at *m/z* 241 (19%) in the mass spectrum arises from cleavage at C-9′-C-11 to give an *N*-methyl flindersine portion.

A second dimer obtained from *P. trifoliata* (*104*) was given the name pteledimeridine; it was shown to be isomeric with pteledimerine and to have a structure with two 2-quinolinone moieties. Pteledimeridine has a similar mass spectrum and ^{1}H-NMR spectrum to those of pteledimerine; the lowest NMR resonance at δ8.07 indicates the proton at C-5 is not deshielded by a 4-carbonyl group.

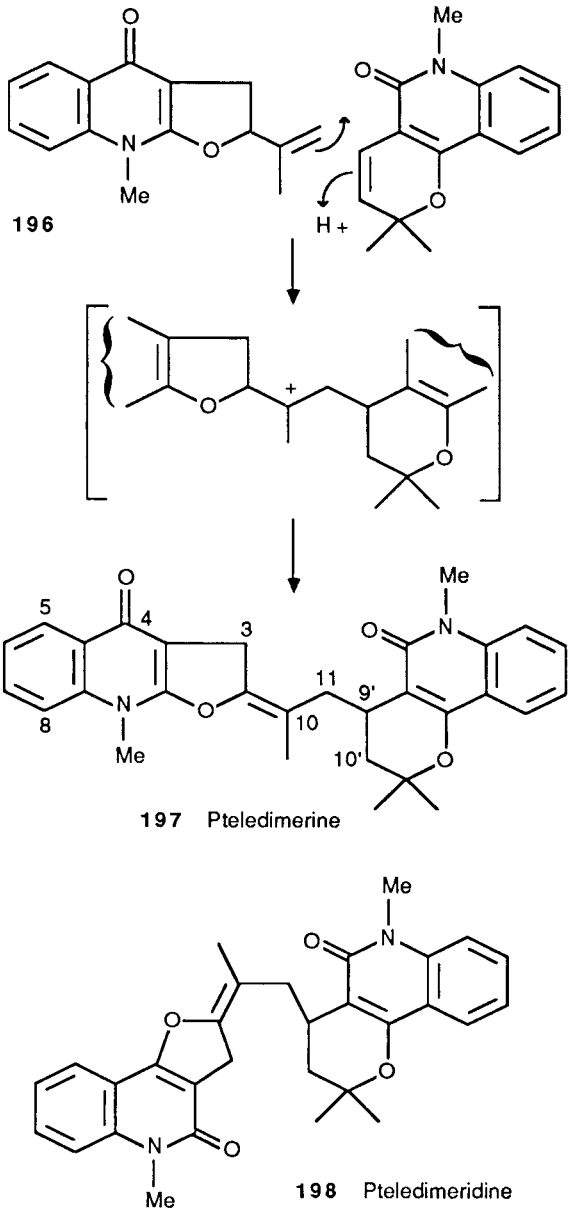
A possible biosynthetic route to pteledimerine involves an ene reaction of the olefin **196** with *N*-methylflindersine (**162**), or more likely, an acid-catalyzed reaction of the two components as shown in Scheme 23. In the case of pteledimeridine (**198**) the corresponding olefin would be almeine (**151**). The co-occurrence in *Ptelea trifoliata* of *N*-methylflindersine and terminal olefins of type **196** provides chemotaxonomic support for the biosynthetic proposal and stimulated a study of the reactions of *N*-methylflindersine with acids (*108*). When *N*-methylflindersine was refluxed with formic acid or trifluoroacetic acid a single dimer was formed and shown to have structure **200** on the basis of its ^{1}H- and ^{13}C-NMR spectra and the mass spectrum and those of the dehydro derivative **199** obtained by reaction of dimer **200** with DDQ. Compound **200** can be regarded as the Markovnikov dimer of *N*-methylflindersine and its formation by the mechanism indicated in Scheme 24 supports the proposed analogous route to pteledimerine (Scheme 23) and pteledimeridine. Jurd *et al.* (*106*) discussed alternative routes.

By analogy, the crystalline dimer formed by heating flindersine (**161**) with aqueous sulfuric acid (Vol. 7, p. 231) may have structure **201.**

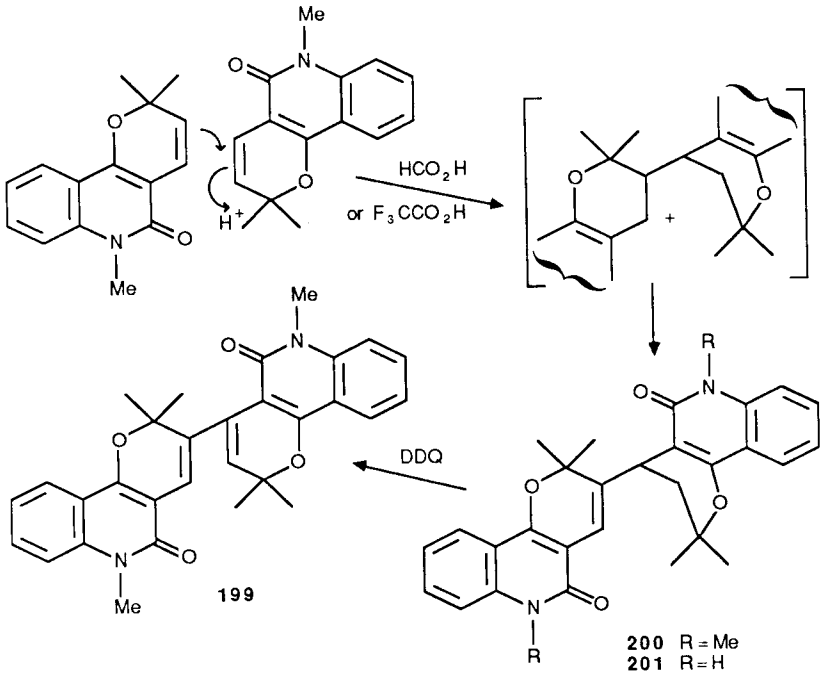
B. Paraensidimerins B and D

In the course of an investigation of the alkaloids of the heartwood of the Brazilian tree *Euxylophora paraensis,* Jurd and co-workers isolated a number of dimeric quinolinones. Paraensidimerin D, $C_{30}H_{30}N_2O_4$, which was the first of these dimers to be studied, was shown to have structure **205** by single-crystal *X*-ray diffraction measurements (*90*). In the ^{1}H-NMR spectrum of paraensidimerin D, four one-proton doublets were assigned as follows: δ2.20 (J = 3 Hz, H_B), 3.92 (J = 9 Hz, H_C), 5.30 (J = 9 Hz, C*H*=CMe$_2$), and 5.56 (J = 3 Hz, H_A). Since the dihedral angle between H_B and H_C is 86.9°, application of the Karplas equation predicts near-zero coupling and the observation of a doublet instead of normal double doublets.

The spectroscopic properties of paraensidimerin B, $C_{30}H_{32}N_2O_4$, indicate that this alkaloid is the hydrate of paraensidimerin D with structure **206.** Thus, the IR spectrum shows absorption at 3450 cm^{-1} (OH) and signals for four aliphatic *C*-methyl groups appear in the ^{1}H NMR spectrum as in dimer **205.** Resonances for an exocyclic olefin group in dimer D at δ_H 5.30 (CH=CMe$_2$) and at δ_C 127.6 (–CH=) were replaced in dimer B (**206**) by resonances for a methylene group at δ_H 1.97 and 1.82 and at δ_C 52.5 ppm and for >C—OH at 70.0 ppm (*107*).



SCHEME 23. Pteledimerine and Pteledimeridine and their possible origin.

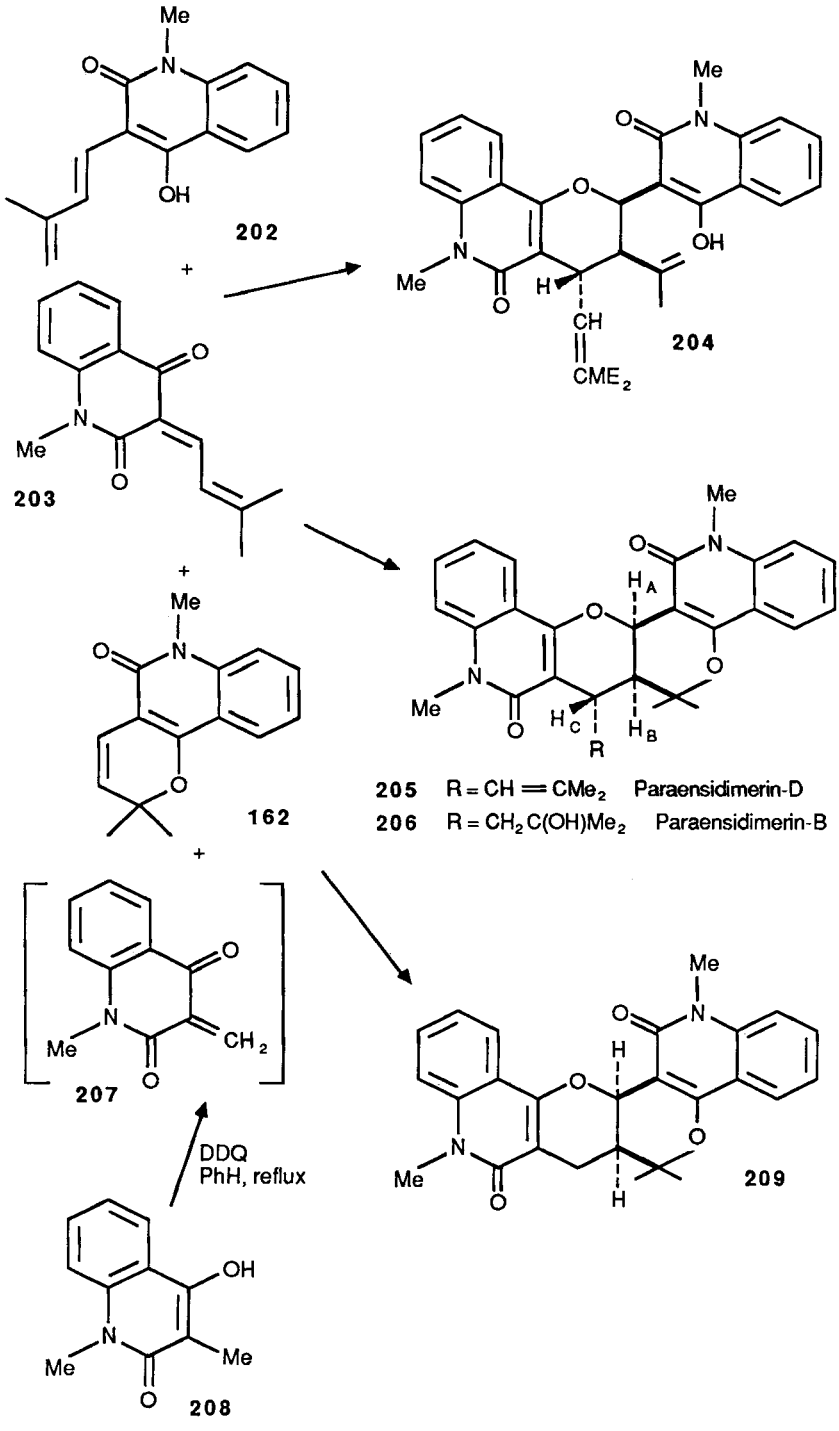


SCHEME 24. Dimerization of *N*-methylflindersine.

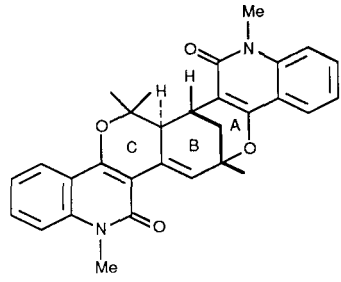
The *cis*-fusion of rings B and C of compounds **205** and **206** suggests that the dimers may originate by Diels–Alder addition of a quinoline–quinone methide **203** to its tautmeric diene **202** followed by cyclization or by addition of the quinone methide to *N*-methylflindersine (**162**) (Scheme 25) (*105, 109*). Support for the latter route is provided by the observation that the electron-deficient quinone methide **207,** generated *in situ* from the 4-hydroxy-3-methylquinolinone **208,** reacts readily with *N*-methylflindersine to give adduct **209,** which contains the same ring system as paraensidimerines B and D (*110*).

C. Vepridimerines A–D, Paraensidimerins A, C, E, F, and G and Geijedimerine

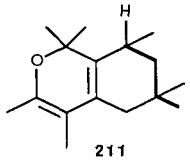
Four isomeric dimeric quinolinone alkaloids, $C_{34}H_{38}N_2O_8$, of a new type were isolated from *Vepris louisii* by Ngadjui, Ayafor, and Sondengam (Yaoundé) and from *Oricia renieri* by Khalid and Waterman (Strathclyde), and their structures were determined on the basis of spectroscopic evidence with the assistance of Connolly and Rycroft (Glasgow) and Brown, Grundon, and



SCHEME 25 . Paraensidimerins D and B and the synthesis of a related compound.



210 Paraensidimerin G



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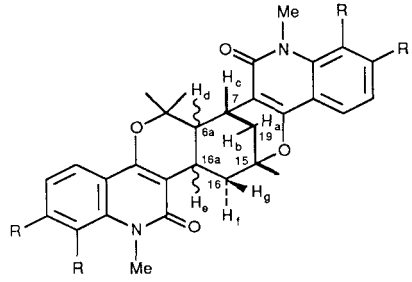
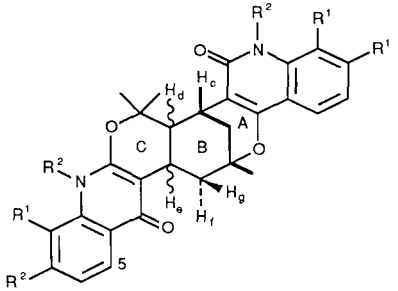
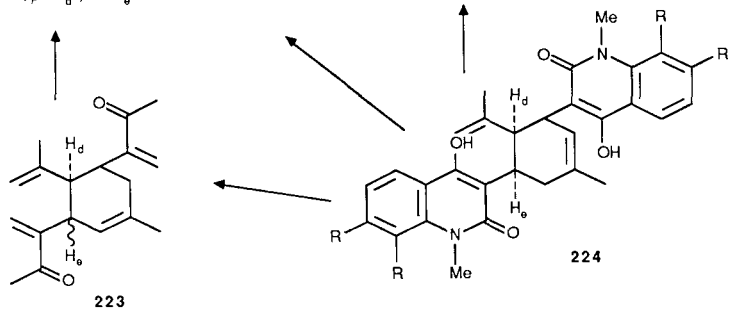


212 R = OMe; α - H_d, α - H_e Vepridimerine A
213 R = H; α - H_d, α - H_e Paraersidimerin A
214 R = OMe; α - H_d, β - H_e Vepridimerine B
215 R = H; α - H_d, β - H_e Paraensidimerin C
216 R = H; β - H_d, β - H_e Paraensidimerin E
217 R = H; β - H_d, α - H_e Paraensidimerin F



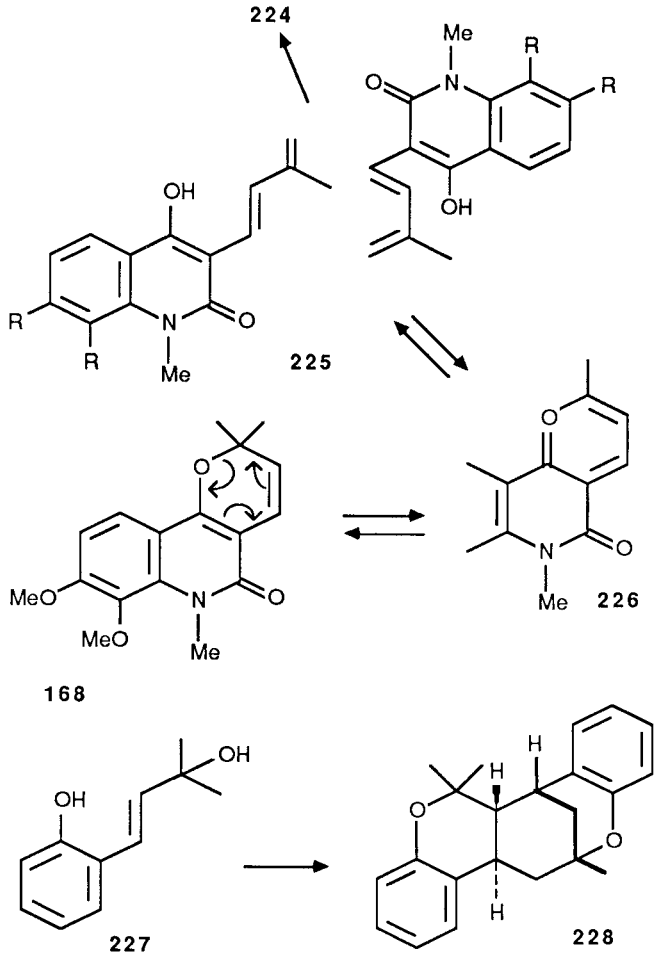
218 R^1 = OMe, R^2 = Me; α - H_d, α - H_e Vepridimerine C
219 R^1 = OMe, R^2 = Me; α - H_d, β - H_e Vepridimerine D
220 R^1 = R^2 = H; β - H_d, α - H_e Geijedimerine
221 R^1 = H, R^2 = Me; β - H_d, α - H_e Dimethylgeijedimerine
222 R^1 = OMe, R^2 = Me; β - H_d, α - H_e Vepridimerine E



223

224

Ramachandran (Coleraine) (*105*). Vepridimerines A–C occur in *V. louisii,* while vepridimerines B-D were isolated from *O. renieri.* Almost simultaneously, Jurd, Benson, and Wong showed by X-ray and spectroscopic studies that dimeric quinolinone alkaloids of *Euxylophora paraensis* have similar structures; paraensidimerin A (**213**) is the demethoxy derivative of vepridimerine A (**212**) and paraensidimerin C (**215**) is the demethoxy derivative of vepridimerine B (**214**) (*106*). Paraensidimerin E (**216**) and paraensidimerin F (**217**) are the remaining two possible racemates (*107*) (Scheme 26).



