

# **Amber, Resinite, and Fossil Resins**



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# Amber, Resinite, and Fossil Resins

**Ken B. Anderson**, EDITOR

*Argonne National Laboratory*

**John C. Crelling**, EDITOR

*Southern Illinois University—Carbondale*

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# Foreword

THE ACS SYMPOSIUM SERIES was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of this series is to publish comprehensive books developed from symposia, which are usually “snapshots in time” of the current research being done on a topic, plus some review material on the topic. For this reason, it is necessary that the papers be published as quickly as possible.

Before a symposium-based book is put under contract, the proposed table of contents is reviewed for appropriateness to the topic and for comprehensiveness of the collection. Some papers are excluded at this point, and others are added to round out the scope of the volume. In addition, a draft of each paper is peer-reviewed prior to final acceptance or rejection. This anonymous review process is supervised by the organizer(s) of the symposium, who become the editor(s) of the book. The authors then revise their papers according to the recommendations of both the reviewers and the editors, prepare camera-ready copy, and submit the final papers to the editors, who check that all necessary revisions have been made.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previously published papers are not accepted.

# Preface

**AMBER IS AN EXTRAORDINARY MATERIAL.** Among sedimentary organic products, it is unique for its exceptional preservation (and the exceptional preservation of materials included within it); for its value as an “organic gemstone”; and for the extraordinary role it has played in human history, especially European history. Until the 18th century, when fossil fuels became important in sustaining the industrial revolution, amber was the single most important organic product from the geosphere. Whether one is discussing Roman invasions, the excesses of the Teutonic Knights, or the secretive and powerful amber guilds, throughout (and undoubtedly before) recorded history, war and intrigue have always surrounded control of the production and distribution of amber. Indeed, at one time gallows stood on the shores of the Baltic, and any unfortunate sole caught collecting amber without proper authority was summarily put to the gibbet!

However, it is not our purpose to describe the remarkably colorful and often bloody history of the amber trade. Rather, this book has been assembled to report, as far as is possible in a rapidly developing field, the current state of the art in scientific (especially chemical) studies of amber. The unique properties of fossil resins make them exceptional candidates as potential markers of paleoenvironments, sedimentary conditions, and geochemical processes. Also, the unparalleled ability of ambers to preserve and protect materials encased within them has opened up opportunities in molecular paleontology that could not previously be addressed, including the recovery of fragmentary DNA from insects more than 100 million years old.

Because of its extraordinary properties, amber is of interest to scientists in a wide range of disciplines. The symposium upon which this book is based was organized to provide a forum for a diverse exchange of information and to present an opportunity for investigators to become familiar with the challenges facing scientists working with amber in a range of disciplines. Likewise, this book was prepared to present a diverse summary of our current knowledge of the nature and properties of fossil resins.

Ambers are derived from biological materials (resins) that have been incorporated into sediments. Their chemical properties are a consequence of both their biological origins and the geological environment into which they were deposited and in which they have subsequently matured. Hence, it is important, when assessing the properties and

characteristics of these materials, to take these factors into account. For this reason, in addition to reporting chemical studies of ambers, including structural characterization, isotopic composition, maturation studies, resinite-derived oils, and amino acid distributions, the reports in this book also discuss aspects of the biology, geology, petrology, and technology of fossil resins.

## **Acknowledgments**

This book and the symposium from which it developed are a result of the efforts and support of a large number of individuals and organizations. Hence, we express our gratitude to the ACS Division of Geochemistry, Inc., that sponsored the symposium, to the speakers and authors whose work is reported in this volume, and to those individuals who generously donated their time to review the papers. The Petroleum Research Fund is also acknowledged for their support by allowing several speakers from outside North America to attend and participate in the symposium (ACS-PRF 29052-SE). The support of the U.S. Department of Energy for K. B. Anderson, under Contract No. W-31-109-ENG-38, is also gratefully acknowledged.

**KEN B. ANDERSON**  
Chemistry Division  
Argonne National Laboratory  
9700 South Cass Avenue  
Argonne, IL 60439

**JOHN C. CRELLING**  
Department of Geology  
Southern Illinois University  
Carbondale, IL 62901

September 5, 1995



# Introduction

Ken B. Anderson and John C. Crelling

Scientific analysis of fossil resins is not new. In fact, the term electron is derived from the Greek word for amber, "*Electra*". In recent years however, scientific analysis of these materials has undergone a renaissance and the pace of new discoveries concerning these materials has quickened considerably. The principle reason for this is the advent and application of modern analytical techniques, which have allowed researchers to address questions which could not be addressed previously. The scope of these discoveries has been exceptionally broad, and significant advances have been achieved in a number of disciplines based on the results of studies of ambers or materials included within it.

This brief introduction has been prepared to assist the reader by defining important terms, discussing some of the broad issues which are presently being discussed among scientists working with fossil resins, and to briefly mention some aspects of work related to ambers which are not covered in the papers in the body of this volume.

## Definitions and Nomenclature:

The title of this book, and the symposium from which it has been developed, "Amber, Resinite and Fossil Resins", reflects from the outset the difficulty with the nomenclature of these materials. To many workers, these terms are largely synonymous and may be used interchangeably. Others however, have insisted that these terms are not synonymous and that each should be used only in specific circumstances. For example, some authors have insisted that the term "*amber*" should only be used to describe Baltic Amber. Other authors contend that the term "*resinite*", which is primarily a coal maceral term and hence is defined by ICCP (1) and ASTM (2) convention, should only be used to describe material which has been petrographically identified (by optical microscopy). It has also been suggested that the terms "*amber*" and "*resinite*", differ only in that "*resinite*" refers to microscopic materials and "*amber*" refers to macroscopic materials.

In the opinion of the present authors, while for some purposes it may be convenient to precisely define these terms, in reality their use is so widespread and so varied that it is futile to attempt to narrowly define their meanings. Therefore, for practical purposes they should be regarded as synonymous. Where a specific or more precise meaning is intended, the writer or speaker should explicitly indicate this. For the purposes of this Introduction, these terms will be treated as synonyms. The term "resin" (used alone) should be used only for modern samples.

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It is also important at the outset to discuss the scope of the terms "Amber", "Resinite" and "Fossil Resins". For the purpose of this discussion, these materials are defined as: "*solid, discrete organic materials found in coals and other sediments as macroscopic or microscopic particles, which are derived from the resins of higher plants.*" This definition is based on the definition given in an earlier report by one of the authors (3). It is not comprehensive, nor do all of the contributors to this volume necessarily adopt this definition in their own reports. It does however, serve as a general working definition and as a basis for further discussion.

The two principle aspects of this definition are (i) fossil resins must be solid, discrete bodies, and (ii) that they must be derived from the resins of higher plants. The first of these two components serves to exclude resin(ite) derived products and material which has become dispersed at a molecular level, such as woody fossils from which resin derived materials can be extracted, and resinite derived oils. This is not to suggest that these materials are not important or worthy of investigation, but, simply that they should not be referred to using the same terms used for discrete macroscopic and microscopic fossil resins.

The second component of this definition, which serves to exclude montan waxes and similar materials, which are sometimes (rather perversely) referred to "wax resinite" (4) and which are clearly not related in either biochemical origin, botanical function, or composition to other forms of fossil resin, is more problematic. The difficulty here centers around the question of what constitutes a resin. Terpenoid resinates, especially polyterpenoid resinates undoubtedly constitute the vast majority of currently known resinates. However, it is entirely possible that some plants may now, or may in the past, have produced non-terpenoid exudates to fulfill the biological and ecological functions now filled in most plants by terpenoid resins, and this definition is certainly not intended to exclude non-terpenoid forms of resinite. Very little is known concerning the evolution of terpenoid resins. It is not even clear when terpenoid resins first appear in the fossil record. The oldest definitively terpenoid resin characterized by the authors is reported to be Triassic, (Anderson, Unpublished results), but information from resinates older than Cretaceous is rare. Resinates are often reported in Paleozoic sediments, especially coals, but little molecular characterization of these materials has been done.

Further complicating the nomenclature of fossil resins is the plethora of geological names given to various samples. Names for ambers based on locality, discoverer etc, abound in the literature, (eg; Glessite, Rumanite, Chemawinite, Burmite, Simetite, Bitterfelderite, Settlingite, Walchowite, Schraufite, Schilerseeite, Simetite, Stantienite, etc ..... ). However, as virtually none of these names is based on any knowledge of the composition of the amber, they are of little or no scientific value in comparing samples. Many of these names are so heavily entrenched in popular usage and in the scientific literature that it is not possible to restrict their usage.