SCHEME 26. Paraensidimerins A, C, E, F, and G, vepridimerines A–D, geijedimerine, and their proposed origins.

Determination of the structures of paraensidimerins A (**213**) and C (**215**) by X-ray diffraction enabled the ^{1}H-NMR spectra to be interpreted. In dimer A the cyclohexane ring adopts a chair conformation so that H-16 eq. and H-19 eq. have an ideal W relationship for the observed long-range coupling constant (J = approx. 2 Hz) and the positions of H-16 equiv and H-7 relative to carbonyl groups accounts for their lowfield chemical shifts: H-16 equiv at δ3.52, and H-7 at 3.87. In dimer C, however, the boat conformation of the cyclohexane ring excludes the possibility of long-range coupling. Similar conclusions were reached for vepridimerines A and B from NMR data alone (*105*). The structures and stereochemistry of paraensidimerine E (**216**) and F (**217**) were also determined by ^{1}H- and ^{13}C-NMR spectroscopy (*107*). In paraensidimerine-E (**216**),

the observed coupling constants of $J_{7,6a} = 0$ Hz, $J_{6a,16a} = 11$ Hz, and $J_{16a,16} =$ 14 and 5 Hz indicate that ring B is twisted so that carbons 6a, 16a, 16, 15, and 19 are approximately coplanar.

The presence of both a 4-quinolinone and a 2-quinolinone unit in vepridimerines C (**218**) and D (**219**) was apparent from the NMR spectra (*105*). Thus, one of the aromatic resonances (δ_H 8.06) and one of the carbonyl carbon resonances (δ_C 176.21) in vepridimerine C is characteristic of a 4-quinolinone unit, whereas the rest of the NMR data are similar to vepridimerine A. Structure **218** was therefore assigned to vepridimerine C, the small chemical-shift difference (approximately 0.2 ppm) of H-16a and H-16 eq showing that the 4-quinolinone unit is adjacent to ring C. Similar arguments led to the allocation of structure **219** to vepridimerine D (*105*).

Paraensidimerin G, $C_{30}H_{28}N_2O_4$, has two hydrogen atoms less than dimers A, C, E, and F and contains a cyclohexene B ring as in structure **210,** which was established by NMR spectroscopy. The ^{1}H-NMR spectrum shows that there are only five protons in ring B, one appearing at δ7.60 (=CH—); in the ^{13}C-NMR spectrum resonances at 130.4 (C-16a) and 128.1 ppm (C-16) replace signals at 26–32 ppm present in other dimers. The double bond must be at 16–16a, since a double bond at 7–19 would not permit an AB ring junction at 7–15. The observation of a near zero value for $J_{6a,7}$ indicated that the protons at C-6a and C-7 had a *trans* orientation, as in dimers A and C. In accord with this configuration, oxidation of dimer C (**215**) with DDQ at position 16–16a gave dimer G quantitatively. A similar reaction of dimer A (**213**) also gave dimer G, but a minor product was formed and was shown by spectroscopy to be isomer **211** (*107*).

Another dimeric quinolinone alkaloid, geijedimerine (**220**) $C_{28}H_{26}N_2O_4$, was isolated recently from *Geijera balansae* (*86*). Fragmentation ions in the mass spectrum at *m/z* 228 (flindersine moiety) and 212, IR absorption at 3450–3200 cm^{-1} (NH), and the absence in the ^{1}H-NMR spectrum of three-proton singlets at δ3.6–3.7 indicated that geijedimerine contained two NH groups instead of the NMe groups present in other dimeric quinolinone alkaloids; this was confirmed by methylation of the alkaloid with methyl iodide and potassium carbonate in refluxing acetone to give dimethylgeijedimerine (**221**). The structure of the alkaloid was established by the ^{1}H NMR spectrum which was similar to that of paraensidimerin F (**217**), thus showing that the stereochemistry of rings A, B, and C in the two alkaloids is identical. The presence of a 4-quinolinone group in geijedimerine is apparent from the large downfield shift of the H-5 resonance at δ8.40 in the dimethyl derivative; the attachment of the group to ring C as in vepridimerine D (**219**), is indicated by the similar values for resonances attributed to H_e, H_f, and H_g in the two alkaloids.

The paraensidimerins A, B, C, E, and F, the vepridimerines, and geijedimerine are optically inactive; paraensidimerin D, although first thought to have a

small optical activity (*90*), was later also shown to be racemic (*106*). The paraensidimerins are not regarded as artifacts of the isolation procedure, since they rapidly crystallized from petroleum extracts without long exposure to air and light, and might be formed in the plant tissue by dimerization of dienes without the participation of enzymes.

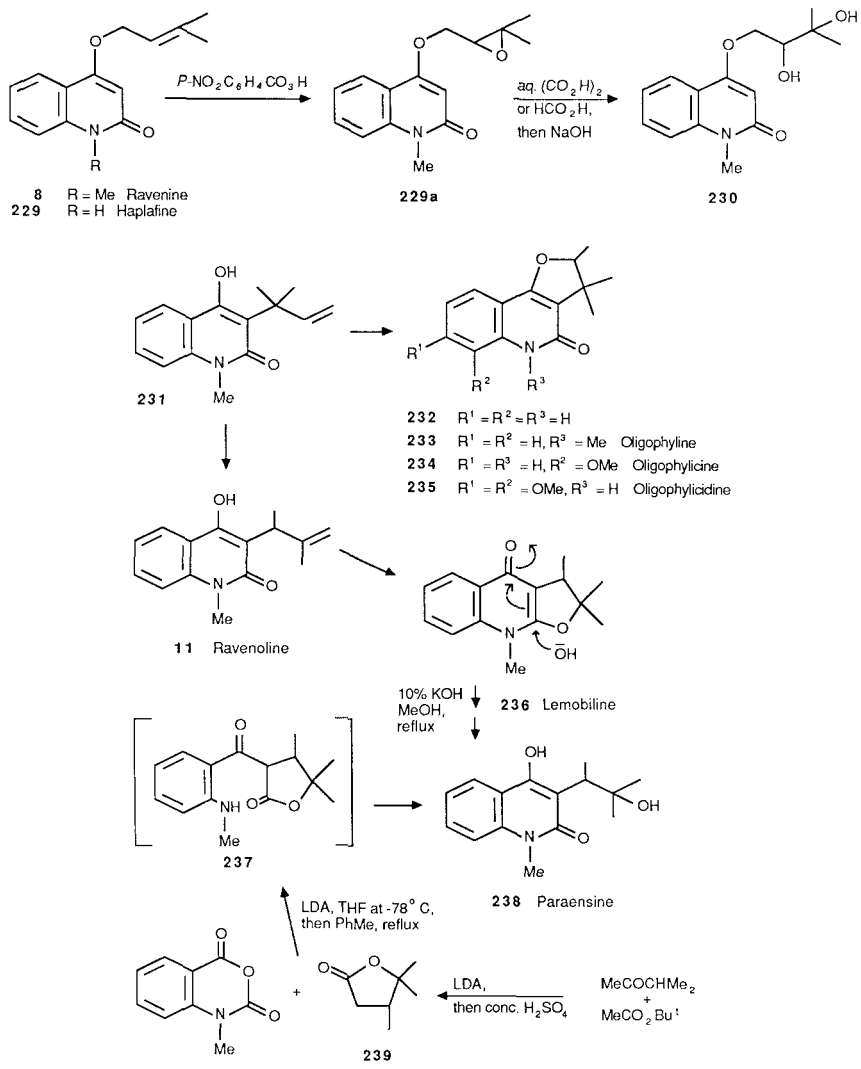
The alicyclic moiety of the paraensidimerins and the vepridimerines is similar to that present in isoalfileramine, the acid-catalyzed cyclization product of the alkaloid alfileramine, and to that found in the dimer **228,** which is formed from the allylic alcohol **227** probably via an *ortho*-hydroxydiene *cf.* (*109*). The biosynthesis of the paraensidimerins and the vepridimerins may occur by a similar route from dienes **225** (Scheme 26) (*109*). Diels–Alder addition of one molecule of diene to the internal double bond of another would be expected to give dimer **224** with a *cis* relationship of H_d and H_e; intramolecular addition of oxygen functions can then lead to dimeric alkaloids with *cis*-fused BC rings. *Trans*-isomers may arise by isomerization of the intermediate **224** to the cyclohexene **223,** which may then epimerize at H_e and then undergo cyclization.

Ayafor *et al.* (*111*) recently reported a synthesis of the vepridimerines. Pyrolysis of the dimethylpyranoquinolinone veprisine (**168**) gave five pure products (total recovery, 42%), four of which were identical with vepridimerines A (**212**), B (**214**), C (**218**), and D (**219**). The fifth isomer was designated vepridimerine E and was assigned structure **222,** largely on the basis of NMR spectra. Thus, resonances of δ_H 8.02 (H-5) and δ_C 176.20 (CO) indicated the presence of a 4-quinolinone ring, although the placing of this ring on the "left-hand side" of the molecule was by analogy with other vepridimerines. The coupling constant of $J = 12.0$ Hz showed that H_d and H_e had a *trans* relationship, and the general similarity of the NMR spectra to those of paraensidimerin F (**217**), in which the alicyclic rings have the same stereochemistry, was apparent. The sequence of reactions (**168**) → (**226**) → (**225**) was proposed for the pyrolysis.

VII 1,1- and 1,2-Dimethylallyl Alkaloids and Related Geranylquinolines

A. Introduction

A group of hemiterpenoid quinoline alkaloids found in *Flindersia ifflaiana* and in *Ravenia spectabilis* can be regarded as derivatives of 3-(1,1-dimethylallyl)quinolinones, *cf.* **231,** or of 3-(1,2-dimethylallyl)quinolinones, *cf.* **11** (Scheme 27). These alkaloids may be derived by Claisen rearrangement or abnormal Claisen rearrangement of *O*-prenylquinolinones; the rearrangement of



SCHEME 27. 1,1-Dimethyl- and 1,2-dimethylallylquinolinones and related alkaloids.

one such compound (**8**), which occurs in *Ravenia spectabilis,* led to the synthesis of several alkaloids of the group and was described previously (Vol. 17, p. 169). A corresponding *O*-geranylquinolinone (**245**) co-occurs in *Haplophyllum bucharicum* with a number of alkaloids that are apparently derived by Claisen or abnormal Claisen rearrangements (Vol. 17, p. 173). This account describes the isolation of new alkaloids from *Euxylophora paraensis,* from *Acronychia*

oligophylebia, and from *Haplophyllum bucharicum* and associated synthetic work.

B. Angular Dihydrotrimethylfuroquinolinones and Related Alkaloids

In accord with the proposed biosynthesis of 1,1- and 1,2-dimethylallylquinolinone derivatives (Scheme 27), *Ravenia spectabilis* contains the *O*-prenyl precursor ravenine (**8**); Kaneko *et al.* (*32*) reported a new synthesis of ravenine in which irradiation of the *N*-oxide (**31;** R=CH_2CH=CMe_2) (Scheme 5) gave the alkaloid in 47% yield. The alkaloid haplafine, which was isolated from *Haplophyllum perforatum,* was shown by spectroscopic studies to be 4-(3-methylbut-2-enyloxy)-2-quinolinone (**229**) (*233*). The diol **230,** mp 94°C $[\alpha]_D^{20}$ + 31.8° ($CHCl_3$), corresponding to ravenine, was isolated from *Euxylophora paraensis* and its structure was determined by spectroscopic studies (*90*) and by a synthesis from ravenine (**8**) involving conversion into an epoxide **229a** which was hydrolyzed with oxalic acid or via the secondary formate ester (*98*). (−)-Lemobiline (**236**), the cyclization product of the 1,2-dimethylallylquinolinone ravenoline (**11**), was isolated previously from *Flindersia ifflaiana* and *Ravenia spectabilis* (Vol. 17, p. 171), and has now been obtained from *E. paraensis* [mp 114–115°C $[\alpha]_D^{20}$ − 2.56° ($CHCl_3$)] (*90*).

Jurd *et al.* (*107*) showed that a major alkaloid of the heartwood of *E. paraensis* was the optically inactive angular furoquinolinone (**233**), mp 90°C; an enantiomer, mp 92–93°C, $[\alpha]_D$ − 13.5° (EtOH), was isolated by Xu and Xue (*42*) from the root of *Acronychia oligophylebia* and given the name oligophyline. This compound was first obtained by heating ravenine (**8**) at 130–140°C in the presence of sodium carbonate; the base inhibits the rearrangement of the Claisen product **231** to ravenoline (**11**) and allows cyclization to compound **233** to occur (*49*). Support for structure **233** was provided by the ^{13}C-NMR spectrum which showed signals for three *C*-methyl groups, a quaternary carbon atom at 144.4 ppm, and a methine carbon linked to oxygen which produced a resonance at 90.8 ppm.

An alkaloid, $C_{14}H_{15}NO_2$, mp 200–201°C, which was isolated in trace amounts from *E. paraensis* (*90*), is the angular furoquinolinone **232.** The ^1H-NMR spectrum is similar to that of alkaloid **233,** except for the absence of a signal due to an *N*-methyl group; in the ^{13}C-NMR spectrum the resonance at 162.8 ppm showed that a 2-quinolinone carbonyl group was present. Methoxy derivatives of alkaloid **232** were obtained from *A. oligophylebia* (*42*). The structures of the monomethoxy alkaloid oligophylicine (**234**) mp 170–171°C $[\alpha]_D$ −21.4 (EtOH), and the dimethoxy derivative oligophylicidine (**235**), mp 233–235°C, $[\alpha]_D$ −59.4° [EtOH], were determined by spectroscopy and by synthesis involving heating of the requisite *O*-prenyl derivatives in the presence of base.

Compound **234** was prepared previously by another procedure (*112*). The optical activity of oligophylicine (**234**) and oligophylicidine (**235**) is significant and indicates that whatever the origin of the presumed 1,2-dimethylallyl precursor, *cf.* (**231**), cyclization to the furans is enzymatically controlled.