### **Classification of Ambers:**

As an attempt to rationalize the nomenclature of these materials and to provide a logical basis for comparison of ambers for the purposes of scientific discussion, a classification system, based on the structural characteristics of the amber itself has been proposed (3,5,6). This classification system has not yet been universally

adopted, however, a number of workers are presently using this system and appear to be finding it useful. The bases for classification using this system are described in Table I below, and summarized in graphical form in Figure 1.

**Table I.** Classification System for Fossil Resins

<b>Class I</b>	The macromolecular structures of all Class I resinites are derived from polymers of labdanoid diterpenes, including labdatriene carboxylic acids, alcohols and hydrocarbons (6)
<b>Class Ia</b>	Derived from/based on polymers and copolymers of labdanoid diterpenes having the <i>regular</i> [1S, 4aR, 5S, 8aR] configuration, normally including, but not limited to, communinc acid and communol and incorporating significant amounts of succinic acid.
<b>Class Ib</b>	Derived from/based on polymers and copolymers of labdanoid diterpenes having the <i>regular</i> [1S, 4aR, 5S, 8aR] configuration, often including but not limited to communinc acid, communol, and biformene (and related isomers). Succinic acid is absent.
<b>Class Ic</b>	Derived from/based on polymers and copolymers of labdanoid diterpenes having the <i>enantio</i> [1S, 4aS, 5R, 8aS] configuration, including but not limited to ozic acid, ozol, and <i>enantio</i> biformenes (and related isomers).
<b>Class II</b>	Derived from/based on polymers of bicyclic sesquiterpenoid hydrocarbons, especially cadinene, and related isomers. (3)
<b>Class III</b>	Natural (fossil) polystyrene (3)
<b>Class IV</b>	Fundamental structural character is apparently non-polymeric, especially incorporating sesquiterpenoids based on the cedrane carbon skeleton. (3)
<b>Class V</b>	Non-polymeric diterpenoid carboxylic acid, especially based on the abietane, pimarane and iso-pimarane carbon skeletons. (5)

### The 'Age' and 'Maturity' of Fossil Resins

One of the most controversial, and still unanswered questions in this field is: "*When does a 'resin' become 'amber'?*" Modern resins are continuously being deposited into sediments all over the world, but many types of modern resins have not been identified in the fossil record. This may be because they had not evolved and hence were never

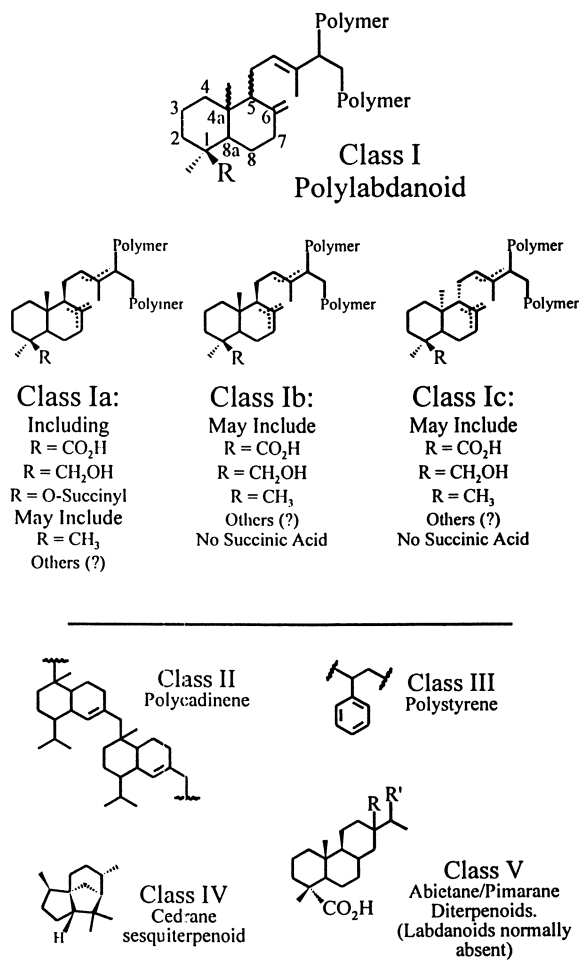


Figure 1. Schematic representation of the classification system for fossil resins proposed by Anderson *et al.* (3,5,6).

deposited, or because they do not survive burial and early diagenesis intact. Clearly, there is a relationship between recently deposited resins and truly fossil resins, but it is also important that these two materials not be confused. This is a highly contentious issue which has not been made easier by the use of terms such as "young amber" and "sub-fossil (resin)" by some researchers and amber dealers. This question also has considerable economic overtones, because the commercial value of amber is related to its scarcity and age. There is no clear consensus on this point, and any age division will necessarily be somewhat arbitrary. But until some consensus is reached, and a standard convention accepted, there will always be some degree of confusion associated with discussions of ambers and their relationship with (modern) resins.

Related to this question, (yet distinct from it) is the question of the use of chemical analyses to determine the "age" of fossil resins. It is certainly true that definite and measurable transformations occur in the chemical structures of fossil resins over time. However, geochemists have known for many years that it is basically futile to attempt to determine the chronological age of sedimentary organic matter by chemical analysis alone. The reason for this is that all chemical transformations, except nuclear decay, proceed at a rate which is determined by (i) the rate constant of the particular reaction and (ii), the *temperature* at which the reaction is allowed to proceed. Normally a reaction will proceed faster as the reaction temperature is increased. Consequently, the vast majority of geochemists rely on the concept of "maturity", which takes into account both age and thermal history. To put this concept in context, two identical resins, deposited at the same time and buried at the same rate in sediments with differing geothermal gradients, will have different maturities due to acceleration of the chemical changes in the resin which has experienced higher temperatures. That is, even though the samples will be the same age at any given point in time, they will have different maturities. Tests to determine the maturity of natural resins are the subject of intensive research in several laboratories, however, none of these tests will be useful for determining the age of any given sample unless a detailed thermal history of the sample is known.

### **Preservation of Biological Materials in Amber:**

Finally, one active area of research related to amber which is not fully covered in this volume concerns the analysis of biological materials in amber, including the retrieval, amplification, and analysis of fragmentary DNA from (primarily) insects entombed in amber. Strictly speaking, since this work relates to inclusions within amber and does not involve analysis of the amber itself, it might be considered to be outside the scope of this volume. However, the extraordinary preservation of biological materials included within amber is undoubtedly due to the properties of amber, and the implications of this work are so important that it merits at least some brief introduction here. Readers interested in further pursuing this subject are referred to the citations given below.

It has been known for some time that amber preserves, better than any other medium, insects and plant tissues entombed within it. A number of microscopic and electron microscopic studies have shown that this exceptional preservation includes preservation of soft tissues (albeit dehydrated) including muscle fibre and delicate internal organs (7). How amber is able to preserve delicate tissues which would

normally decay very rapidly is unclear, although it is likely related at least in part to rapid dehydration of the tissue by the resin. This question is the subject of ongoing research by a number of workers.

It has also been shown that this high degree of preservation extends, at least to some extent, to the molecular level. The amplification of DNA preserved in amber was first reported virtually simultaneously by DeSalle et. al., (8) and Cano et. al.,(9) in 1992. Since those initial reports, amplification of DNA from amber as old as 125-130 million years (10) has been reported, as has amplification of DNA fragments from bacteria entombed in amber (11).

This field is still in its infancy, but as new information becomes available, and as the genetic sequences which are recovered are placed in the evolutionary context, it is likely that these kinds of analyses will contribute very significantly to our understanding of evolutionary processes.

It must also be mentioned that recently, it has been reported that bacterial spores recovered from amber have been successfully cultured (12). This work is very recent and has not yet been independently reproduced by other laboratories, and there are serious questions about the true age of the samples from which the spores were recovered (13). If correct however, this work has very significant implications for molecular paleontology. Of course, this work has also (appropriately) generated significant public and scientific discussion concerning the implications of "resurrecting" extinct microbes (or other organisms).

### **Future Research and New Directions**

Guessing at the direction of future research is always an exercise fraught with peril. However, recent advances in our understanding of the characteristics of fossil resins, many of which are described in this volume, have raised significant new questions, and it is likely that research over the next several years will attempt to address these issues.

In order for structural changes in fossil resins to be useful as geochemical indicators, a much more precise understanding of the structural characteristics of fossil resins, and the structural changes which occur in resinates with increasing maturity will be necessary. Although much has been learned, more still remains unknown and it is likely that molecular characterization will continue to be a major focus of fossil resin research for the foreseeable future. The potential of fossil resins as paleobotanical/paleoenvironmental indicators is now apparent and the value of these materials as potential characteristic fossils is likely to be further explored in the future. It is also apparent that interest is growing in Mesozoic and Paleozoic resinates. Characterization of these rare materials will help to address questions concerning the evolution of terpenoid resins and the role these resins played in shaping the evolution of various types of early ecosystems. Finally, recovery of DNA from organisms entombed in amber will certainly continue, and it is likely that this work will become more systematic, for example, examining the genetic changes which occur in related organisms across geologic time.