C. Paraensine

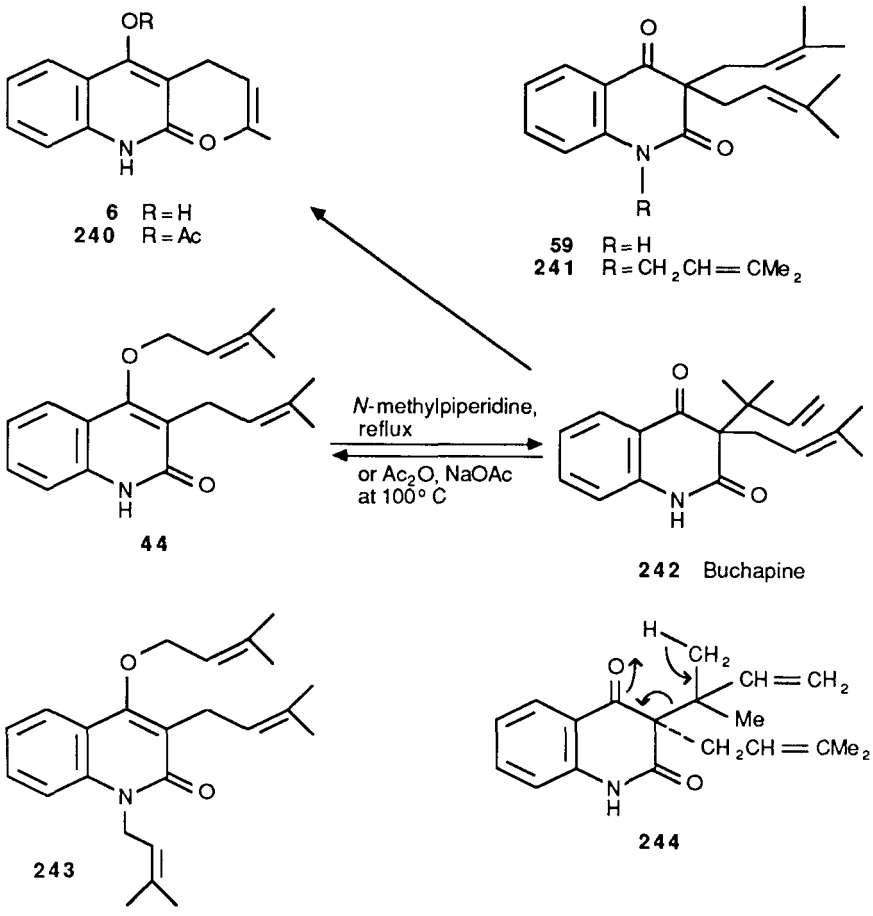
Jurd *et al.* (*107*) showed that *Euxylophora paraensis* contained the alkaloid paraensine (**238**), $C_{15}H_{19}NO_3$, mp 217–218°C, $[\alpha]_D$ −0.54°, $[\alpha]_{436}$ −0.88° and $[\alpha]_{365}$ −2.35° ($CHCl_3$). The structure of paraensine was determined by spectroscopic methods and by single-crystal X-ray diffraction and can be regarded as the hydrate of the 1,2-dimethylallyl quinolinone ravenoline (**11**). In fact, paraensine was obtained first by Talapatra and co-workers (*113*) in 1971 (Vol. 17, p. 172) as one of the products of cleavage of lemobiline (**236**) with base; the probable mechanism is indicated in Scheme 27.

Coppola and Schuster (*114*) synthesized paraensine by reaction of the lithium enolate of lactone **239** with *N*-methylisatoic anhydride; the lactone was prepared from isopropyl methyl ketone and tertiary-butyl acetate followed by cyclization of the product (Scheme 27).

D. Buchapine

Two hemiterpenoid quinoline alkaloids obtained recently from *Haplophyllum bucharicum* by Yunusov and co-workers (*45*) are the 3-prenyl-4-prenyloxy quinolinone **44** and its Claisen rearrangement product, buchapine (**242**). The *C*-,*O*-diprenylquinolinone alkaloid **44** was isolated first from *H. tuberculatum* and was prepared by Reisch and co-workers as one of six products of the allylation of 4-hydroxy-2-quinolinone (Scheme 3). The structure of buchapine, mp 134–135°C, which was not reported to be optically active, was established mainly by ^{1}H-NMR spectroscopy.

A synthesis of buchapine (**242**) and a study of the interconversion of buchapine and the *C*-,*O*-diprenylquinolinone **44** was carried out by Grundon and Ramachandran (*115*) (Scheme 28). Reaction of 4-hydroxy-3-prenyl-2-quinolinone (**6**) with 3,3-dimethylallyl bromide and potassium carbonate gave the *C*-,*O*-diprenyl derivative **44** in 10% yield; the major product of the reaction was the 3,3-bis-prenyl derivative **59** and two minor products were shown by ^{1}H-NMR spectroscopy and by their mass spectra to be the triprenyl compounds **241** and **243.** When the *C*-,*O*-diprenylquinolinone **44** was refluxed in *N*-methylpiperidine (bp 106°C) (±)-buchapine (**242**) was obtained and its structure was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy; the synthetic compound has mp 126°C, the lower value compared to the natural alkaloid indicating that the latter is probably an optically active enantiomer.



SCHEME 28. Synthesis and reactions of buchapine.

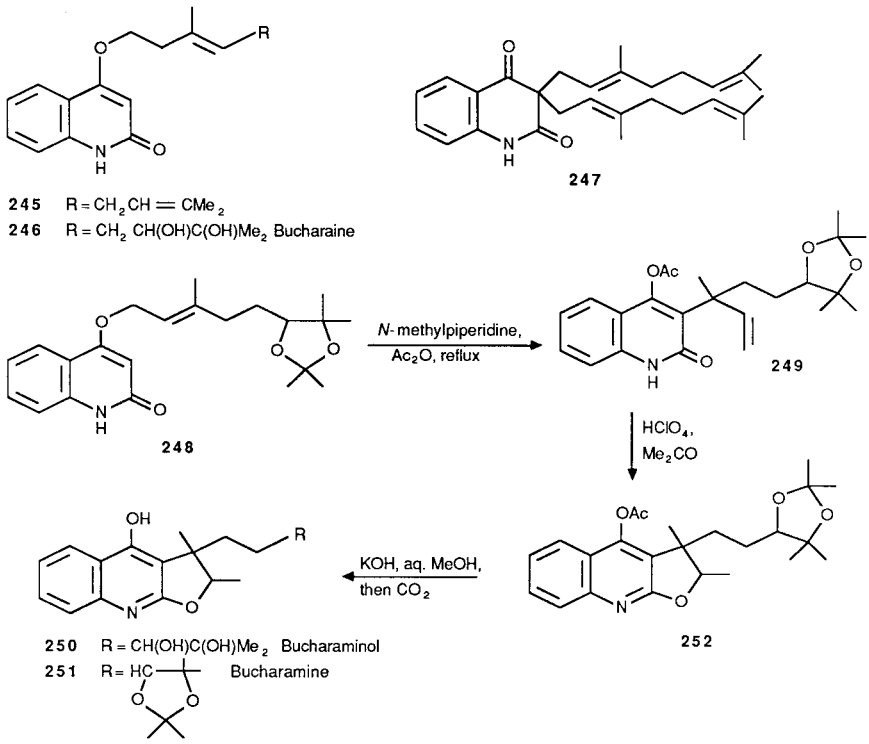
When the rearrangement of compound **44** was carried out for 3 hours, buchapine was obtained in 43% yield and the starting *C*-,*O*-diprenyl derivative **44** in 47% yield; since this ratio was not affected by prolonged reflux, it appeared that a reversible rearrangement occurred. Reversibility was confirmed by the observation that buchapine was converted into compound **44** (47%) under the same conditions. The same equilibrium of compounds **44** and **242** (approximate ratio 1 : 3) was obtained by heating either compound with acetic anhydride and sodium acetate at 100°C for 3 hr. A minor product (6%) of the forward reaction in *N*-methylpiperidine was the 4-hydroxy-3-prenylquinolinone **6** which was obtained in 20% yield in the reverse reaction; the corresponding figures for production of acetate **240** when Ac_2O-NaOAc was used were 46% and 66%.

The reversibility of the Claisen rearrangement was attributed to steric crowding at the C-3 quaternary carbon of buchapine. The driving force for cleavage of the 1,1-dimethylallyl group of buchapine may be loss of isoprene as indicated in **244;** support for this mechanism in the reaction of buchapine with acetic anhydride was provided by identification of isoprene by GLC analysis and by trapping the compound as its maleic anhydride adduct (*116*). The stability of the bisprenylquinolinone **59** to prolonged treatment with acetic anhydride and sodium acetate at 100°C emphasized the importance of the 1,1-dimethylallyl group of buchapine for the rearrangement reaction and for loss of the allyl group.

The same method of synthesis of buchapine was reported later by Bellino and Venturella (*230*).

E. Bucharaine, Bucharaminol, and Bucharamine

The structures of the monoterpenoid quinoline alkaloids bucharaine (**246**), bucharamine (**251**), and bucharidine of *Haplophyllum bucharicum* were discussed previously, and a synthesis of bucharaine was reported briefly (Vol. 17,



Scheme 29. Synthesis of bucharaminol and bucharamine.

p. 173). A full account of the synthesis of bucharaine (*117*) indicates that reaction of 4-hydroxy-2-quinolinone with geranyl chloride gave the *O*-geranyl ether (**245**) (12%) and the bis-geranyl derivative (**247**) (7%). The three-stage synthesis of bucharaine (**246**) from the geranyl ether (**245**) by means of epoxidation and then conversion into a formate ester occurred in an overall yield of 60% and was more efficient than hydroxylation of the geranyl ether with osmium tetraoxide.

Bucharamine is the acetonide **251,** possibly derived by Claisen rearrangement of the *O*-geranyl ether **245** followed by cyclization of the product. The diol bucharaminol (**250**) corresponding to bucharamine has now been obtained from *H. bucharicum* (*118*) and biomimetic syntheses of the two alkaloids have been carried out by Grundon and Ramachandran (*119*) (Scheme 29). Geranyloxyquinolinones undergo abnormal Claisen rearrangements readily, but when bucharaine acetonide (**248**) was heated with *N*-methylpiperidine the presence of acetic anhydride resulted in trapping of the initially formed Claisen rearrangement product as the acetate **249.** Cyclization with perchloric acid in acetone then gave the dihydrofuroquinoline **252,** which was hydrolyzed to the trihydroxy derivative **250** (bucharaminol); three crystalline diasteroisomers have been isolated and converted into the acetonides (stereoisomers of bucharamine), but the configurations of the natural alkaloids are not yet known.

VIII. Furoquinoline Alkaloids

Furoquinoline alkaloids can be regarded as derivatives of dictamnine (**10**) or isodictamnine (**253**) and are the most widely distributed of quinoline alkaloids. Known members of the group obtained from new sources since 1976 are listed in Table VI, which supplements earlier data (Vol. 9, p. 227, Table I; Vol. 17, p. 146, Table VI and p. 151, Table VII). Dictamnine, γ-fagarine (8-methoxydictamnine), kokusaginine (6,7-dimethoxydictamnine), and skimmianine (7,8-dimethoxydictamnine) continue to be the most frequently isolated.

Furoquinoline alkaloids are typical constituents of the Rutales and particularly of the Rutaceae and the isolation of γ-fagarine and skimmianine from *Tylophora asthmatica* (Asclepiadaceae) (*147*) and skimmianine from *Datura stramonium* (Solanaceae) (*161*) is therefore of considerable taxonomic interest.

A. Further Structural Studies and New Alkaloids

The occurrence of furoquinoline alkaloids first isolated since 1976 and their physical properties are indicated in Table VII (*12, 92, 122, 134, 135, 150, 156, 157, 177, 187–194, 229, 236–241*). In the following account, the alkaloids are grouped in accord with the pattern of oxygenation in the homocyclic ring.

TABLE VI

OCCURRENCE OF FUROQUINOLINES OF THE DICTAMNINE GROUP[a]

Name	Plant source	Reference
Dictamnine (**10**)	*Amyris pinnata*	*120*
	Boronella aff. *verticillata*	*121*
	Dutaillyea drupacea	*122*
	D. oreophila	*122*
	Esenbeckia flava	*46*
	E. litoralis	*46*
	Euodia lanu-ankenda	*15*
	Feronia limonia	*123*
	Flindersia fournieri	*124*
	Geijera balansae	*86*
	Glycosmis bilocularis	*125*
	G. mauritiana	*40*
	Haplophyllum dauricum	*17*
	H. obtusifolium	*237*
	H. perforatum	*24*
	H. robustum	*118*
	Hortia longifolia	*13*
	Melicope indica	*36*
	Myrtopsis macrocarpa	*127*
	M. myrtoidea	*127*
	M. novae-caledoniae	*127*
	M. sellingii	*18*
	Sarcomelicope argyrophylla	*128*
	S. glauca	*229*
	Toddalia aculeata	*129*
	T. aculeata var. *gracilis*	*130*
	Zanthoxylum alatum	*131*
	Z. budranga	*132*
	Z. cuspidatum	*20*
	Z. inerme	*133*
	Z. integrifoliolum	*22*
	Z. simulans	*97*
Isodictamnine (**253**)	*Dictamnus angustifolius*	*43*
Confusameline (7-Hydroxydictamnine)	*Melicope lasioneura*	*134*
	Myrtopsis sellingii	*18*
Haplophydine (**266**)	*Haplophyllum glabrinum*	*238*
Robustine (8-Hydroxydictamnine)	*Haplophyllum dauricum*	*17*
	H. obtusifolium	*237*
	H. vulcanicum	*135*
	Thamnosa montana	*136*
	Toddalia aculeata	*129*
	Zanthoxylum alatum	*131*
	Z. cuspidatum	*20*
	Z. integrifoliolum	*22*

(*continued*)

TABLE VI (*Continued*)

Name	Plant source	Reference
Pteleine (6-Methoxydictamnine)	*Dutaillyea drupacea*	122
	D. oreophila	122
Isopteleine (6-Methoxyisodictamnine)	*Dictamnus angustifolius*	43
Evolitrine (7-Methoxydictamnine)	*Acronychia oligophylebia*	42
	A. pedunculata	137
	Almeidea guyanensis	138
	Dutaillyea drupacea	122
	D. oreophila	122
	Esenbeckia flava	46
	E. litoralis	46
	Euodia lanu-ankenda	15
	Glycosmis cyanocarpa	139
	Melicope indica	36
	M. lasioneura	134
	Sarcomelicope argyrophylla	128
	S. glauca	229
γ—Fagarine (8-Methoxydictamnine)	*Aegle marmelos*	140
	Dictamnus angustifolius	43
	Erythrochiton brasiliensis	141
	Flindersia fournieri	124
	Geijera balansae	86
	Haplophyllum buxbaumii	92
	H. dauricum	142
	H. dubium	143
	H. glabrinum	238
	H. leptomerum	241
	H. obtusifolium	237
	H. suaveolens	87
	H. tenue	144
	H. tuberculatum	145
	H. vulcanicum	135
	Monnieria trifolia	146
	Myrtopsis myrtoidea	127
	M. novae-caledoniae	127
	M. sellingii	18
	Toddalia aculeata	129
	T. aculeata var. *gracilis*	130
	Tylophora asthmatica	147
	Vepris stolzii	97
	Zanthoxylum alatum	132
	Z. cuspidatum	20
	Z. oxyphyllum	131
	Z. rubescens	148
	Z. simulans	94

(*continued*)

TABLE VI (*Continued*)