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## Chapter 1

# Biology of Amber-Producing Trees: Focus on Case Studies of *Hymenaea* and *Agathis*

Jean H. Langenheim

Department of Biology, Sinsheimer Laboratories, University of California,  
Santa Cruz, CA 95064

A survey of resin-producing trees is presented, including evidence for those that have produced amber, with a discussion of possible botanical confusion regarding the use of common and commercial terminology for resins. Comparisons of chemotaxonomy based on terpenoid resins of extant and fossil plants as well as for ecological roles are discussed relative to the different chemical fractions of the resin. The importance of distinguishing between constitutive and induced resin, and the organ in which the resin is produced is emphasized in making comparisons from modern to fossil resins—particularly in determining botanical source. Case studies of the tropical genera *Hymenaea* (Leguminosae) and *Agathis* (Araucariaceae) relevant to understanding amber production are emphasized, including such aspects as: 1) the nature and location of resin secretory tissue in different organs, 2) variation in resin properties (composition and yield) among species in the genera, 3) the forest habitats in which the most copious production occurs and where the resin accumulates, 4) chemical and environmental factors influencing accumulation and polymerization, and 5) evidence for their being the botanical source for amber deposits.

Research on amber (fossil resin) is undergoing a renaissance. This is particularly true with regard to its chemistry because of recently available techniques that enable greater in-depth analysis of the material. Often, however, when amber is compared to resin from extant trees, the geochemist may have difficulty in interpreting the comparison, in part because of lack of knowledge about resin-producing trees. This paper is thus a broadly conceived overview of some of the important aspects of the biology of amber-producing trees that may be useful for the geochemists, as well as a variety of other amber researchers, some of whose work is included in this Symposium volume.

Various definitions of resin have been used (1-2); in this paper resin is defined as a complex mixture composed primarily of either terpenoid and/or phenolic compounds from living higher plants. Terpenoid resins, which comprise the majority of known natural resins, will be emphasized. Although terpenoids predominate in these resins, they may be accompanied by some volatile phenolics such as phenylpropenoids (e.g.,

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cinnamic acid) or phenylpropenes (e.g., eugenol), acetogenins, fats, and in some cases traces of amino acids. As most biologists do, I shall use "amber" to refer to "fossil resin," but assume it to be synonymous with "resinite," which is more commonly used by geochemists (3). The time required for resins to become fossilized is a controversial topic and as yet no feasible empirical means have been established to determine it.

With the focus on terpenoid resins, the importance of these resins being comprised of mixtures of a volatile fraction (mono- and sesquiterpenoids that provide fluidity and act as plasticizers) and an essentially nonvolatile diterpenoid or sometimes triterpenoid fraction will be emphasized (4). No resins are known to contain both di- and triterpenoids. Most chemosystematic (and ecologic) studies of extant terpenoid-producing plants have utilized mono- and sesquiterpenoid fractions with a focus on the monoterpenoids in leaves, where easily identified and quantified by MS and GC (e.g., 5-10). On the other hand, chemosystematic studies of amber have mainly focused on the non-volatile diterpenoid fractions, the majority of which have polymerized from the trunk resin of trees (although some volatile compounds that have not evaporated may be occluded in the resin). Polymerization also occurs in sesquiterpenoid moieties with a cadanane-type skeleton found in triterpenoid resins (3, 11). All terpenoid resin-producing trees contain resin in their leaves, which is another reason they have been studied by chemosystematists and ecologists in extant plants. Resin in the tree trunk is generally more complex chemically and has been investigated primarily in species in which the resin is of economic importance; however, even in these cases, monoterpenes have been emphasized (e.g., 12-14). It is important to consider both the terpenoid fraction and the organ in which the resin has occurred in assessing results from resin studies, as both of these factors can significantly affect understanding and interpretation of various aspects of amber.

### Raison d'être of Resins

**Multifarious Ecological Roles.** In some amber studies, the question has arisen as to why trees began to secrete resins, particularly since they do not play physiological roles in the plants (1-2). With the development of the field of chemical ecology, which has in many ways paralleled the continued improvement in chemical techniques of analysis, attention has been directed to the ecological roles of chemicals, such as resins (15). The most relevant of these ecological roles to amber research are those regarding resins sequestered in the tree. Generally as a result of wounding, resins are exuded outside the plant in direct defense against herbivores and pathogens (e.g., fungi). Physical damage, such as breakage of limbs in storms, can also result in exudation. The resins then can accumulate on the trunk or limbs and thence fall to the soil; if sufficiently polymerized, they can be washed into swamps or into streams where they can be transported to depositional sites. Some resins, however, remain within the secretory tissue of the tree and are found preserved in coal deposits.

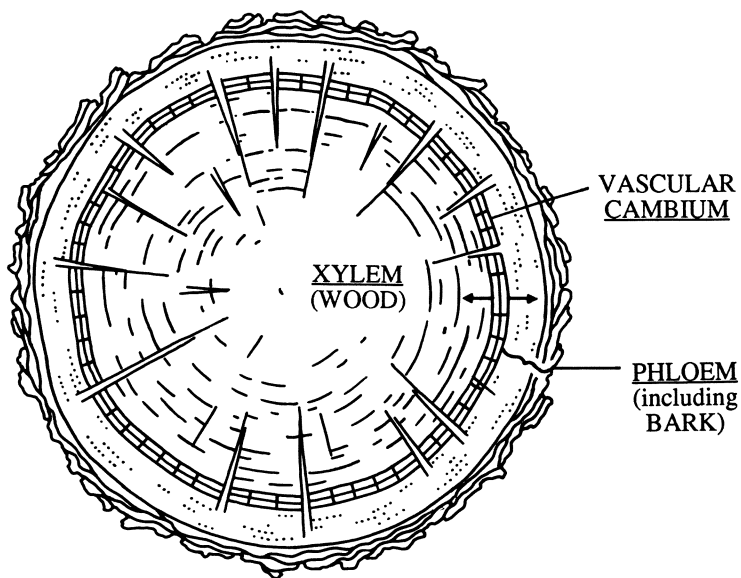
Other roles of resin components that are emitted into the environment have been significant in the evolution of resin-producing trees. For example, some trees or shrubs exude resins that coat leaves and hence prevent desiccation and damage from UV, especially in dry environments, as well as deter herbivory. Resin can also influence the time of release of seeds. For example, resin coats the cones of some species of pine, aiding in keeping the cones closed until opened by fire. This adaptation leads to the seed germinating in the early stages of the forest community following fire. Volatile terpenoids are involved both in direct defense and indirect defense by attracting predators of herbivores; they can also attract pollinators, and can affect plant-plant and plant-soil microbe interactions (4). In the latter case, components of resins can be either leached from the plant or released by decomposition of the plant parts. With these multifarious roles, the components of resins, both those sequestered in the plant, and those exuded from it or otherwise emitted into the environment, are important

ecologically, not only to the individual plant producing the resin but in their effects on plant populations, communities and ecosystems (4). It is further assumed that these roles, which have been significant in the survival of the plant and are of value to other members in the ecosystem, have occurred throughout long periods of geologic time; in fact, one of the roles, chemical defense of plants, may have led to the appearance of resins in trees (1, 2, 4).

**Different Concepts Regarding the Terpenoid Mixture.** Because resins are a mixture of compounds, chemical ecologists use different concepts to refer to their qualitative and quantitative effects, such as: a) composition [the relative proportion (%) of compounds within the terpenoid class or among them within an organ], b) concentration (total quantity of a single component terpenoid in the mixture within an organ), c) yield or total concentration (total quantity of resin produced within an organ). Because of the useful characterization these concepts provide for all resin studies, they will be used throughout the paper.

The distinction between constitutive and induced resins additionally has considerable consequence for resin and amber investigations. Constitutive or preformed resin is that synthesized in the plant prior to either natural or artificial wounding. Induced or reaction resin is that produced following wounding and may be similar to or different in composition from the constitutive resin. In some cases the induced resin is synthesized *de novo* close to the site of wounding. The relative amount of the monoterpene fraction produced in constitutive vs. induced resin greatly varies among conifer taxa (16). Those taxa with resin canals (Figure 1) tend to occur in early, pioneer stages of forest development and, upon wounding, appear to transport resin from other parts of the interconnected canal system to the wound site; hence there is not short-term induction by *de novo* synthesis. However, those taxa that occur in mature, old-growth stages of forest development often have isolated blister or pocket secretory tissues (Figure 1); upon wounding, the secretory area around the pocket is increased or *de novo* synthesis of resin commences at the site of wounding, if away from the pockets (14). It has been hypothesized that constitutive or preformed resin is produced copiously in healthy trees with a canal system, and generally may be adequate for defense, except under epidemic outbreaks of herbivorous insects (14, 17). Therefore, little may be induced and increased resin flow is similar to the constitutive resin. However, constitutive resin produced in the isolated pockets by healthy trees may be insufficient for adequate defense; hence induced or reaction resin in these trees may be produced copiously following wounding by herbivores or infection by microbes. Furthermore, the composition is different from the constitutive resin (4, 14). Apparently a close relationship may also exist between the kind of secretory tissue (i.e., its anatomical complexity) and the quantity of synthesis of either constitutive or induced resin. At least this has been suggested for monoterpenes, as indicated by availability of monoterpene cyclases in different taxa of conifers (17).

**Relevance of the Type of Secretory Tissue.** Because the kind of secretory tissue may be correlated with the quantity of constitutive vs. induced resins, two extreme secretory tissue types are briefly described, as these appear to be of primary significance in amber research. The secretory tissues are differentiated from cells produced in the actively dividing meristematic region, the vascular cambium, during growth (Figure 1). Cells are produced either inward into the xylem or wood tissues or outward into the phloem tissues, which are included in the bark (18). Interconnected canals, often vertical and horizontal that may span both xylem and phloem, represent one extreme of secretory structures (Figure 1). Spheroidal or ovoid pockets (sometimes called "blisters") are the other extreme (Figure 1). They are unconnected and much more localized in their occurrence, i.e., tending to occur either in xylem or in phloem (but can occur in both at different stages of development of the organ). In both



CROSS SECTION OF TREE TRUNK

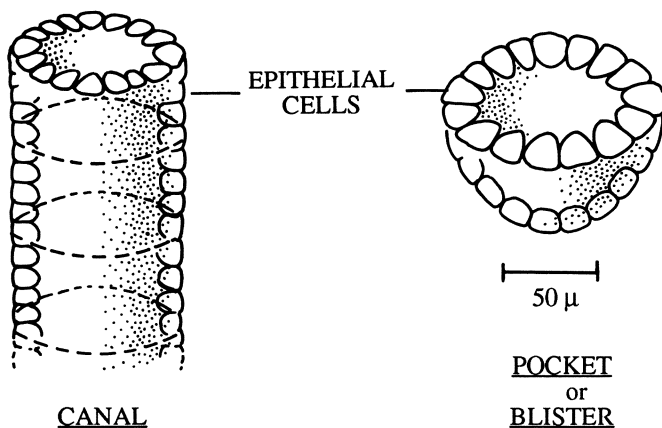


Figure 1. Diagrammatic cross section of a tree trunk (secondary stem tissue), indicating vascular cambium, cells from which two kinds of resin secretory structures (canals and pockets or blisters—shown greatly enlarged to distinguish them) are differentiated in xylem or phloem tissue.

of these cases, however, the terpenoids are synthesized in epithelial cells lining an intercellular lumen into which they are moved. In this manner, the resin is stored and sequestered such that the plant's cells are protected from autotoxicity of the resin. The xylem contains the cells that transport water and minerals from the roots and the phloem contains cells that transport sugars from the photosynthesizing leaf tissue. Upon wounding, resin that exudes from these structures could then encounter either of those aqueous solutions, thus possibly incorporating some of their contents into the resin. If the resin is produced by lysis with consequent breakdown of the epithelial cell walls, then polysaccharides and amino acids common to the cell wall (hydroxyprolines) can enter the resin mixture, the latter as reported by S. Halpine in a subsequent chapter in this volume. The location within the tree trunk and the kind of secretory tissue in which the resin is synthesized and stored can have considerable influence on the quantity of resin and the included "extraneous" material, which is exuded to the outside of the trunk. Hence these factors may influence the quantity and composition of ambers.

**Chemical and Physical Ecological Effects of Resin.** In terms of resin composition, both the effects of concentrations of individual components and the ratio between volatile and non-volatile components can be ecologically significant. Resins provide a versatile defense in being able to act either chemically or physically. For example, single compounds at different concentrations, or several together acting either additively or synergistically can have toxic or deterrent effects. A survey of recently recorded ecological effectivity of terpenoids has shown them universally to be concentration- or dosage-dependent (4, 19) and thus high yields of resins indirectly promote the toxic or deterrent effects through increasing the concentration of individual compounds. Schuck (20) indicates that one resin fraction, such as monoterpenes, may act toxically, whereas another, such as diterpenoids, may act as a physical barrier in defending against pathogens. On the other hand, Lerda and Penuelas (21) suggest a toxic role for the diterpenoids and emphasize the solvent properties of the monoterpenes. Thus the different fractions may act chemically and/or physically in varying ways in different trees and in different environments. In addition, the ratio of volatile to non-volatile compounds determines the fluidity, viscosity and may influence oxidation rates, which increase the capacity to physically engulf organisms, coat wounds, etc. (4, 22-23). This ratio further affects the accumulation of resins that polymerize to form amber.

### General Biological Background of Resin-producing Trees

**Tree Resin- and Amber-producing Plant Families.** Table I presents the coniferous and most prominent angiospermous families of resin-producing trees with those indicated for which evidence has been presented for the botanical source of amber.

**Conifers.** Amber derived from conifers and their progenitors, of course, appears earlier in the geologic record, as they were present before the angiosperms evolved (1-2, 24-25). Five families in the order Coniferales synthesize terpenoid resins but only members in the families Pinaceae and Araucariaceae produce appreciable quantities today. Among the conifers, the Pinaceae is the largest family, primarily distributed today in middle latitude temperate zones—particularly in the boreal forests that span the Northern Hemisphere and in the mid- to higher elevations of North America and Eurasia. They include the pines (*Pinus*), firs (*Abies*), spruces (*Picea*), Douglas fir (*Psuedotsuga*), and larches (*Larix et al.*), so familiar to most temperate zone inhabitants. In many cases, they assume dominance or codominance of these

Table I. Important Tree Resin-Producing Plant Families

Conifers (Gymnosperms)	Flowering Plants (Angiosperms)	
*Pinaceae	*Leguminosae	Zygophyllaceae
*Araucariaceae	*Burseraceae	Euphorbiaceae
*Taxodiaceae	*Dipterocarpaceae	Rubiaceae
†Cupressaceae	*Hamamelidaceae	Guttiferae
†Podocarpaceae	†Anacardiaceae	Palmae
	†Styraceae	Betulaceae

\*Good evidence and †limited evidence for family as source of amber.

forests, e.g., the spruce-fir forests across northern North America, and in the Rocky Mountains, and the ponderosa pine-Douglas fir of the Rocky Mountains and Sierra Nevada. Thus, there are more individual trees of temperate zone conifers than tropical angiosperms, but not a greater diversity of taxa. These northern coniferous trees produce sufficient quantity and variable constitutive resin for defense against many enemies (e.g., 26-27), but insufficient to have been exploited commercially. Today subtropical species in Asia (e.g., *P. merkusii* and *P. khasya*) are the leading commercial producers of pine resin, with China the world's most important exporter of it (28). In most members of the Pinaceae, trunk resins are dominated by abietane and pimarane diterpenoid acids and are low in labdatrienes that polymerize. Resins with high abietane or pimarane compositions tend to be subject to oxidative degradation or dehydrogenation and hence are not good candidates for the formation of amber. However, the production of amber by a small pinaceous genus, *Pseudolarix*, will be documented by Anderson and LePage in a later chapter of this volume.

The Araucariaceae only contains two genera, *Agathis* and *Araucaria*, but *Agathis* (with the most tropical distribution of all conifers) produces constitutive resin in great quantity and it, or a relative of it, appears to have been a very important amber-producer in many deposits from the early Cretaceous to Pliocene (1-2, 25). *Agathis*, and its possible relations to some members of the Pinaceae, will be discussed in more detail in later sections of this paper.