Name	Plant source	Reference
Kokusaginine (6,7-Dimethoxydictamnine)	*Acronychia oligophylebia*	42
	A. pedunculata	137
	Bauerella simplicifolia ssp. *neo-scotica*	149
	Dutaillyea drupacea	122
	D. oreophila	122
	Esenbeckia litoralis	46
	E. pilocarpoides	150
	Glycosmis bilocularis	151
	Haplophyllum buxbaumii	92
	H. vulcanicum	152
	Melicope lasioneura	134
	M. leptococca	153
	Monnieria trifolia	146
	Oricia renieri	99
	O. suaveolens	154
	Sarcomelicope glauca	229
	Sargentia greggii	155
	Teclea ouabanguiensis	12
	T. verdoorniana	156,157
	Tinospora malabarica	158
	Vepris pilosa	159
	Zanthoxylum bungeanum	160
Maculosidine (6,8-Dimethoxydictamnine)	*Acronychia oligophylebia*	42
	Esenbeckia flava	46
	Oricia renieri	99
Isomaculosidine (6,8-Dimethoxyisodictamnine)	*Dictamnus dasycarpus*	236
Skimmianine (7,8-Dimethoxydictamnine)	*Acronychia oligophylebia*	42
	Aegle marmelos	140
	Almeidea guyanensis	138
	Amyris pinnata	120
	Datura stramonium	161
	Dictamnus hispanicus	71
	Esenbeckia flava	46
	E. litoralis	46
	Euxylophora paraensis	90
	Flindersia founieri	124
	Geijera balansae	67
	Glycosmis bilocularis	151
	G. mauritiana	40
	Haplophyllum albertii	162
	H. buxbaumii	92
	H. dauricum	142
	H. glabrinum	238

(*continued*)

TABLE VI (*Continued*)

Name	Plant source	Reference
	H. leptomerum	*241*
	H. tuberculatum	*145*
	H. vulcanicum	*135*
	Hortia longifolia	*13*
	Melicope lasioneura	*134*
	M. leratii	*3*
	Murraya paniculata	*163*
	Myrtopsis macrocarpa	*127*
	M. myrtoidea	*127*
	M. novae-caledoniae	*127*
	M. sellingii	*18*
	Oricia gabonensis	*95*
	O. renieri	*99*
	Sarcomelicope glauca	*229*
	Teclea sudanica	*164*
	T. verdoorniana	*165*
	Toddalia aculeata var. *gracilis*	*130*
	Tylophora asthmatica	*147*
	Vepris stolzii	*97*
	Zanthoxylum acanthopodium	*239*
	Z. arnottianum	*166*
	Z. bouetense	*167*
	Z. bungeanum	*94*
	Z. caribaeum	*168*
	Z. culantrillo	*169*
	Z. cuspidatum	*20*
	Z. fagara	*168*
	Z. integrifoliolum	*22*
	Z. leprieurii	*170*
	Z. limoncillo	*168*
	Z. nitidum	*171*
	Z. ovalifolium	*172*
	Z. sp. Sévenet-Pusset 1345	*173*
	Z. simulans	*240*
	Z. tessmanii	*66*
	Z. williamsii	
Maculine (6,7-Methylenedioxydictamnine)	*Esenbeckia litoralis*	*46*
	E. pilocarpoides	*150*
	Helietta parvifolia	*174*
	Sargentia greggii	*155*
	Teclea simplicifolia	*164*
	T. sudanica	*164*
Kokusagine (7,8-Methylenedioxydictamnine)	*Esenbeckia pilocarpoides*	*150*
	Euodia lanu-ankenda	*15*

(*continued*)

TABLE VI (*Continued*)

Name	Plant source	Reference
Haplopine (7-Hydroxy-8-methoxydictamnine)	*Afraegle paniculata*	*33*
	Geijera balansae	*86*
	Haplophyllum dauricum	*17*
	H. ferganicum	*175*
	H. latifolium	*176*
	H. obtusifolium	*177*
	Melicope lasioneura	*134*
	Monnieria trifolia	*178*
	Sarcomelicope glauca	*229*
	Zanthoxylum bungeanum	*160*
	Z. cuspidatum	*20*
	Z. integrifoliolum	*22*
7-(3-Methylbut-2-enyloxy)-γ-fagarine	*Haplophyllum ferganicum*	*175*
	H. glabrinum	*238*
	H. latifolium	*176*
	Melicope lasioneura	*134*
Heliparvifoline (7-Hydroxy-6-methoxydictamnine)	*Melicope confusa*	*179*
Evoxine [7-(2,3-Dihydroxy-3-methylbutyl)oxy-8-methoxydictamnine]	*Haplophyllum alberti*	*162*
	H. ferganicum	*175*
	H. glabrinum	*238*
	H. tuberculatum	*145*
	Monnieria trifolia	*178*
	Orixa japonica	*180*
	Teclea boiviniana	*181*
Evoxine acetate [7-(2-Acetoxy-3-hydroxy-3-methylbutyl)oxy-8-methoxydictamnine]	*Haplophyllum ferganicum*	*175*
Methylevoxine [7-(2-Hydroxy-3-methoxy-3-methylbutyl)oxy-8-methoxydictamine]	*Haplophyllum glabrinum*	*238*
	H. obtusifolium	*177*
Anhydroevoxine [7-(2,3-Epoxy-3-methylbutyl)oxy-8-methoxy-dictamnine]	*Haplophyllum ferganicum*	*175*
	H. perforatum	*182*
Evodine [7-(2-Hydroxy-3-methylbut-3-enyl)oxy-8-methoxydictamnine)	*Haplophyllum ferganicum*	*175*
	H. glabrinum	*238*
	H. obtusifolium	*177*
	H. perforatum	*126*
Evoxoidine [7-(3-Methylbutan-2-onyl)oxy-8-methoxydictamnine]	*Haplophyllum perforatum*	*126*
Glycoperine (7-*O*-Rhamnosyl-8-methoxydictamnine)	*Haplophyllum ferganicum*	*175*
	H. latifolium	*187*
Perfamine (**295**)	*Haplophyllum glabrinum*	*238*
Halfordinine (6,7,8-Trimethoxydictamnine)	*Diphasia angolensis*	*183*
	Oricia suaveolens	*154*
	Teclea verdoorniana	*165*

(*continued*)

TABLE VI (*Continued*)

Name	Plant source	Reference
Flindersiamine (8-Methoxy-6,7-methylenedioxydictamnine)	*Esenbeckia flava*	*46*
	E. pilocarpoides	*150*
	Oriciopsis glaberrima	*184*
	Teclea ouabanguiensis	*12*
	T. sudanica	*164*
	T. verdoorniana	*156,157*
Acronydine (5,11-Dimethoxy-3,3-dimethyl-3H-furo-[2,3-*b*]pyrano[3,2-*b*]quinoline)	*Melicope leptococca*	*153*
	Sarcomelicope leicarpa	*185*
Acronycidine (5,7,8-Trimethoxydictamnine)	*Bauerella simplicifolia* subsp. *neoscotica*	*186*
	Melicope leptococca	*153*
	Sarcomelicope argyrophylla	*128*
	S. leicarpa	*185*

[a] This table is supplementary to Table I in Chapter 6 of "The Alkaloids," Vol. 9, and to Tables VI and VII in Chapter 2 of "The Alkaloids," Vol. 17.

1. Melineurine, 7-Hydroxy-8-(3-methylbut-2-enyl)dictamnine, Haplophydine, Taifine, Isotaifine, 8-Methoxytaifine, O-Ethylnordictamnine, O-Ethylnor-γ-fagarine, O-Ethylnorskimmianine, and Dutadrapine

The optically inactive alkaloid melineurine (**254**), $C_{17}H_{17}NO_3$, was isolated from *Melicope lasioneura* (*134*). Melineurine was shown to contain a prenyloxy and a methoxy group by fragment ions in the mass spectrum at *m/z* 215 and 200. This was confirmed by the ^{1}H-NMR spectrum which showed a three-proton singlet at δ4.36 characteristic of a methoxy group at C-4 in a furoquinoline; the substitution pattern in the aromatic ring was apparent from the signals at δ8.09 (J = 9 Hz, H-5), 7.29 (J = 2 Hz, H-8), and 7.04 ppm (J = 9 and 2 Hz, H-6). Melineurine displayed the typical rearrangement with methyl iodide at 105–110°C to give isomelineurine (**255**).

7-Hydroxy-8-(3-methylbut-2-enyl) dictamine (**256**), which is a constituent of *Sarcomelicope glauca* (*229*), is a member of the rare group of *C*-prenylfuroquinolines; its structure was established by spectroscopy and by its formation by treatment of the synthetic diprenyl derivative **257** (cf. (*234*)) with hydrobromic acid in acetic acid.

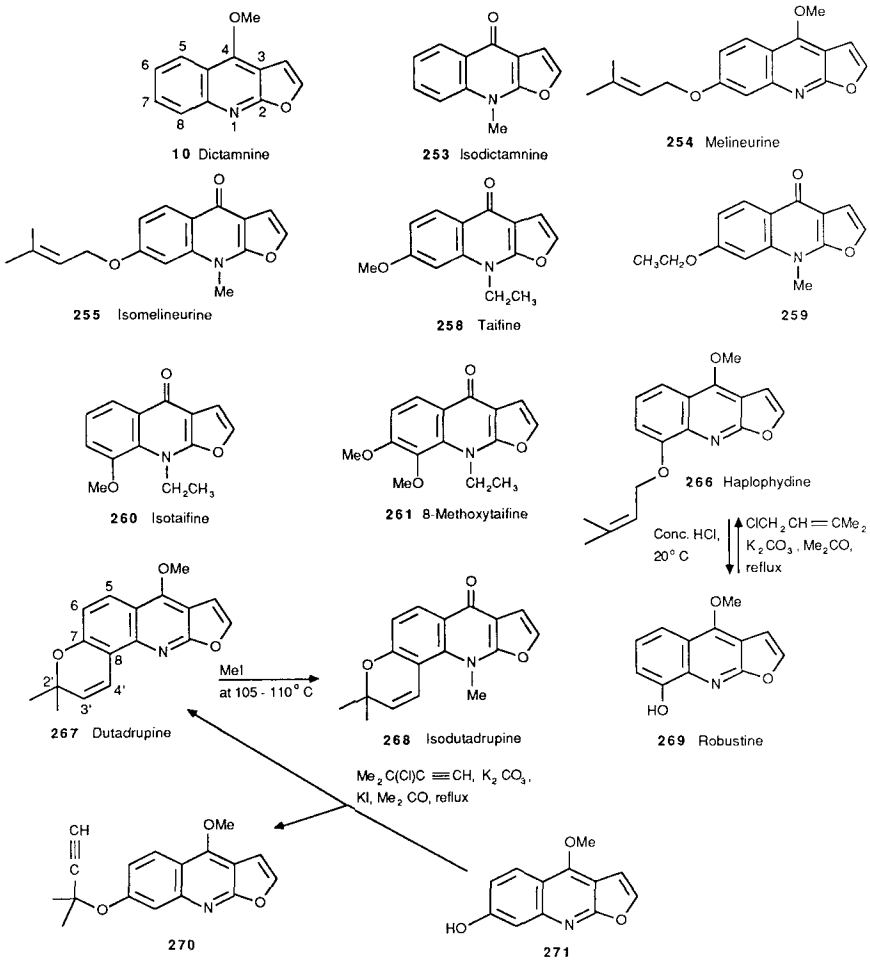
Structure **266** for haplophydine, which was proposed earlier on the basis of spectroscopic studies (Vol. 17, p. 158), has now been confirmed by chemical studies (*189*). Hydrolysis of haplophydine with hydrochloric acid gave 8-hydroxydictamnine (robustine) (**269**) and, on reaction of robustine with isopropenyl chloride, haplophydine was formed.

TABLE VII

OCCURRENCE OF FUROQUINOLINE ALKALOIDS FIRST ISOLATED 1976–1985

Name	Structure number	Source	Melting point (°C)	$[\alpha]_D$ (Solvent)	Ref.
Delbine	**300**	*Monnieria trifolia*	229–231		*188*
		Haplophyllum vulcanicum	—		*135*
Diacetylglycoperine		*H. perforatum*	—		*189*
Dihydroperfamine	**297**	*H. glabrinum*	179–183	$[\alpha]_{546}$ −640($CHCl_3$)	*238*
5,6-Dimethoxydictamnine	**309**	*H. buxbaumii*	amorphous		*92*
5,7-Dimethoxydictamnine	**310**	*H. buxbaumii*	amorphous		*92*
Dutadrupine	**267**	*Dutaillyea drupacea*	107–109		*122*
O-Ethylnordictamnine	**263**	*Dictamnus dasycarpus*	95–96		*236*
O-Ethylnor-γ-fagrine	**264**	*Dictamnus dasycarpus*	142–143		*236*
O-Ethylnorskimmiamine	**265**	*Dictamnus dasycarpus*	135–136		*236*
Glycohaplopine	**279**	*H. perforatum*			*190*
Haplatine acetate	**281**	*H. obtusifolium*			*177*
Haplobine	**282**	*H. obtusifolium*	151–153		*237*
8-Hydroxy-7-methoxydictamnine	**272**	*Zanthoxylum arborescens*	129–130		*191*
7-Hydroxy-8-(3-methylbut-2enyl)dictamnine	**256**	*Sarcomelicope glauca*	—		*229*

Isomaculine	**303**	*Esenbeckia pilocarpoides*	269		*150*
Isotaifine	**260**	*Ruta chalepensis*	123–125		*192*
Melineurine	**254**	*Melicope lasioneura*	98–99		*134*
7-Methoxy-8-(3-Methylbut-2-enyloxy)dictamnine	**273**	*Zanthoxylum arborescens*	120.5–121.5		*191*
8-Methoxytaifine	**261**	*Ruta chalepensis*	122–123		*192*
Monoacetylglycoperine	**277**	*Haplophyllum perforatum*	120–121		*189*
Montrifoline	**298**	*H. vulcanicum*	190–192	+23°(MeOH) +7°($CHCl_3$)	*135* *188*
		Monnieria trifolia	191–193	—	
		Teclea ouabanguiensis	190–191	—	*12*
		T. verdoorniana	—	−16.5°(MeOH)	*193*
Nigdenine	**274**	*Haplophyllum vulcanicum*	177–180	+5.3°(MeOH)	*135*
Taifine	**258**	*Ruta chalepensis*	110		*194*
Tecleamine	**313**	*Teclea ouabanguiensis*	112–113		*12*
Tecleaverdine	**316**	*T. verdoorniana*	218–219		*157*
Tecleaverdoornine	**315**	*T. ouabanguiensis*	191–192		*12*
		T. verdoorniana	191		*156,157*
		T. ouabanguiensis	256–257		*12*
Tecleine	**311**	*T. verdoorniana*	257–258		*157*
Triacetylglycoperine	**278**	*Haplophyllum perforatum*	181–182	−91°(EtOH)	*195*



An unusual group of *N*-ethyl-4-quinolinones was isolated by Budzikiewicz and co-workers from *Ruta chalepensis* and shown by spectroscopic studies to be the monomethoxy derivatives taifine (**258**) (*194*) and isotaifine (**260**) (*192*), and the dimethoxyfuroquinolinone 8-methoxytaifine (**261**) (*192*). Taifine, for example, was apparently either the *N*-ethyl-*O*-methylquinolinone **258** or the *N*-methyl-*O*-ethylquinolinone **259;** structure **258** was preferred as a result of comparing the ^{1}H-NMR spectrum in chloroform and trifluoroacetic acid and observing a significant downfield shift of 0.26 ppm in the acidic solvent due to the methylene group, as would be expected from protonation of the nitrogen atom in structure **258.** The method of isolation of the alkaloids of *R. chalepensis* which involved refluxing in ethanol and heating with ethanolic potassium hydroxide, may be responsible for the presence of ethyl groups. Kuo *et al.* (*235*) prepared the *N*-

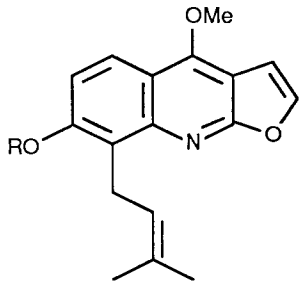
ethylquinolinone **258** by the modified Tuppy-Böhm procedure (*see* Scheme 31) and showed that it did not have the same melting point or spectroscopic properties as taifine. Although the 4-ethoxyfuroquinoline **262,** which was also synthesized, has the same melting point as taifine and the ^{1}H-NMR data recorded for the two compounds are similar, a final decision on the structure of the alkaloid must await further investigation.

Three *O*-ethylfuroquinoline alkaloids, *O*-ethylnordictammine (**263**), *O*-ethylnor-γ-fagarine (**264**) and *O*-ethylnorskimmianine (**265**), which were identified recently from the roots of *Dictammus dasycarpus* (*236*) were reported not to be artifacts.

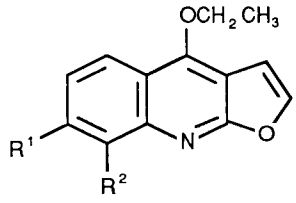
Acronidine and medicosmine, which are furoquinolines containing a 2,2-dimethylpyrano ring, have been known for many years, and a third member of the group, dutadrupine (**267**), has now been isolated from the stem bark of *Dutaillyea drupacea* (*122*). The structure of the alkaloid was determined mainly by the ^{1}H-NMR spectrum in comparison with that of isodutadrupine (**268**), obtained in the usual way by heating the alkaloid with methyl iodide. Thus, the presence of *ortho*-coupled aromatic protons was apparent from the spectra and displacement of the H-5 signal in dutadrupine to lower field (δ8.34) in isodutadrupine because of the influence of the 4-carbonyl group was observed. The resonance at 7.44 ppm (H-4′) in dutadrupine appeared at a lower field (−0.71 ppm) in isodutadrupine because of the steric effect of the neighboring *N*-methyl group, thus confirming the position of the attachment of the pyrano ring at C-7 and C-8 as in structure **267.** In a synthesis carried out by Tillequin *et al.* (*196*), 7-hydroxydictamnine (confusameline) (**271**) reacted with 3-chloro-3-methylbut-1-yne to give a mixture of dutadrupine and the ether **270.**

2. *8-Hydroxy-7-methoxydictamnine, 8-Isopentenyloxy-7-methoxy-dictamnine, and Nigdenine*

An alkaloid isolated by Stermitz and his collaborators (*191*) from *Zanthoxylum arborescens* was shown to be 8-hydroxy-7-methoxy-dictamnine (**272**) by meth-



256 R = H
257 R = $CH_2CH=CMe_2$



262 R^1 = OMe, R^2 = H
263 $R^1 = R^2$ = H *O*-Ethylnordictamine
264 R^1 = H, R^2 = OMe *O*-Ethylnor-γ-fagarine
265 $R^1 = R^2$ = OMe *O*-Ethylnorskimmianine

ylation with diazomethane to 7,8-dimethoxy-dictamnine (skimmianine) and by its nonidentity with 7-hydroxy-8-methoxydictamnine (haplopine) (**296**). A second new furoquinoline alkaloid from *Z. arborescens* was assigned structure **273** on the basis of spectroscopic studies and its formation from alkaloid **272** by reaction with 3,3-dimethylallyl bromide (*191*).

The structure of the 7,8-dimethoxyfuroquinoline alkaloid, nigdenine (**274**), which was isolated recently from *Haplophyllum vulcanicum,* was determined by the formation of a monoacetate and by spectroscopic studies (*135*). Thus, the presence of a (2,3-dihydroxy-3-methylbutyl)oxy substituent was apparent from the mass spectrum in which loss of the side-chain gave a fragmentation ion at *m/z* 244 and from the ^{1}H-NMR spectrum; the position of the side-chain was indicated by the absence in the ^{1}H-NMR spectrum of a signal at approximately 4.4 ppm, which is characteristic of a methoxy group at C-4 in furoquinoline alkaloids. Maculosine (**275**) is the only other known furoquinoline alkaloid containing a bulky alkoxy side-chain at C-4.

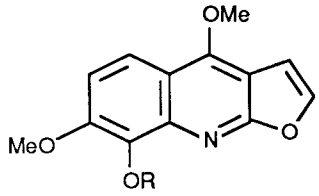
3. *Mono- and Triacetylglycoperine, Glycohaplopine, Haplatine, Haplatine Acetate, and Haplobine*

Yunusov and co-workers have obtained several new derivatives of 7-hydroxy-8-methoxydictamnine (haplopine) from *Haplophyllum* species.

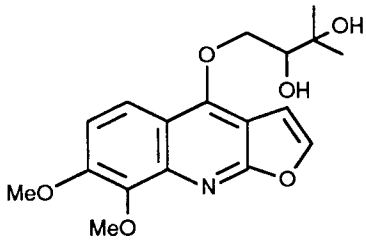
Haplophyllum perforatum was shown previously to contain glycoperine (**276**), the rhamnoside of haplopine; chromatography of the glycoperine fraction resulted in the isolation of triacetylglycoperine (**278**); its structure was established by spectroscopy and by its formation through acetylation of glycoperine (*195*). Chromatography of the skimmianine fraction from this species gave monoacetylglycoperine (**277**), which was converted into triacetylglycoperine by reaction with acetic anhydride and pyridine; a fraction containing a mixture of mono- and diacetylglycoperine was also obtained, but a pure sample of diacetylglycoperine was not isolated (*189*). Glycohaplopine (**279**) is also a constituent of *H. perforatum* and is believed to be the β-glycoside of haplopine (*190*).

A further study of haplatine (**280**), a furoquinoline of *Haplophyllum latifolium,* has shown that heating the alkaloid with methyl iodide gives the expected isohaplatine (**283**) and also results in methylation of the allylic hydroxy group to furnish a second product (**284**). Reductive cleavage of haplatine gave a mixture of the hexahydro derivative (**286**) and 4,8-dimethoxy-3-ethyl-7-hydroxy-2-quinolinone (**285**) (*187*). Haplatine acetate (**281**) has now been isolated from *H. obtusifolium* (*177*).

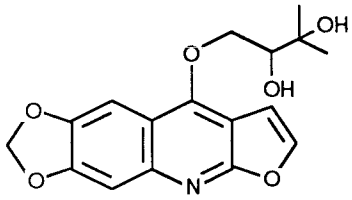
The structure of the chlorine-containing alkaloid haplobine (**282**), which was isolated from the root of *Haplophyllum obtusifolium,* was determined from its ^{1}H-NMR spectrum, from the mass spectrum, and by its hydrolysis to 7-hydroxy-8-methoxydictammine (haplopine) (*237*).



272 R = H
273 R = $CH_2CH{\equiv}CMe_2$



274 Nigdenine



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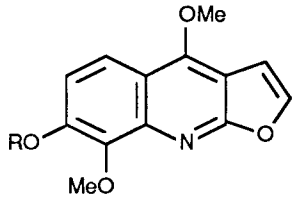
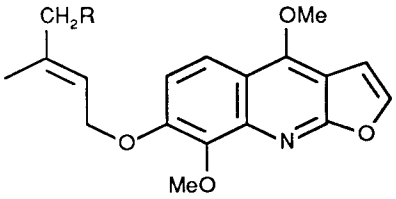
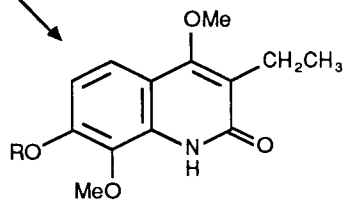


276 R = *O*-rhamnose Glycoperine
277 R = *O*-rhamnose monoacetate
278 R = *O*-rhamnose triacetate
279 R = *O*-glucose Glycohaplopine



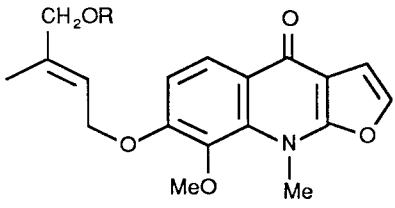
280 R = OH Haplatine
281 R = OAc Haplatine acetate
282 R = Cl Haplobine

(R = OH)



285 R = H
286 R = $CH_2CH_2CH(Me)CH_2OH$

(R = OH) MeI



283 R = H Isohaplatine
284 R = Me

4. Haplophyllidine, Perforine, Perfamine, and Dihydroperfamine

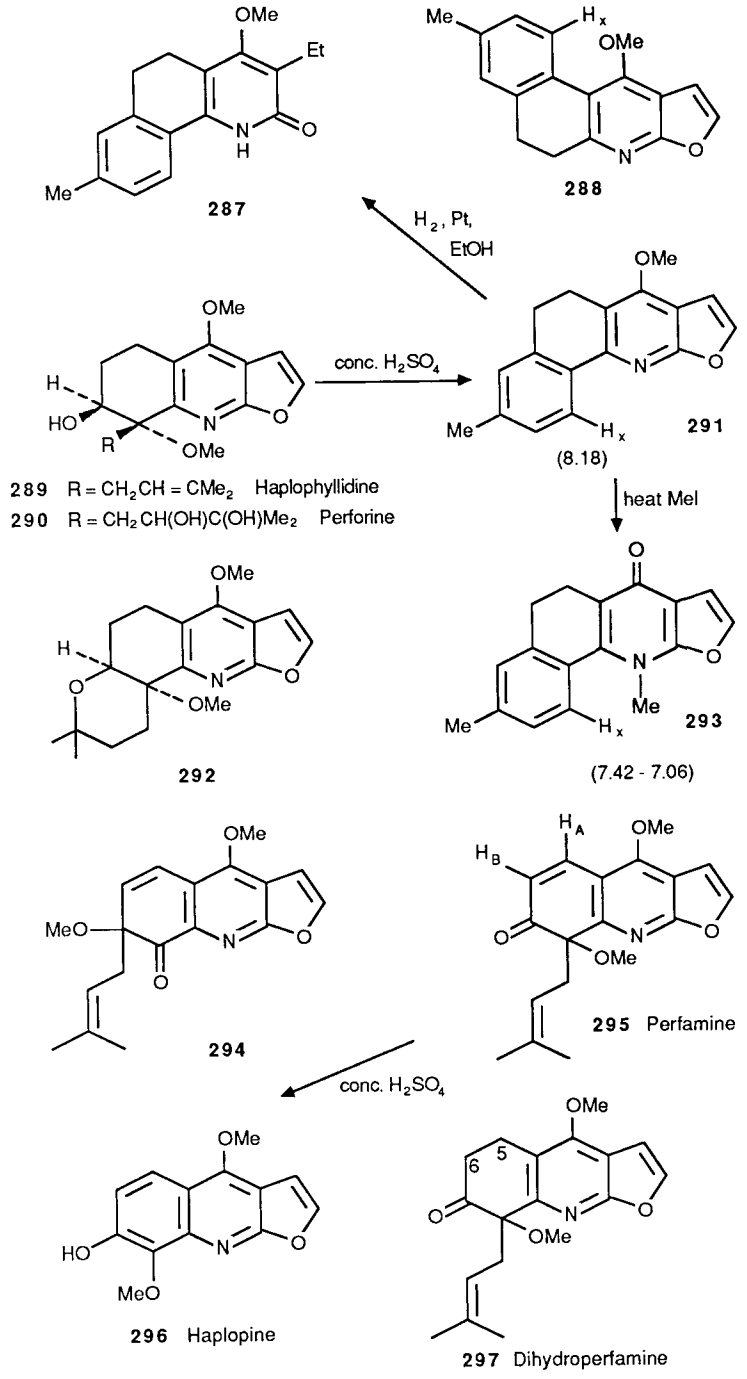
The unusual furoquinoline alkaloids haplophyllidine, perforine, and perfamine from *Haplophyllum perforatum* were previously assigned structures (**289**), (**290**), and (**294**), respectively (*see* Vol. 17, p. 155); as a result of further investigation during the last decade the structures of haplophyllidine and perforine have been confirmed and a new structure (**295**) for perfamine has been established.

Treatment of perforine and haplophyllidine with concentrated sulfuric acid (Scheme 30) gives the same compound, $C_{17}H_{15}NO_2$, which was assigned structure **291** on the basis of chemical and spectroscopic evidence. Thus, reductive cleavage with hydrogen and platinum gave the 3-ethyl-2-quinolinone **287** showing carbonyl absorption in the IR spectrum at 1647 cm^{-1}; the ^{1}H-NMR spectrum indicated the presence of one *C*-methyl and one *O*-methyl group, two benzylic methylene groups, and three aromatic protons. The alternative structure **288** was excluded by the NMR spectrum, which showed no NOE between the methoxy group and H_X and by the spectrum of the iso compound in which a substantial diamagnetic shift for H_X is due to the proximity of the *N*-methyl group as in structure **293** (*197*). Identification of product **291** supports the structures proposed for haplophyllidine and perforine; X-ray analysis of the furoquinoline derivative **292,** which was formed from both alkaloids by reaction with zinc and acid, confirms the structures and establishes the stereochemistry shown (*198*).

Earlier work on perfamine did not distinguish between structures **294** and **295** but the reported formation of 8-hydroxy-7-methoxydictamnine on treatment of perfamine with acid clearly favored structure **294** (Vol. 17, p. 157). The subsequent isolation of 8-hydroxy-7-methoxydictamnine and its non-identity with the product from perfamine led Stermitz and co-workers (*191*) to propose structure **295** for the alkaloid; the ^{1}H-NMR resonances at 8.80 (H_A) and 6.2 ppm (H_B) were assigned by comparison with model compounds and provide support for the new structure. A new investigation of the action of concentrated sulfuric acid on perfamine showed that the product was the sulfate salt of 7-hydroxy-8-methoxydictamnine (haplopine) (**296**), as expected from structure **295** (*199*).

A study of the alkaloids of *Haplophyllum glabrinum* by Reisch and his coworkers (*238*) resulted in the isolation of a range of 7,8-dioxygenated dictamnine derivatives typical of *Haplophyllum* species (Table VI) including perfamine and the new alkaloid dihydroperfamine (**297**). The spectroscopic data for dihydroperfamine are similar to those for perfamine except for a multiplet at 2.5–3.3 ppm in the ^{1}H-NMR spectrum, which was attributed to protons at C-5 and C-6; the constitution of dihydroperfamine was established by X-ray analysis, which also confirms the structure assigned to perfamine.

The relationship between the partially reduced furoquinoline alkaloids of *Haplophyllum perforatum* is now clear; perfamine appears to be formed by *C*-

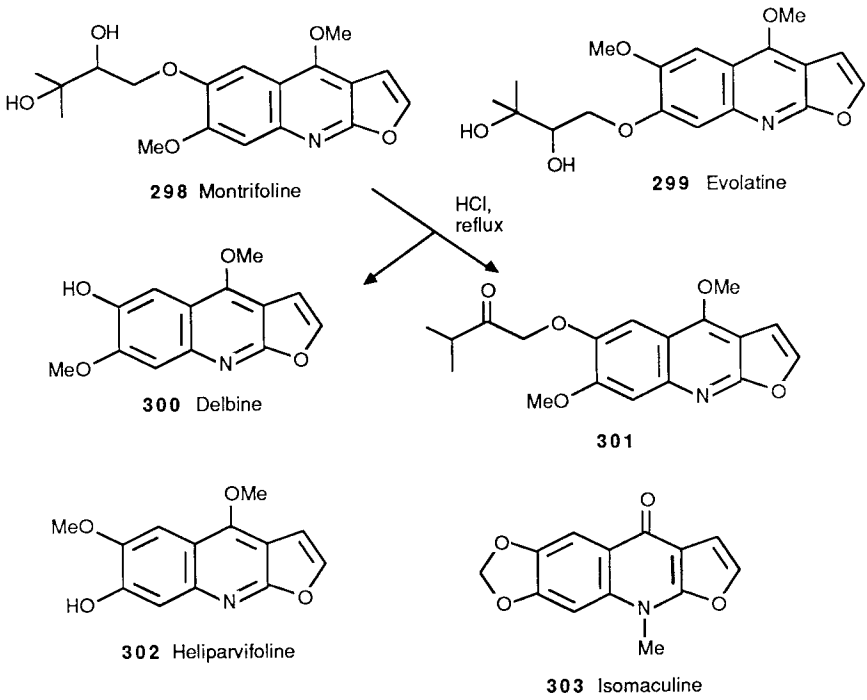


SCHEME 30. Structures of haplophyllidine, perforine, and perfamine.

prenylation of haplopine, halophyllidine is a tetrahydro derivative of perfamine, and perforine is the hydrate of haplophyllidine.