The Taxodiaceae, a small warm-temperate family, includes the redwoods (*Sequoia*, *Metasequoia*, *Sequoiadendron*) and *Taxodium*. It has constitutive resin occurring in canals in the leaves, but only produces resin traumatically in the wood. Some small amounts also appear to be produced in the phloem. There is some evidence that small pieces of late Cretaceous and early Tertiary amber found amid an abundance of *Sequoia*, *Metasequoia* and *Sequoiadendron*-like remains may be derived from these taxodiaceous plants. IR spectra of late Cretaceous amber from the Alaskan-Arctic Coastal Plain are variable and seem to indicate more than one source. IR, <sup>13</sup>C NMR and Py-MS have indicated a close relationship to *Agathis*-type amber (1, 29-30). Although the IR spectra of other pieces of this amber do not closely match those of modern taxodiaceous resins, some of them may be derived from this source because these resins are known to be chemically heterogeneous (1). On the other hand, both megafossil remains and IR spectra suggest that *Metasequoia* is the only source of mid-Eocene amber from the state of Washington and British Columbia (31). Furthermore, Mustoe (31) presents experimental evidence that burial temperature may affect the amber and hence could be another source of the variability of IR spectra so evident in the Alaskan amber.

All trees of the Cupressaceae, with primarily a temperate distribution, are resinous (especially the leaves), but resin is not copiously produced from the trunk. Cupressaceae has the largest number of genera of any coniferous family, including prominent ones such as *Cupressus*, *Juniperus*, *Callitris* and *Tetraclinis*. Plant fossils of the Cupressaceae have demonstrated the wide distribution of members of the family in conifer forests during the Cretaceous and Tertiary, but only sesquiterpenoids characteristic of the family (with the cedrane skeleton) have been found in amber at one Cretaceous (32) and two Tertiary sites (33). It should be pointed out that the "amber" in the latter sites consists of "resinous earthy material." Anderson *et al.* (3) have questioned whether to assign these materials to another category of their classification system because Mangoni and Belardini (34) have shown that the resin of *Cupressus sempervirens* is composed primarily of communic acid and related diterpenoids. In fact, communic acid and other diterpenoid acids apparently are present in leaves of numerous genera of the Cupressaceae, with considerable quantitative differences between young and mature leaves (Gough, L., unpublished manuscript). However, different genera of the Cupressaceae, such as *Juniperus* (35), contain sesquiterpenoids with the cedrane skeleton, which are particularly common in the heartwood. When these sesquiterpenoids accumulate in abundance, they are the source of "cedarwood oil," which occurs in species of various cupressaceous genera (36). Thus, terpenoid evidence of cupressaceous taxa may occur in two forms and hence may ultimately have to be represented in two categories of the classification system of Anderson *et al.* (3). Amber from the early Tertiary European brown coals have been reported to be either cupressaceous or taxodiaceous through association with wood specimens such as *Cupressinoxylon*, *Juniperoxylon* and *Taxodioxylon* (37), but there has been no chemical substantiation of these sources.

Similar to the Cupressaceae, the Podocarpaceae, with numerous species occurring in warm temperate-subtropical-tropical forests, produce small quantities of trunk resin; nonetheless, several characteristic sesquiterpenes and a predominant diterpene acid have been identified from an extinct podocarp *Dacryiamites* from mid-Eocene coals in New Zealand (38).

**Angiosperms.** Among the flowering plants or angiosperms, the resin-producing Leguminosae (subfamily Caesalpinioideae), Dipterocarpaceae (subfamily Dipterocarpoideae), Burseraceae, Anacardiaceae, Euphorbiaceae, Guttiferae, and Palmae are all prominent tropical families. Members of the leguminous subfamily Caesalpinioideae comprise one of the most important and largest tree taxa in New World and African rainforests. Resins only occur within the large tribe Detariae (with 50 genera—more than half of the Caesalpinioideae) (39, 40). Cowan and Polhill (40) have further noted the particularly interesting adaptations for plant-animal interactions in this tribe. Perhaps the evolution of resin in this group is related to these interactions. The well-known African "copal" producing genera of considerable importance include *Copaifera*, *Daniellia*, and *Guibourtia*. *Copaifera* has been reported to produce amber (41) and the exemplary leguminous amber-producing genus *Hymenaea* will be discussed in detail later.

Similarly, the Dipterocarpaceae, with the subfamily Dipterocarpoideae alone having over 500 species and 13 genera, are significant components of rainforests over wide areas in tropical Asia and Malaysia (42-43). Although all genera in the subfamily produce resins, only some produce it copiously (e.g., *Shorea*, *Hopea*, *Vatica*, *Anisoptera* and *Dipterocarpus*). The resins contain sesquiterpenoids and triterpenoids produced in resin canals in wood and bark (44). The proportion of sesquiterpenoids to triterpenoids varies among genera (45). The genus *Shorea* has been suggested as the source of Miocene amber at two localities in Sumatra, based on IR spectra (46) at one site and <sup>13</sup>C NMR indicating characteristic sesqui- and triterpenoids at the other site

(47). The amber from the latter Sumatran coal deposit was investigated because it occurs in relatively large amounts in finely dispersed veins and lenses (47). Van Aarssen *et al.* (11) compared Miocene amber from Brunei, Southeast Asia with resin from an extant member of the Dipterocarpaceae (genus not stated). A polymer consisting of a linear chain of unsaturated cadinenes was demonstrated in both the fossil and modern resin. Thermal stress induced a disintegration of this polycadinene chain into monomeric, dimeric and trimeric cadinenes. It was further hypothesized that naturally occurring bicadinanes result from dimeric cadinenes upon cyclization. Evidence for the structure of the polymer and its structural relationship with bicadinanes was primarily derived from different pyrolysis techniques (11).

Meuzelaar *et al.* (48) found a very high resin content in central Utah coals, with sesqui- and triterpenoid components determined to be similar to those in the Dipterocarpaceae by Curie-point pyrolysis/evaporation combined with isobutane chemical ionization mass spectrometry. These resins contain four terpenoid fractions: 1) a sesquiterpenoid polymer, 2) sesqui- and triterpenoid monomers and dimers, 3) triterpenoid alcohols, ketones and acid, and 4) more or less highly aromatized terpenoids. The pollen floras of these coal deposits have suggested a warm temperate to subtropical climate with *Sequoia* dominating in an acidic swamp environment (49). However, chemistry of extant *Sequoia* resin is quite different from that in the coal. Furthermore, some amber from Eocene lignites from the Arkansas coastal plain has chemistry (shown by IR) similar to *Shorea* (50) but also has no megafossils supporting this source. In fact, the fossil pollen indicates the presence of *Pinus* and legumes that are not known to produce resin. Thus, mystery exists regarding the similarity of the chemistry of the Utah and Arkansas ambers to that distinctive of some Asian dipterocarps in that there are no associated plant macro- or microfossils in this family associated with the amber.

Even though today members of the Dipterocarpaceae are restricted to the Southern Hemisphere, a few dipterocarpaceous macrofossils have been reported in the Northern Hemisphere. For example, poorly preserved winged fruits in Upper Cretaceous sediments along the eastern coast of the United States and the Lower Cretaceous in England have been questionably compared to *Woburnia* (42). A wood specimen of *Woburnia* has been thought to be "distinctly dipterocarpaceous" but also has been questioned because of its isolated occurrence (42). Eocene leaf impressions of *Anisoptera*, which grows today in Asian peat swamps, as well as *Parashorea* have been reported from a (para-) tropical Eocene flora in Alaska (51-52). Lack of identifiable dipterocarpaceous pollen may be explained because their thin walls do not preserve well. Moreover, the percentage of pollen is always low (perhaps due to being devoured by thrips) even in extant stands of peat completely dominated by *Shorea* (42). A possible explanation for general lack of fossil pollen, and the appearance of poorly preserved macrofossils of genera that occur today in swampy peat forests, tantalize in providing some corroborative evidence for the appearance of amber chemically characteristic of members of the Dipterocarpaceae in similar habitats in the past.

Recently, however, the polycadinene structure previously thought to be distinctive for the Dipterocarpaceae has appeared in resin canals of Eocene fossil fruits from Germany and England (53). These fossil fruits were produced by representatives of mastixioid Cornaceae, taxa that were widespread in the Tertiary of Europe and North America and are not related to the Dipterocarpaceae. Interestingly, the extensive Eocene floras of Europe, which contain many elements whose closest relatives occur in Southeast Asia, questionably contain members of the Dipterocarpaceae (54). Thus, these studies turn attention to possible sources other than the Dipterocarpaceae for the North American ambers. If members of the Dipterocarpaceae are not the source of the Utah and Arkansas ambers, the question still remains as to what other resin-producing plants were present that had this distinctive chemistry—not at all similar to the conifers reported from plant remains associated with these ambers.

The Burseraceae is another large (600 species) pantropical family noted for its balsam-type resins (Table I). The elemi-type resin producers include the pantropical genera *Protium* and *Dacryodes*, the New World *Bursera* and Old World *Canarium*. A burseraceous origin for Tertiary amber from three localities has been reported (55-56) based on X-ray diffraction analyses demonstrating the presence of the crystalline triterpenoid alcohol,  $\alpha$ -amyryn, characteristic of various species of *Canarium*, *Protium*, and *Bursera*. The presence of this alcohol in some specimens of "Highgate Copalite" from the London Clay Flora along the southeast coast of England, with its associated burseraceous seeds, has suggested either *Protium* or *Canarium* as its source. The affinities of the Paleocene-Eocene London Clay Flora are predominantly tropical with most of the flora being allied to Indo-Malaysian rainforests where *Protium* and *Canarium* produce large quantities of resin today (1). However, Anderson and Botto (57) recently have reported that the sample of Highgate Copalite they analyzed by GC-MS was a simple mixture of diterpenoid resin acids and n-alkyl materials similar to modern pine resin. On the other hand, Beck and Shannon (58) have reported that IR data indicate that resinous materials described as "Highgate Copalite," from a number of collections, may be derived from several botanical sources. Therefore, the results of Anderson and Botto may not invalidate the conclusions from X-ray diffraction (55-56), since the samples may not have been the same. This study raises the importance of reporting in the literature the sample number of amber from museum specimens that were analyzed so that data may be compared from different analyses.

Another problem exists with regard to concluding that Glessite, a less common amber found in the Baltic region, has a burseraceous origin.  $^{13}\text{C}$  NMR, FTIR and Py-GC-MS data all indicate a high degree of similarity between Glessite and Succinite (57, 59-61). Hence the burseraceous origin of Glessite has been seriously questioned. Furthermore, the botanical origin of Guayaquillite is being currently investigated and considered open to question (57).

The warm temperate and subtropical genus *Liquidambar*, or more probably its immediate ancestor in the warm temperate-subtropical family Hamamelidaceae, is considered the source of amber from several localities along the Atlantic Coastal Plain near the Cretaceous-Tertiary boundary, based on the presence of polystyrene and its derivatives determined through IR (62) and FTIR and PY-MS analysis (63). Py-GC-MS studies have further shown no evidence for significant alteration of the original polystyrene structure in a late Cretaceous amber sample (3). Although Kuprianova (64) has indicated, based on palynology, that modern lineages of *Liquidambar* have not arisen until the Oligocene, these North American ambers are similar to Sieburgite, which had also been related chemically to *Liquidambar* by isolation of cinnamic acid and styrene. Sieburgite occurs in Eocene-Miocene Rhineland brown coals where *Liquidambar* was considered to be a prominent component in the *Myrica-Cyrilla* moor and *Sequoia* woods that formed these coals (1). Cronquist (44) has reported evidence for *Liquidambar* occurring in Upper Cretaceous sediments in Europe. The Hamamelidae to which the family belongs is a very old lineage (Wolfe, T. A., U.S. Geological Survey/University of Arizona, personal communication, 1994). However, Wolfe indicates that *Liquidambar* leaves have not been unequivocally reported until the earliest Eocene. Thus, Cretaceous amber in North America could well be derived from an immediate *Liquidambar* ancestor.

*Pistacia* has also been reported as the source of amber from Pleistocene sediments in Israel from the very large tropical family, Anacardiaceae (65). Phenolic resin from *Styrax* in the small tropical family Styraceae has been questionably suggested in the older literature (1) as the source of some of the rarer ambers in the Baltic region. Both of these sources, however, are based on somewhat questionable evidence (1).

It is noteworthy that not only are there more angiosperm tree taxa (family, genera and species) producing resin than conifers, but also that the copiously-producing ones



**Table II. Common/Commercial Resin Names for Resin from Various Tree Taxa**

NAME	PLANT GENERA/FAMILY
I. OLEO-RESINS (relatively fluid — high proportion of volatile terpenes)	Many spp. Pinaceae, Dipterocarpaceae
Rosin (colophony) = nonvolatile fraction after distillation of resin from <i>Pinus</i> spp.	
II. BALSAMS (relatively soft; malleable initially)	
A. Balsam	
Canadian balsam	<i>Abies balsamea</i> (Pinaceae)
Tolu & Peru balsam	<i>Myroxylon balsamum</i> (Leguminosae; Papilionoideae)
Malaysian balsam	<i>Canarium</i> spp.; <i>Dacyrodes</i> spp. (Burseraceae)
B. Elemi (highly scented, semisolid initially)	<i>Protium</i> spp., <i>Canarium</i> spp., <i>Dacyrodes</i> spp. (Burseraceae) <i>Amyris</i> spp. (Rutaceae) <i>Calophyllum</i> , <i>Symponia</i> (Guttiferae)
C. Incense (highly scented)	
Frankincense	<i>Boswellia</i> spp. (Burseraceae)
Myrrh	<i>Commiphora</i> spp. (Burseraceae)
Mexican incense	<i>Bursera</i> spp. (Burseraceae)
D. *Storax	<i>Liquidambar</i> spp. (Hamamelidaceae)
E. *Styrax	<i>Styrax</i> spp. (Styraceae) except <i>S. benzoin</i> = Benzoin
III. DAM(M)ARS (Malay word for all resins)	Many genera Dipterocarpaceae; sometimes Burseraceae, <i>et al.</i>
IV. SANDARACS	<i>Callitris</i> , <i>Tetraclinus</i> (Cupressaceae)
V. MASTIC	<i>Pistacia</i> spp. (Anacardiaceae)
VI. COPALS (extremely hard with high melting point)	
Brazilian copal	<i>Hymenaea</i> spp. (Leguminosae; Caesalpinioideae)
African copal	<i>Copaifera</i> spp., <i>Daniellia</i> spp., <i>H. verrucosa</i>
Manila copal	<i>Agathis alba</i> (Araucariaceae)
Kauri copal	<i>A. australis</i>

\*Resin comprised primarily of aromatic phenolics.

listed in Table I are concentrated in the tropics. Only the Betulaceae in this list occurs in North Temperate regions, and its resin is secreted over the surface of leaves and young stems. In fact, the most copious resin production among both conifer and angiosperm trees occurs in the tropics or subtropics. Furthermore, resins that readily polymerize tend to occur in tropical and subtropical trees. The reason for this is unknown, but given the rate organic material is decomposed under tropical conditions, this kind of hardening would be necessary for the persistence of the resin. The hardened coating of wounds may have been selected for, since direct fungal infections or those vectored by insects are particularly lethal in the tropics. The fact that evidence is currently available for amber being predominantly produced by a small number of genera within relatively few copious resin-producing tropical-subtropical families probably is related to: 1) lack of the resin components with the requisite structures for polymerization, and 2) location where the resin can either be immediately deposited or transported to adequate depositional sites. Nonetheless, keeping a tropical perspective may be valuable in amber studies for determining not only the plant source but the environment in which the tree may have existed as well.

Because of structural similarities of resin among taxa and possible effects of maturation, Anderson *et al.* (3) have proposed a classification system of resinites (ambers) based on structural characteristics and composition of the amber rather than solely on botanical origin. They have found that even up to high levels of maturity (including age and thermal history) the degree of structural character retained allows reasonable establishment of the nature of the amber. They further have pointed out that ambers that are by far the most widespread and abundant in the geosphere are derived from resins based primarily on labdatriene diterpenoids. Thus, genera in the tropical-subtropical families Leguminosae (Caesalpinioideae) and Araucariaceae, which have resins with these acids, are particularly prominent amber producers and exemplary genera that will be emphasized in later sections of this paper.

### **Commercial Resin Terminology: Confusion Regarding Plant Source.**

An attempt to clarify the semantic morass, or at least expose the problem regarding the so-called common names of resins that have been perpetuated by the resin industry, is useful for amber studies for several reasons (Table II). First, varied usages of the names in reference to the botanical sources in the scientific literature have produced confusion. Perhaps even more serious for amber studies involving comparisons with modern resins is that it is possible to obtain mixed samples from commercial dealers; thus, it is important, if possible, to obtain samples from botanical collectors who can authenticate the samples and assign scientific names to them. This is an analog of the care with which geologists demand that stratigraphic data be as accurate as possible.