5. Delbine, Montrifoline, and Isomaculine

Bhattacharyya and Serur (*188*) isolated delbine (**300**) and montrifoline (**298**) from leaves of the Brazilian herb, *Monnieria trifoliata*. Spectroscopic examination showed that delbine was a hydroxymethoxydictamnine. Since methylation with diazomethane gave 6,7-dimethoxydictamnine (kokusaginine) and delbine was not identical with 7-hydroxy-6-methoxydictamnine (heliparvifoline) (**302**), the alkaloid was clearly 6-hydroxy-7-methoxydictamnine (**300**). Montrifoline was shown by spectroscopic studies to be a derivative of dictamnine containing a methoxy and a 2,3-dihydroxy-3-methyl-butyloxy group in the homocyclic ring; since the properties of the alkaloid did not correspond to those of evolatine (**299**), structure **298** was proposed and confirmed by alkaline fusion to give delbine (**300**). At about the same time, Moulis *et al.* (*178*) also investigated the alkaloids of *M. trifoliata* and isolated alkaloids with melting points almost identical to those of delbine and montrifoline, for which the alternative structures (**302**) (heliparvifoline) and (**299**) (evolatine) were assigned. Further evidence for the original structures for delbine and montrifoline was subsequently provided (*200*). An optical rotation for montrifoline was not recorded and this applies to a sample

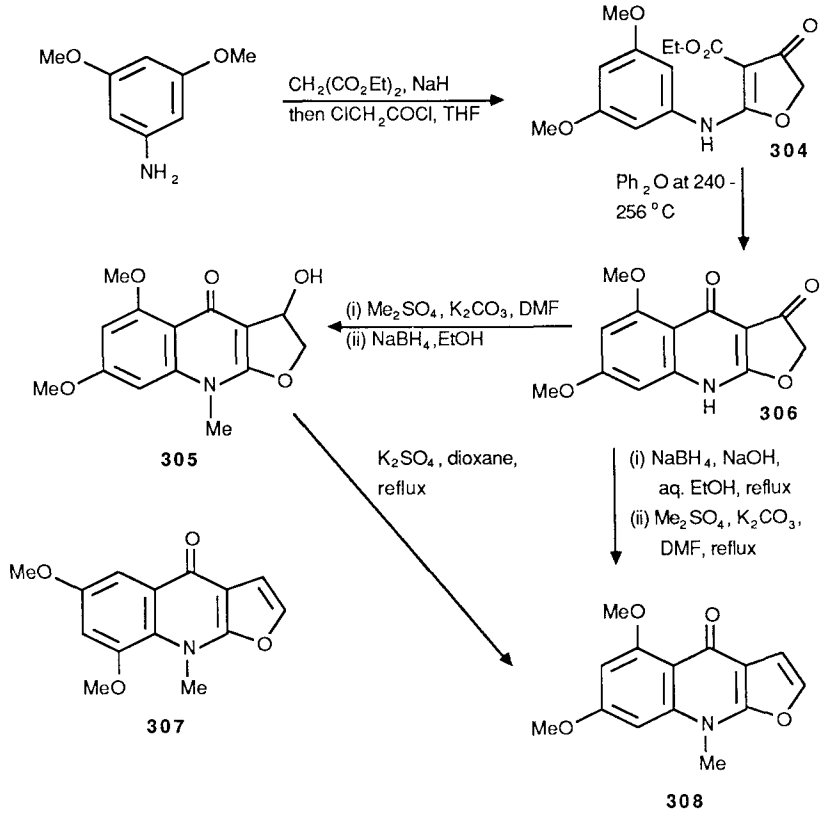


obtained from *Teclea ouabanguiensis* (*12*), but *T. verdoorniana* was reported to contain (−)-montrifoline (*193*); the name nkolbisine was given to the alkaloid of *Teclea* species, but the original name montrifoline should be used in future. The constituents of *Haplophyllum vulcanicum* include delbine (**300**) and (+)-montrifoline; the latter alkaloid was shown to give ketone **301** as well as delbine on reaction with hydrochloric acid (*135*).

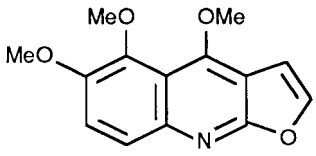
Isomaculine (**303**), which was known previously only as a synthetic compound, was isolated from the root bark of *Esenbeckia pilocarpoides* (*150*).

6. *Glycarpine, 5,6-Dimethoxydictamnine, and 5,7-Dimethoxydictamnine*

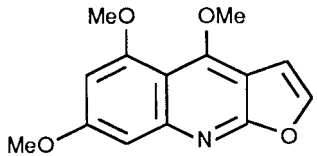
A furoquinolinone alkaloid of *Glycosmis cyanocarpa* was given the name glycarpine and assigned structure **308** (5,7-dimethoxyisodictamnine) on the basis of spectroscopic studies (*139*). In an attempt to confirm this structure, Kuo *et al.* (*201*) prepared 5,7-dimethoxyisodictamnine by the Tuppy–Böhm procedure and



SCHEME 31. Synthesis of 5,7-dimethoxyisodictamnine.



309 5, 6-Dimethoxydictamnine



310 5, 7-Dimethoxydictamnine

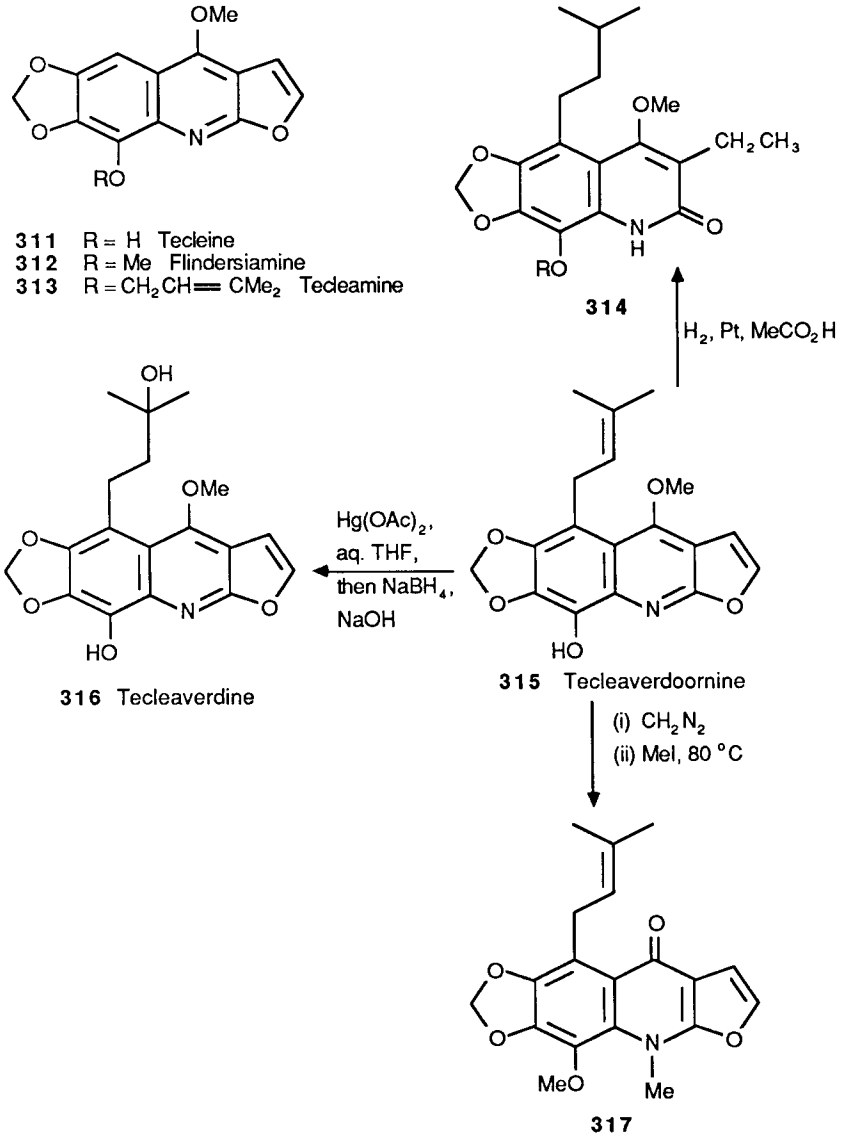
by the modification of this method introduced by Govindachari and co-workers (*see* Vol. 17, p. 162) (Scheme 31). Cyclization of the tetronic ester **304** gave the dione **306,** which after methylation was reduced to the alcohol **305;** dehydration then gave the required quinolinone **308,** which was also obtained from dione **306** by treatment with excess borohydride and alkali and then methylation of the furoquinolinone. The structure of synthetic 5,7-dimethoxyisodictamnine (**308**) was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy. The melting point (171°C) of compound **308** corresponds to that given for glycarpine (170–171°C) but the spectral data for the two compounds were not identical. Although the structure of glycarpine remains in doubt, it was pointed out (*202*) that 6,8-dimethoxyisodictamnine (isomaculosidine) (**307**) (*203*) also has a similar melting point (170–172°C) and the chemical shifts of the aromatic protons (7.24 and 6.99 ppm) resemble those of glycarpine (7.28 and 7 ppm).

Two new furoquinoline alkaloids isolated recently from *Haplophyllum buxbaumii* were shown to be 5,6-dimethoxydictamnine (**309**) and 5,7-dimethoxydictamnine (**310**) (*92*). The structures were established by mass spectrometry in which the lack of M^{+}-1 and M^{+}-29 fragmentation ions indicated the absence of a methoxy group at C-8 and by ^{1}H-NMR spectra in which the aromatic protons of 5,6-dimethoxydictamnine appeared as *ortho*-coupled doublets at δ7.65 and 7.40 (J = 8–9 Hz) and those of 5,7-dimethoxydictamnine as *meta*-coupled doublets at 7.65 and 7.08 (J = 2.5 Hz); they were clearly not identical with the well-known 6,7-, 6,8-, or 7,8-dimethoxydictamines.

7. Tecleine, Tecleamine, Tecleaverdoornine, and Tecleaverdine

An investigation of the alkaloids of *Teclea ouabanguiensis* and *T. verdoorniana* by Ayafor and co-workers (*12, 156, 157*) led to the isolation of a new group of dictamnine derivatives with three or four-substituents in the homocyclic ring.

A hydroxymethylenedioxydictamnine was obtained from *Teclea sudanica* in 1959 (*204*), but not in sufficient quantity for a full structural investigation. The same alkaloid, now given the name tecleine, has been isolated from *T. verdoor-*



niana and shown to be 8-hydroxy-6,7-methylenedioxydictamnine (**311**) by spectroscopic studies and by methylation with diazomethane to flindersiamine (**312**) (*157*).

Tecleamine (**313**) was isolated from *Teclea ouabanguiensis* and shown to be the *O*-prenyl derivative of tecleine (**311**) by the ^{1}H- and ^{13}C-NMR spectra (*cf.*

Scheme 3) and by refluxing the alkaloid with hydrochloric acid and acetic acid in ethanol to give tecleine (*12*).

Tecleaverdoornine (**315**) is another constituent of *Teclea oubanguiensis* and *T. verdoorniana* and is the first furoquinoline alkaloid to contain a fully substituted homocyclic ring (*156, 157*). The presence of a phenolic hydroxy group was indicated by IR absorption at 3410 cm^{-1} and by the formation of a monomethyl ether and a monoacetate. The formation of a tetrahydro derivative on catalytic reduction with palladium and the ^{1}H-NMR spectrum showed that tecleaverdoornine was a furoquinoline containing a *C*-prenyl group; this conclusion was supported by reduction with hydrogen and a platinum catalyst to give hexahydrotecleaverdoornine (**314**) (IR absorption at 1638 cm^{-1}).

Since the alkaloid failed to react with hydrochloric acid to give a dihydropyrano derivative, the prenyl and OH groups were not attached to adjacent carbon atoms. The prenyl group was assigned to C-5 because of the low-field chemical shift of the methylene group in the alkaloid (3.8 ppm) and in isotecleaverdoonine methyl ether (**317**) because of deshielding by the methoxy and carbonyl groups, respectively, at C-4. The nonbasicity of tecleaverdoornine and its failure to form an iso derivative with methyl iodide was attributed to hydrogen bonding of the OH group at C-8 to the nitrogen atom.

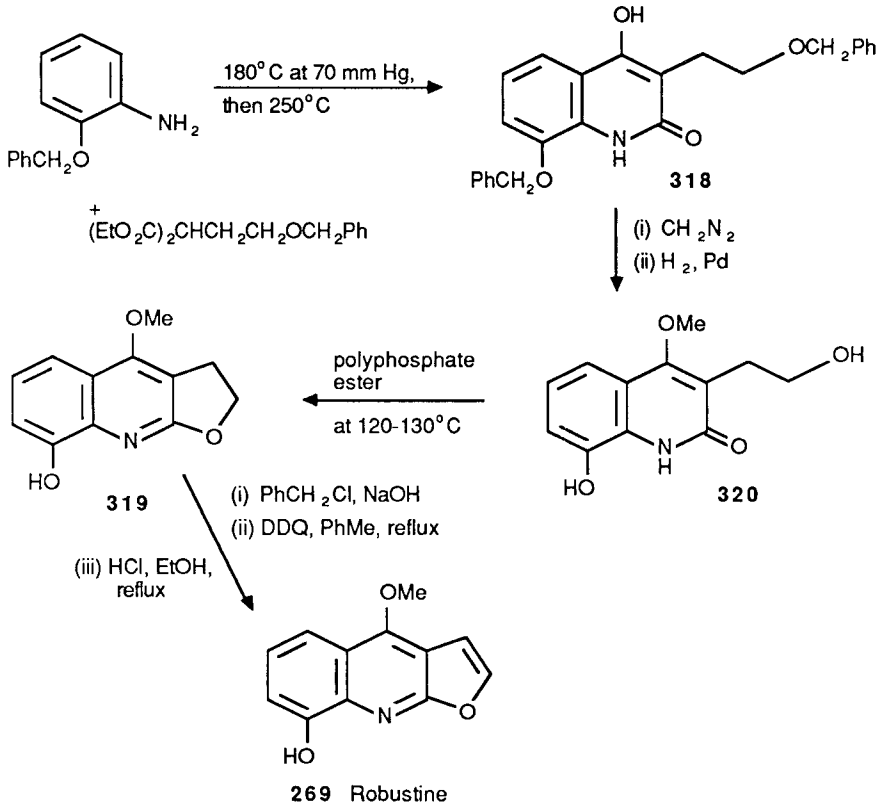
Tecleaverdine (**316**), another alkaloid of *T. verdoorniana,* is the hydrate of tecleaverdoornine; its structure was determined by spectroscopy and through its formation by oxymercuration of tecleaverdoornine (**315**) (*157*).

B. Synthesis

In the last ten years, established methods of synthesis of furoquinoline alkaloids have been refined and extended to new examples.

1. Modified Grundon–McCorkindale Method

Phenolic furoquinoline alkaloids that were first synthesized by the Tuppy–Böhm procedure (*see* Vol. 17, p. 162) have now been prepared by Sekiba from aromatic amines and diethyl (2-benzyloxy-ethyl)malonate. 7-Hydroxydictamnine (confusameline) (*205*), 7-hydroxy-8-methoxy-dictamnine (haplopine) (*205*), 7-hydroxy-6-methoxydictamnine (heliparvifoline) (*206*), and 8-hydroxydictamnine (robustine) (*205*) were synthesized in this way; the synthesis of the latter alkaloid is illustrated in Scheme 32. Methylation of the dibenzyloxy-4-hydroxy-quinoline **318** followed by removal of the protecting groups gave the dihydroxy derivative **320,** which was cyclized with polyphosphate ester to a dihydrofuran **319.** Dehydrogenation to a furoquinoline was accomplished by reaction of the benzyl ether of dihydrofuran **319** with DDQ.



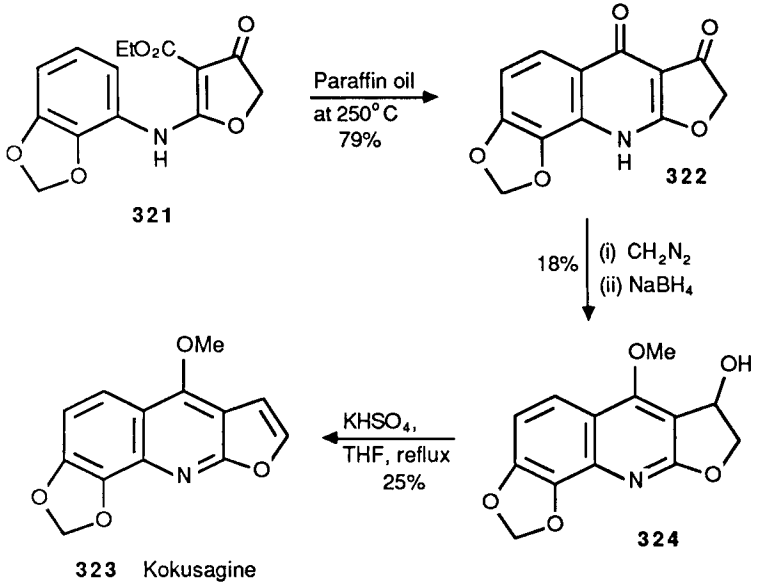
SCHEME 32. Synthesis of robustine.

2. *Modified Tuppy–Böhm Procedure*

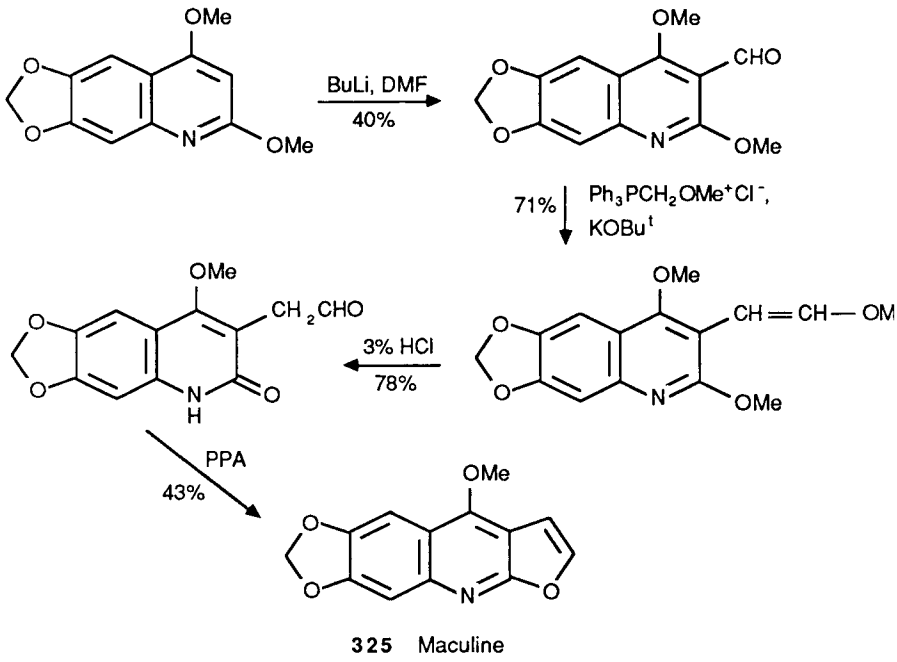
Synthesis of 5,7-dimethoxyisodictamnine from ester **304** has already been described (Scheme **31**) and Yazima and Munakata (*207*) used a similar method to prepare kokusagine (**323**), maculine (**325**), and dictamnine. For example, cyclization of compound **321** gave the ketone **322** which, after successive reaction with diazomethane and borohydride, furnished the 4-methoxyquinoline alcohol **324;** kokusagine was produced by dehydration of the alcohol (Scheme 33). Isomaculosidine (6,8-dimethoxyisodictamnine) was also prepared by the Tuppy–Böhm method (*236*).

3. *Narasimhan–Mali Method from 3-Formyquinolines*

The synthesis of furoquinoline alkaloids from 2,4-dimethoxyquinoline derivatives by the lithiation–formylation–Wittig reaction method (*see* Vol. 17, p. 168)



SCHEME 33



SCHEME 34. Synthesis of maculine.

has been applied to the synthesis of 6,7-dimethoxydictamnine (kokusaginine), which was obtained from 2,4,6,7-tetramethoxyquinoline in an overall yield of 13% (*208*). A modification in which formylation was carried out with *N,N*-dimethylformamide resulted in a synthesis of maculine (**325**) (13% overall yield) from 2,4-dimethoxy-6,7-methylenedioxyquinoline (Scheme 34) (*209*).

IX. 2-Alkyl- and 2-Aryl-4-quinolinones and Related Alkaloids

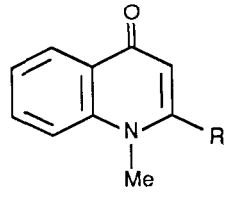
2-Alkyl- and 2-aryl-4-quinolinone alkaloids and related 4-methoxyquinolines discovered during the last decade are listed in Table VIII (*69, 143, 210–214, 241, 242*).

A. 2-Alkyl-4-quinolinone Alkaloids

1. Structural Studies

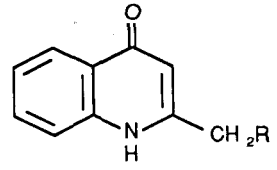
4-Quinolinones containing a long unbranched alkyl chain in the 2 position (pseudans) continue to be isolated from rutaceous plants as well as from microorganisms.

A full account has now been published (*215*) of the determination of the structure of the 2-nonylquinolinone **326** found in *Ruta graveolens* (*see* Vol. 17,



326 $R = (CH_2)_8Me$

327 $R = CH_2CH_2Me$



328 $R = (CH_2)_4(CH{=}CHCH_2)_3Me$ Hapovine

329 $R = CH_2CH{=}CH(CH_2)_6Me$

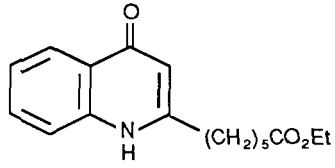


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TABLE VIII

2-ALKYL AND 2-ARYL-4-QUINOLINONE ALKALOIDS FIRST ISOLATED 1976–1985[a]

Compound	Structure number	Molecular formula	Melting point (°C)	Source	Ref.
Leptomerine	**327**	$C_{13}H_{15}NO$	147–148	*Haplophyllum leptomerum*	*241*
Malayamine[b]	**330**	$C_{15}H_{17}NO$	—	*H. cappadocicum*	*210*
Norgraveoline	**345**	$C_{16}H_{11}NO_3$	288–289	*H. dubium*	*143*
				H. foliosum	*69*
3-Heptyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione	**331**	$C_{16}H_{21}NO_3$	142	*Pseudomonas aeriginosa*	*211*
3′-Hydroxygraveoline	**346**	$C_{17}H_{13}NO_4$	amorphous	*Ruta chalepensis*	*242*
3-Nonyl-3-hydroxy-1,2,3,4-tetrahydroquinoline-2,4-dione	**332**	$C_{18}H_{25}NO_3$	146	*P. aeriginosa*	*211*
2-(Undec-3-enyl)-4-quinolinone	**329**	$C_{20}H_{37}NO$		*P. aeriginosa*	*214*
Hapovine	**328**	$C_{24}H_{31}NO$		*H. popovii*	*213*
Melovinone	**336**	$C_{24}H_{29}NO_4$	134–136	*Melochia tomentosa*	*212*

[a] This table is supplementary to Table VIII in Chapter 2 of *The Alkaloids* (*17*).

[b] Isolated as amorphous ethyl ester.

p. 180); the same compound has been isolated from *Euodia rutaecarpa* (*216*). A new 4-quinolinone alkaloid was isolated by Akhmedzhanova *et al.* (*241*) from *Haplophyllum leptomerum;* it was given the name leptomerine and shown to be the 2-propyl derivative **327** by spectroscopic analysis.

Razakova and Bessonova (*213*) isolated hapovine (**328**) from *Haplophyllum popovii* and showed by spectroscopic studies that the alkaloid had a pentadeca-6,9,12-trienyl chain at C-2.

A 2-alkyl-4-quinolinone isolated from *Haplophyllum carpadocicum* was shown by spectroscopic studies to have structure **330** and was given the name malatyamine ethyl ester (*210*); since the compound was obtained by extraction of the plant with ethanol, the authors suggested that the corresponding acid is probably the true natural product.

Another bacterial pseudan isolated from *Pseudomonas aeruginosa* was shown by spectroscopy to be 2-(undeca-3-enyl)-4-quinolinone (**329**) (*214*).

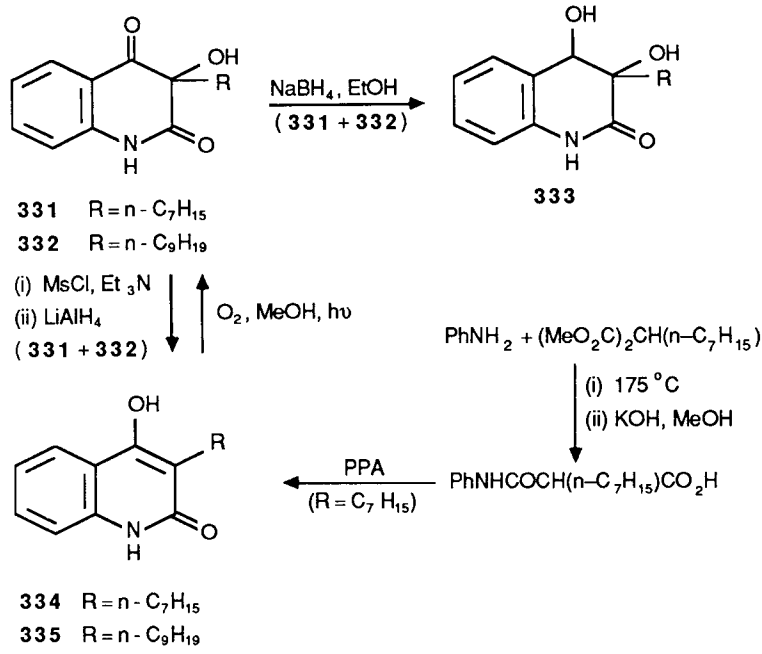
Pseudomonas aeruginosa also contains the unusual 3-alkyl-3-hydroxyquinolinones (**331**) and (**332**) (*211*). The metabolites gave molecular ion peaks in their mass spectra, and the presence of ketonic and amidic carbonyl groups was indicated by IR absorption at 1710 and 1665 cm^{-1}, respectively. The ^{1}H-NMR resonances at δ3.16 (OH) were not affected by the addition of D_2O, but the presence of a hydroxy group was confirmed by reaction of alkaloid **331** with $Cl_3CCONCO$ to give a derivative showing a new NH signal in the ^{1}H-NMR spectrum. Further structural evidence was provided by reduction of a mixture of alkaloids **331** and **332** with borohydride to compounds **333,** and reduction of a mixture of the mesyl derivatives with lithium aluminium hydride to the 4-hydroxy-2-quinolinones **334** and **335.** 3-Heptyl-4-hydroxy-2-quinolinone (**334**) was prepared from aniline and dimethyl heptylmalonate, hydrolysis of the resultant anilide and then cyclization with polyphosphoric acid; photochemical oxidation then furnished alkaloid **331** (Scheme 35 (*211*).

The tricyclic 3-methoxy-4-quinolinone melochinone is a constituent of *Melochia tomentosa* (sterculiaceae) (*see* Vol. 17, p. 181), and a structurally related alkaloid, melovinone (**336**), has since been isolated from the same species (*212*). The ^{1}H NMR spectrum showed that the alkaloid contained three methoxy groups and a single aromatic proton in the quinolinone portion of the molecule. The structure of the side-chain at C-5 was supported by the mass spectrum which showed fragment ion peaks arising from loss of $PhCH_2$, $PhCH_2CH_2$, and $PhCH_2CH_2CH_2$ groups.

2. *Synthesis*

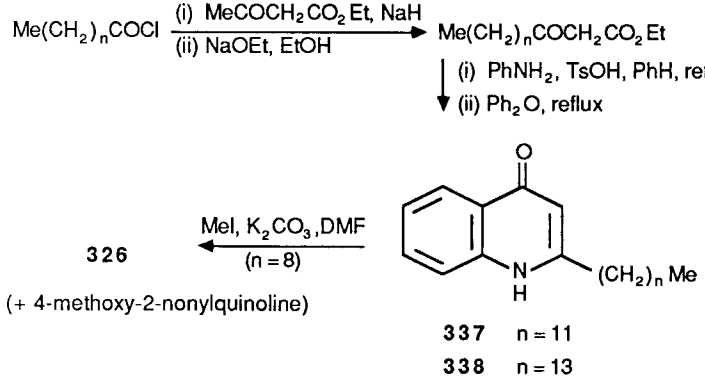
An earlier method of preparing 2-alkyl-4-quinolinones from β-ketoesters (*see* Vol. 17, p. 177) has been applied by Somanthan and Smith (*217*) to the first synthesis of the alkaloids **326, 337** and **338** (Scheme 36).

A new synthesis of 2-alkyl-1-methyl-4-quinolinones devised by Coppola (*218*)

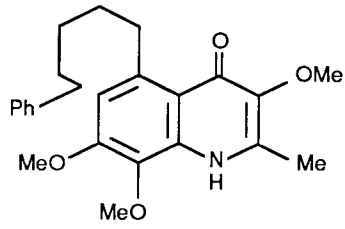


SCHEME 35. Structure and synthesis of 3-alkyl-3-hydroxyquinoline-2,4-diones.

(Scheme 37) involves treatment of *N*-methylisatoicanhydride with the enolates of methylketones (2 mol) to give alkaloids **326** and **341** in yields of 74–81%; an advantage over the earlier procedures is that methylation to give a mixture of *N*- and *O*-methyl derivatives is not required. The new method was applied to the synthesis of evocarpine (**342**) (Scheme 37). The ketone **339** was prepared from hex-1-yne and reacted with *N*-methylisatoic anhydride to give the quinolinone

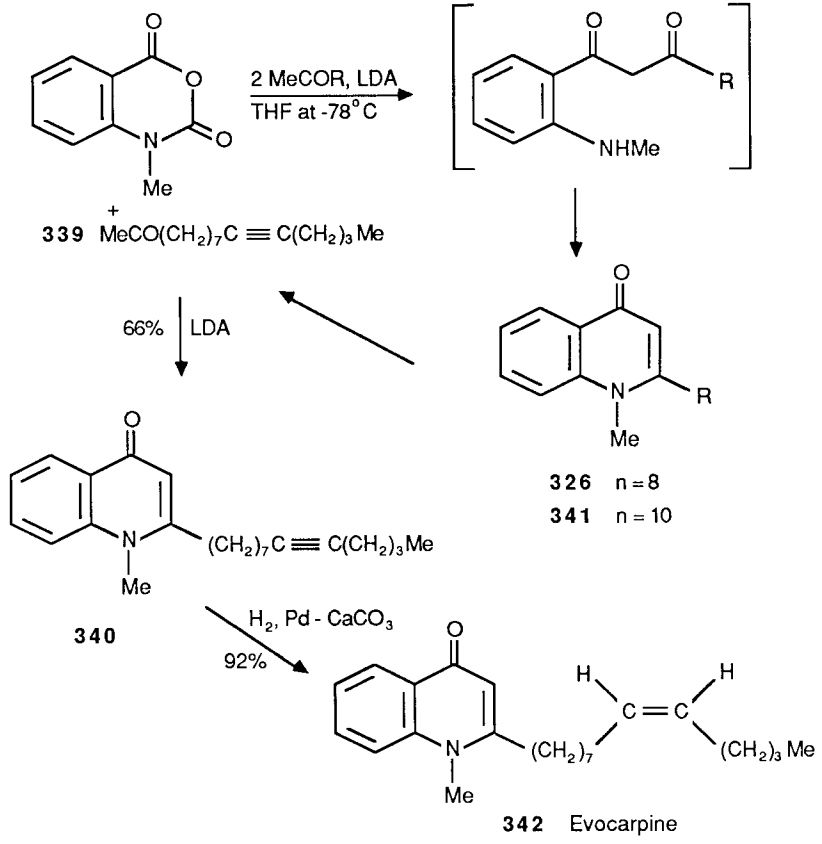


SCHEME 36. Synthesis of 2-alkyl-4 quinolinones form β-ketoesters.