Some "common" names result in more possible confusion than others for amber studies. For example, oleoresins and "true balsams" have not as yet unequivocally been reported to produce amber. Oleoresins are relatively fluid, with a high proportion of volatile terpenes, which generally are secreted into an interconnected canal system (Figure 1). Many genera in the Pinaceae and Dipterocarpaceae produce oleoresins. In commercially important species of *Pinus*, the nonvolatile diterpenoid fraction, especially that left after commercially distilling off the volatile fraction as "turpentine," is common known as "rosin" or "colophony" (Table II). Relatively soft, initially malleable, and generally very fragrant resins are called balsams. The term "balsam" as such (and hence sometimes considered as "true balsam") is used for four widely dispersed genera. Diversity of sources is demonstrated in that Canadian balsam comes from a northern species of *Abies*, whereas Tolu and Peru balsam come from a tropical papilionoid legume, *Myroxylon*, and Malaysian balsam from at least two genera in the Burseraceae. In *Abies* and members of the Burseraceae, the resin is produced in "blisters" scattered through the phloem (Figure 1). Four other kinds of balsams have distinctive names and some have been used to refer to the source of amber. For

example, the elemis attributed as a source of some amber (55-56), come from various species in the three tropical families—Burseraceae, Rutaceae and Guttiferae. The commercial incenses are all derived from various genera in the Burseraceae, although local people often use other fragrant plant resins as incenses. Storax comes from *Liquidambar* in the Hamamelidaceae, and styrax from various species of *Styrax* in the Stryaceae, except *S. benzoin*, which is called "benzoin." However, storax has sometimes been equated with styrax in the scientific literature (e.g., 3).

Dam(m)ar is a case where confusion has resulted for several reasons. First, it is the Malay word for *all* resins, but may be used locally for different members of the Burseraceae and Dipterocarpaceae in particular (66). Further semantic problems revolve around the genus *Dammara*, a name obviously derived from the plant's producing resin. Two species previously put in the genus *Dammara* are now considered to be *Protium* (Burseraceae) in one case and *Shorea* (Dipterocarpaceae) in another (67). To add to this complexity, numerous species now assigned to the genus *Agathis*, which also occurs in mixed stands in Malaysian forests, were initially thought to be in the genus *Dammara* (67). Thus, when obtaining resins from commercial dealers, it is possible to obtain "damar" in which resins are either not properly identified as to the botanical source or are from various sources that have been mixed (a practice common among local collectors) (68). Nonetheless, "damar," in some scientific articles, is used synonymously with dipterocarpaceous resins (e.g., 11, 48). Furthermore, *Shorea* resin, most often reported to have been obtained from dealers, has been the resin most commonly compared with ambers (11, 47, 48); it may thus be assumed that all dipterocarps are similar to *Shorea*. However, it should be recognized that chemical variation in the resin occurs among the numerous genera in the Dipterocarpaceae (45, 66).

Sandarac is the commercial name, generally, for the cupressaceous genera *Callitris* and *Tetraclinus* and mastic for *Pistacia* in the Anacardiaceae (Table II). Less confusion exists in the use of these terms, probably because the resins are much less common than the damars.

Although the term "copal" may be derived from the Aztec word "copalli," meaning "resin" or "incense," usage throughout the commercial literature has restricted "copal" to resins that are very hard and durable with a high melting point (68, 69) (Table II). This characterization makes them likely candidates for the formation of amber and hence important for consideration by amber investigators. Outside of Mexico and in the commercial literature, "copal" has generally been used for the hard resins from various genera (e.g., *Hymenaea*, *Copaifera*) in the Caesalpinioideae and for various species of *Agathis* in the Araucariaceae. For genera in both families copals are distinguished geographically; only a few examples of the geographic sources are given in Table II. Since the most common usage, including that in commerce, has been to consider that only genera in two families of trees produce "copal," it seems that using this term to refer to any "moldable exuded resin" (no matter the botanical source) that is less than 3-4 million years old (25) only creates semantic confusion for future amber studies. This is not to deny the value of an acceptable term to refer to exuded resin before it is considered sufficiently polymerized to become "fossilized."

### Case Study of *Hymenaea* (Leguminosae: Caesalpinioideae)

*Hymenaea* is a member of the resin-producing tribe Detarieae in the legume subfamily Caesalpinioideae (70). In fact, all leguminous resin-producing trees now belong to this tribe (39). *Hymenaea* is emphasized in a detailed discussion of the biology of amber-producing trees because: 1) it is the probable source of amber from Mexico, Dominican Republic and Brazil—possibly Colombia—if this resin is considered to have become fossilized, 2) polymerization of labdatriene diterpenoids in *Hymenaea* resin occurs soon after it exudes from the tree, especially if exposed to sunlight, thus

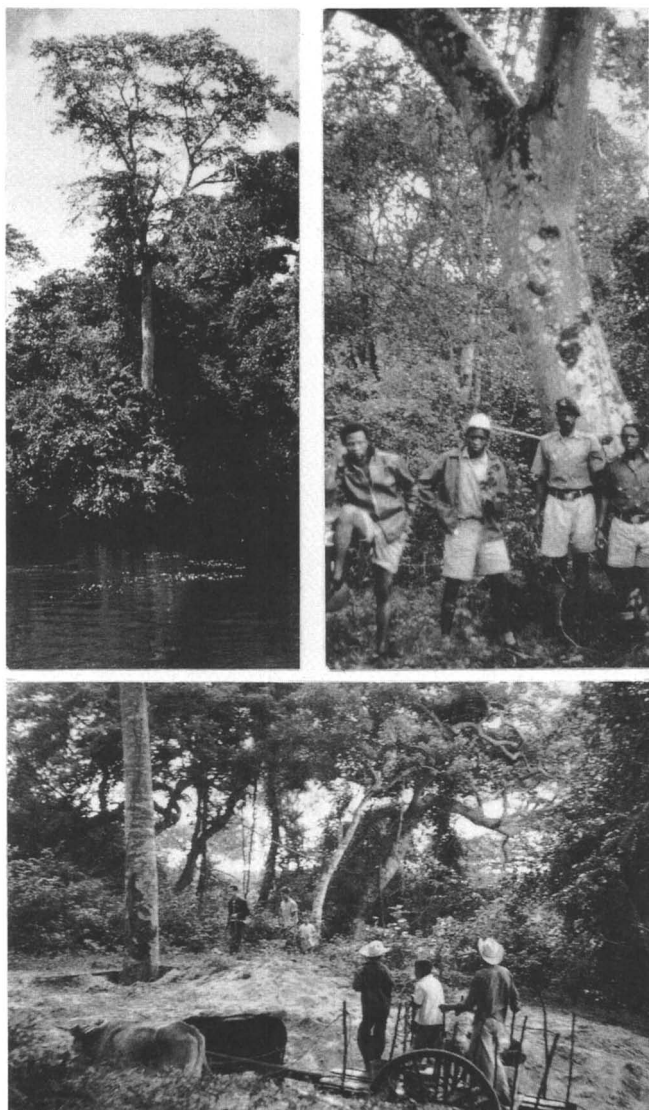
forming a highly durable material, 3) some extant species occur in habitats where resin can enter good depositional sites, thus providing additional evidence useful in identifying modern species related to the amber trees, and 4) chemosystematics and chemical ecology of some organs of *Hymenaea* have been studied in detail—contributing to a better understanding of factors controlling variation in composition and yield of the resin and hence of amber.

**Distribution of *Hymenaea* and Relation of Species to Amber Trees.** The revised systematics of the genus indicates that one species occurs in eastern coastal Africa and Madagascar and 13 species in the New World, which span the tropics from northern Mexico to southern Brazil—from evergreen rainforests to desert thorn forests (70). The center of distribution is in Amazonia with species having radiated into drier forest systems. A number of varieties have been described, based primarily on differences in foliar characteristics. Several species occur in habitats that represent particularly good depositional sites for resin but two, possibly three, species have been suggested to be related to the source of the amber. *H. verrucosa* (Figure 2) occurs in eastern coastal rainforests of Kenya and Madagascar; it produces enormous masses of resin that have been utilized commercially (69), and it or an ancestor is thought to be the closest relative to the tree that produced the Tertiary Dominican amber (71). Poinar (72) has described this amber-producing tree, named it *H. protera*, and further concurred with its relationship to *H. verrucosa*.