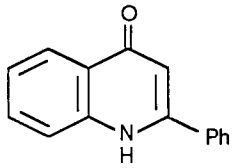


336 Melovinone

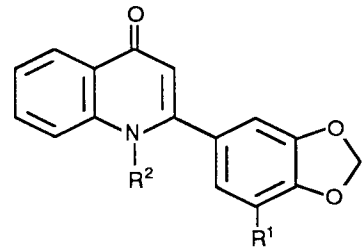
340, which on partial catalytic reduction was converted into the (*Z*)-isomer **342;** the corresponding (*E*)-isomer was obtained from the ketone **339** by a four-step sequence. The (*Z*)-isomer **342** was identical with evocarpine, thus establishing the stereochemistry of the alkaloid for the first time.



SCHEME 37. Synthesis of 2-alkyl-1-methyl-4 quinolinones and evocarpine from *N*-methylisatoic anhydride.



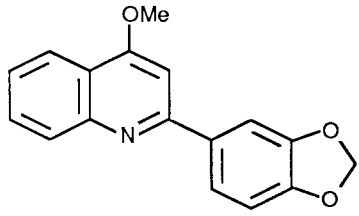
343



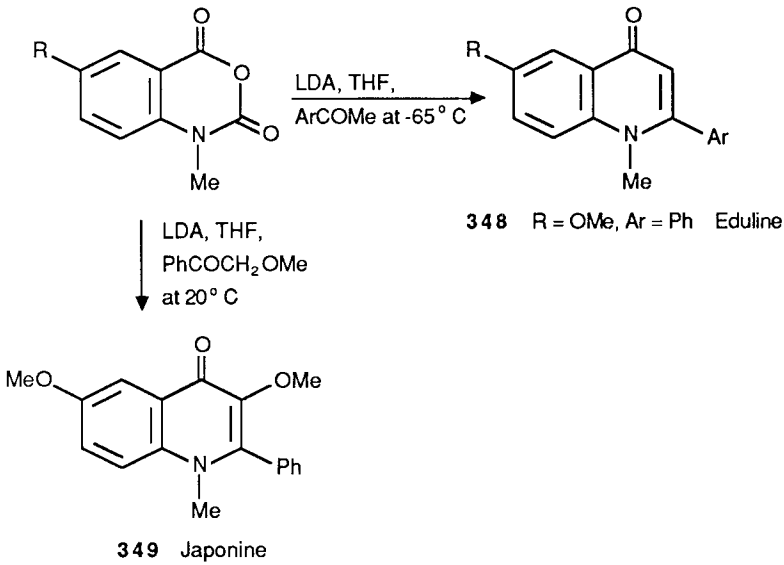
344 $R^1 = H$, $R^2 = Me$

345 $R^1 = R^2 = H$ Norgraveoline

346 $R^1 = OH$, $R^2 = Me$ 3′-Hydroxygraveoline



347



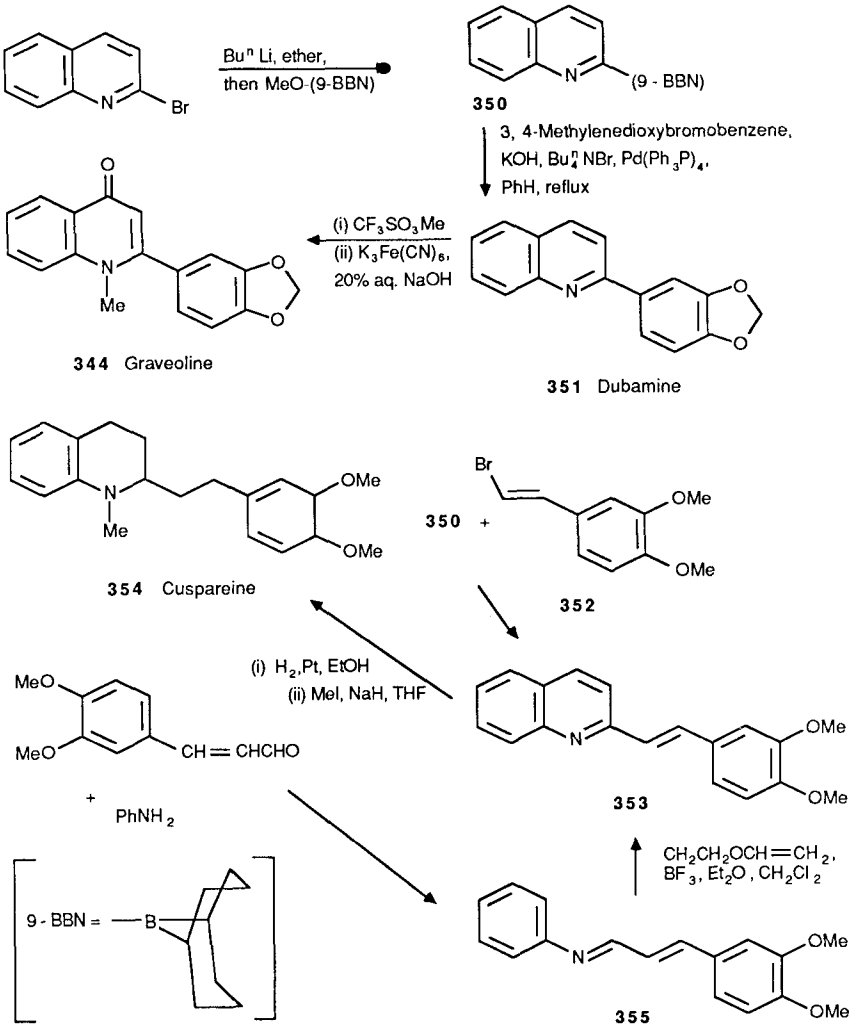
348 R = OMe, Ar = Ph Eduline

349 Japonine

SCHEME 38. Synthesis of 2-aryl-4-quinolinone alkaloids from *N*-methylisatoic anhydrides.

B. 2-Aryl-quinoline and -quinolinone Alkaloids

Known alkaloids of this group obtained from new sources include 1-methyl-2-phenyl-4-quinolinone (**343**) from *Casimiroa edulis* (*219*), *Flindersia fournieri* (*63*), and *Haplophyllum perforatum* (*24*), graveoline (**344**) from *H. dubium* (*143*), *H. foliosum* (*220*), and *Ruta chalepensis* (*244*) and the 4-methoxy-quinoline derivative graveolinine (**347**) from *R. chalepensis* (*71*).



SCHEME 39. Synthesis of dubamine, graveoline, and cuspareine.

The only new 2-arylquinolinone alkaloids found during the last 10 years are norgraveoline (**345**), which was isolated from *H. dubium* (*143*) and from *H. foliosum* (*69*) and 3′-hydroxygraveoline (**346**) from *R. chalepensis* (*242*).

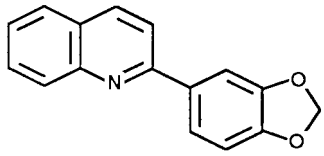
A high-yield synthesis of 2-aryl-1-methyl-4-quinolinone alkaloids similar to that used for the 2-alkyl analogues, *cf.* Scheme 37, has been described by Coppola (*221*) (Scheme 38). Reaction of a methoxy *N*-methylisatoic anhydride with the lithum enolate of acetophenone gave eduline (**348**) and with the enolate of methoxymethyl phenyl ketone gave japonine (**349**); graveoline (**344**) was prepared similarly.

Terashima and co-workers (*243*) have described a new synthesis of 2-arylquinoline alkaloids from the boron derivative **350** (Scheme 39). Thus, reaction with 3,4-methylenedioxybromobenzene using a Pd° catalyst and under phase-transfer conditions gave dubamine (**351**) (42%), which was converted into graveoline (**344**) in good yield by methylation followed by oxidation. Reaction of the boroquinoline **350** with the vinyl bromide **352** gave the styryl derivative **353** (50%), which on reduction and then methylation furnished the alkaloid cuspareine **354** in good yield. Kametani *et al.* (*244*) adopted another approach to the synthesis of 2-arylquinoline alkaloids (Scheme 39); a compound **355** (prepared from aniline and 3,4-dimethoxycinnamaldehyde) reacted with ethyl vinyl ether and a Lewis acid catalyst to give the styryl derivative **353,** also in 50% yield.

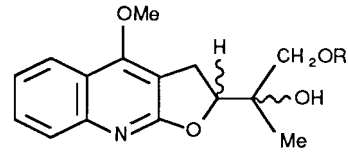
X. Pharmacology

The pharmacology of quinoline alkaloids was last reviewed in this series in Volume 9 (pp. 233, 244, and 254) and included studies on *Lunasia* alkaloids and on the furoquinoline alkaloids, skimmianine, dictamnine, and haplophyllidine found in *Haplophyllum* species. Quinoline alkaloids found more recently to be biologically active are listed in Scheme 40.

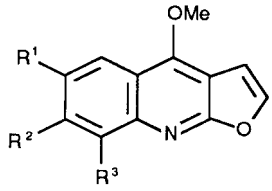
Russian workers have continued the pharmacological investigation of *Haplophyllum* alkaloids. Thus, an iron complex of the furoquinoline alkaloid skimmiamine (**360**) and the hydrochloride salt of the 2-arylquinoline dubamine (**356**) were shown to have strong antimicrobial activity but evoxine (**363**) and dubinidine (**357**) were inactive (*222*). When fed orally to mice or rats a number of constituents of *Haplophyllum* species exhibited sedative and hypothermic effects, including the furoquinolines haplophyllidine (**367**) (*223a*) and perforine (**368**) (*223b*) and the alkaloid dubinidine (**357**) (*224*); haplophyllidine and dubinidine, but not perforine, had antidiuretic properties. A pharmacological study of the monoterpenoid quinoline bucharaine (**369**) (*225*) indicated that the alkaloid supressed aggressive responses to electrical stimulation in rats; the alkaloid had a significant hypothermic effect but no antidiuretic properties.



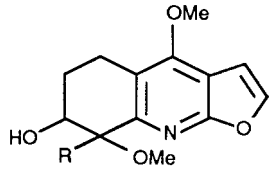
356 Dubamine



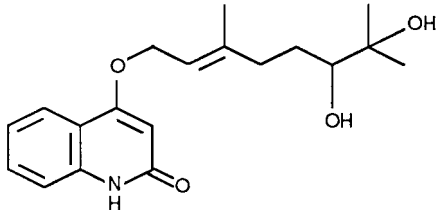
357 R = H Dubinidine
358 R = Ac Dubinine



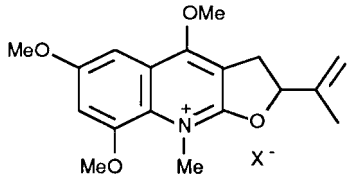
359 $R^1=R^2=R^3=H$ Dictamnine
360 $R^1=H$, $R^2=R^3=OMe$ Skimmianine
361 $R^1=H$, $R^2=OH$, $R^3=OMe$ Haplopine
362 $R^1=R^3=OMe$, $R^2=H$ Maculosidine
363 $R^1=H$, $R^2=OCH_2CH(OH)C(OH)Me_2$, $R^3=OMe$ Evoxine
364 $R^1,R^2=OCH_2O$, $R^3=H$ Maculine
365 $R^1=H$, $R^2,R^3=OCH_2O$ Kokusagine
366 $R^1,R^2=OCH_2O$, $R^3=OMe$ Flindersiamine



367 $R=CH_2CH=CMe_2$ Haplophyllidine
368 $R=CH_2CH_2C(OH)Me_2$ Perforine

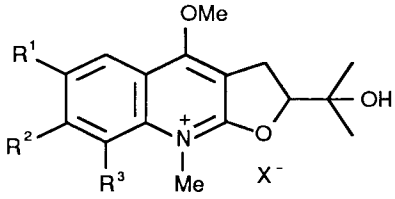


369 Bucharaine

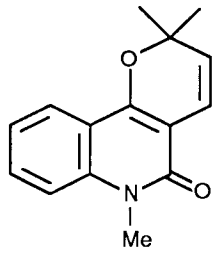


370 *O*-Methylptelefolonium salt

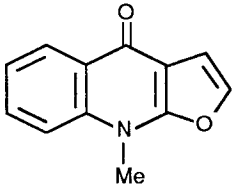
The antimicrobial activity of alcoholic extracts of the American hop tree, *Ptelea trifoliata,* was traced to the quinolinium derivative pteleatinium salt (**371**), which was found to be active against *Mycobacterium smegmatis* and against *Candida albicans* (*226, 227*). In a later study of alkaloids of *Ptelea trifoliata* growing in Europe, pteleatininium salt was not detected, but *O*-methylptelefolonium salt (**370**), another quinolinium constituent, was found to have antimicrobial and antifungal properties and to show marked cytotoxicity against plant and animal tumors (*62*). *O*-Methylptelefolonium salt (**370**) and *O*-methylbalfourodinium salt (**373**), which has been isolated from *Balfourodendron*



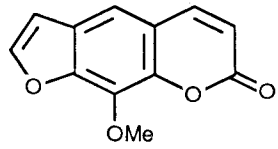
371 $R^1=R^2=H$, $R^3=OH$ Pteleatinium salt
372 $R^1=R^2=R^3=H$ *N*-Methylplatydesminium salt
373 $R^1=R^2=H$, $R^3=OMe$ *O*-Methylbalfourodinium salt
374 $R^1=OH$, $R^2=R^3=H$ Ribalinium salt
375 $R^1=H$, $R^2=R^3=OMe$ Veprisinium salt



376 *N*-Methylflindersine



377 Isodictamnine



378 8-Methoxypsoralen

SCHEME 40. Pharmacologically active quinoline alkaloids.

riedelianum, Choisya ternata, and *Orixa japonica,* have growth-inhibitory properties with tomato and pea seedlings but the related quinolinium derivatives (+)-ribalinium salt (**374**) and (−)-*N*-methylplatydesminium salt (**372**) are inactive (*62*). Veprisinium salt (**375**), the 7,8-dimethoxy derivative of *N*-methylplatydesminium salt, is responsible for the antibacterial properties of the bark of *Vepris louisii* (*72*).

Extracts of the root barks of *Fagara chalybea, F. holstii* (Rutaceae), and *Xylocarpus granatum* (Meliaceae) were shown to have antifeedant activity in tests with the African Army worms *Spodoptera exempta* (on the leaves of *Zea mays*) and *S. littoralis* (on the leaves of *Ricinus communis*). The same activity was present in the case of the Mexican bean beetle, *Epilochna verivestis,* on leaves of Bountiful stringbeans. The 2,2-dimethylpyranoquinolinone alkaloid *N*-methylflindersine (**376**) was responsible for the antifeedant activity in each case (*91*). *N*-Methylflindersine was also observed to have antimicrobial activity against the yeast *Canadida albicans,* the fungi *Rhizopus delemay,* and *Trichophyton mentagrophytes,* but was inactive against Gram positive and Gram negative bacteria (*91*). The minor insect antifeedant properties of the leaves of *Orixa japonica* are due to the presence of the furoquinoline alkaloids kokusagine (**365**) and evoxine (**363**) (*180*).

Towers *et al.* (*228*) studied the phototoxicity of eight furoquinoline alkaloids and showed that dictamnine (**359**), isodictamnine (**377**), and maculine (**364**) were active to most yeasts and bacteria tested in long wave length UV light; haplopine (**361**) and maculosidine (**362**) were active to the yeast *Saccharomyces cerevisiae*, but flindersiamine (**366**), the dihydrofuroquinoline derivatives dubinidine (**357**) and dubinine (**358**), skimmianic acid, and 4-methoxyquinoline were not phototoxic. Dose–response curves with dictamnine and the furocoumarin 8-methoxypsoralen (**378**) using *S. cerevisiae* and the Gram-negative bacteria *Escherischia coli* showed that dictamnine was less active than the furocoumarin. These two compounds are constituents of *Skimmia japonica* and the authors point out that it is remarkable that a single plant species should elaborate the furoquinolines (deprived from anthranilate) and the furocoumarins (derivatives of cinnamate) that show the same phototoxic activity.

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