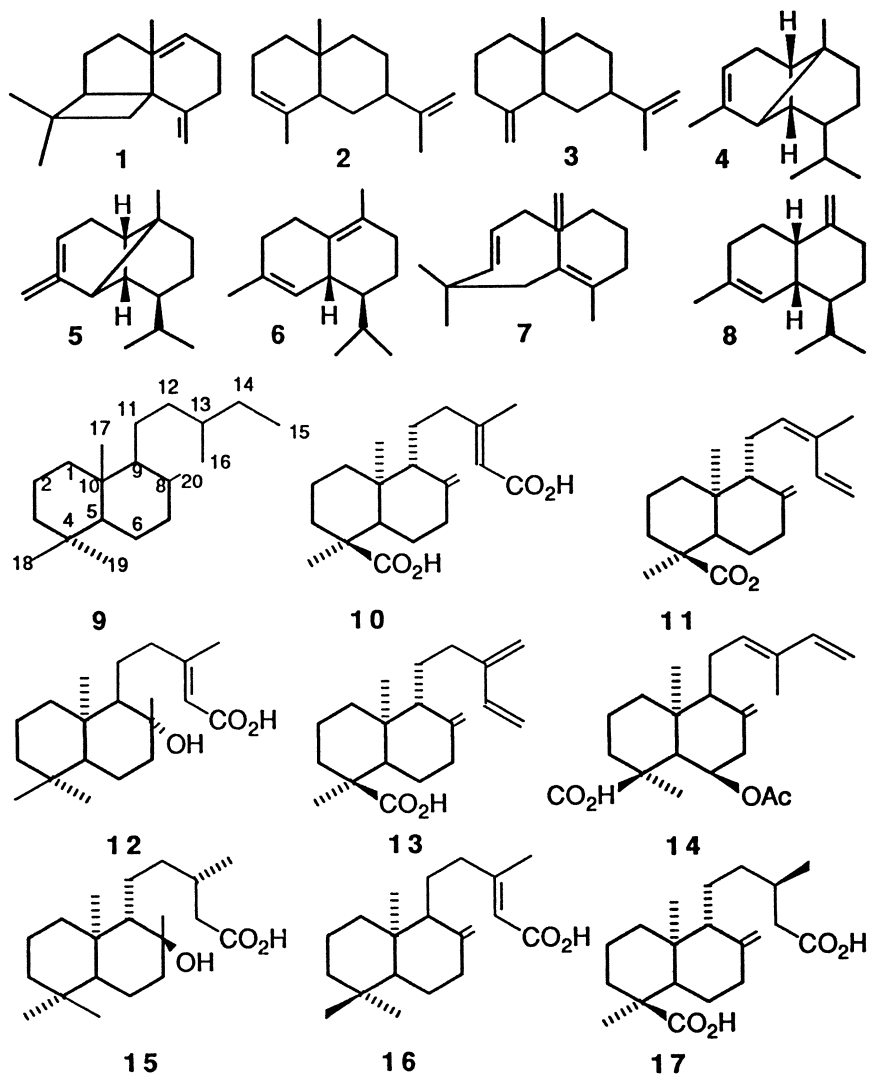
On the other hand, a relative of *Hymenaea courbaril*—*H. intermedia* has been thought to be the source tree of the Oligo-Miocene amber from Chiapas, Mexico (73). *H. courbaril* (Figure 2) has an extremely wide distribution, i.e., essentially that of the entire genus. *H. intermedia*, which today only occurs in central Amazonia, has characteristics intermediate between *H. courbaril* and *H. oblongifolia*. Although early workers questioned whether *H. intermedia* was a hybrid between the two species, others have suggested that it may represent the transitional species in the evolution of *H. courbaril* from *H. oblongifolia* stock (74). This latter concept would explain the intermediate characteristics found in *Hymenaea* inclusions in the Chiapas amber (73), which in turn would also support phyletic conclusions reached in a monographic study of the genus (70). The massive amounts of Brazilian copal from Amazonia are often attributed to *H. courbaril* (75) but probably also comes from *H. oblongifolia* var. *palustris* in the periodically inundated areas there (Figure 2).

*Hymenaea* species in moist forests and along streams apparently synthesize the greatest quantity of resin; those in dry forests produce less, but in this case they produce relatively more during the wet season than in the dry season. It has also been found that conifers produce less trunk resin when water stressed (76).

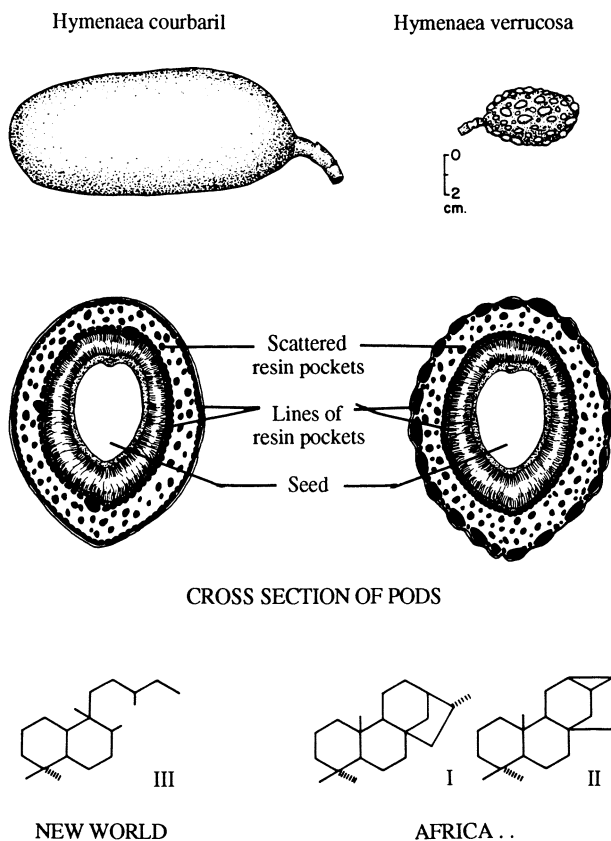
**Studies of Chemical Ecology of *Hymenaea* Resins.** Current chemical defense theories suggest that both composition and yield (total quantity) of the resin produced could be influenced by both biotic selection pressures and by abiotic environmental conditions (4). However, it is also important to consider that differences exist among the organs in the plant (e.g., 22). For example, the ratio of predominantly sesquiterpene hydrocarbons and diterpenoid acids in *Hymenaea* varies among three organs studied and even during their ontogeny (39). Resin in the young stem and leaves primarily contain sesquiterpene hydrocarbons produced in schizogenously-formed pockets (Figure 3) with only traces of diterpenoids (39). However, resin in secondarily-developed wood in the trunk, although probably in part due to induction through lysigeny of initially produced pockets, consists primarily of labdane diterpenoid acids (Figure 3). Sesquiterpenes provide the fluidity and the relative amount of sesquiterpenes and diterpenoids determines the viscosity of the resin. Similar to the trunk resins, the pod resin is comprised primarily of diterpenoids (Figure 4). Only the structures of diterpenoid resin acids from the soluble part of the



**Figure 2.** Upper right, *Hymenaea verrucosa* in coastal Kenyan rainforest; upper left, *H. oblongifolia* v. *palustris* in periodically inundated eastern Amazonian rainforest; below, *H. courbaril* along stream in subdeciduous forest in southern Mexico. Local residents have gashed large tree in foreground for resin.



**Figure 3.** Predominant leaf sesquiterpene hydrocarbons in all *Hymenaea* species and representative bicyclic diterpenoid acids from trunk resins. Sesquiterpene hydrocarbons: 1) Caryophyllene, 2)  $\alpha$ -Selinene, 3)  $\beta$ -Selinene, 4)  $\alpha$ -Copaene, 5)  $\beta$ -Copaene, 6)  $\delta$ -Cadinene, 7)  $\beta$ -Humulene, 8)  $\gamma$ -Muulolene. Diterpenoid acids: 9) Labdane skeleton, 10) Guamaïc, 11) Ozic, 12) Lab-13-en-8-ol-15-oic, 13) Enantio-8(20),13(16),14 labdatriene-18-oic (an isomer of ozic—as indicated by (3)), 14) Zanzibaric, 15) Enantio-13-epilabdanic, 16) Copalic, 17) Enantiopinifolic.



**Figure 4.** Comparison of the pods of the African *Hymenaea verrucosa* and *H. courbaril* as a representative of the New World species. Basic morphology of the pod is shown along with a cross section, which depicts the arrangement of the secretory pockets in the pod wall. Skeletal structures of the predominant resin diterpenoids are contrasted. Adapted from (22).

resin formerly have been elucidated in both trunk and pods (39). However, other acids as well as alcohols and hydrocarbons are reported from Py-GC-MS studies by Anderson in a later chapter in this volume.

The light, moisture and temperature conditions that prevail in tropical and subtropical rainforests are, essentially, continuously favorable for plant metabolism, including photosynthesis, which provides the carbon for terpene synthesis. However, carbon availability alone cannot account for increased terpene synthesis (77) and does not account for the incredible compositional variation found among tropical rainforest plants. Also there are relatively few seasonal changes, at least compared to either the dry tropics or more northern latitudes with definite changes, especially in the temperature regimes. It is in these favorable rainforest conditions that the diversity of organisms also flourishes against which the trees must defend, and hence to which they may respond by increased yield and/or compositional variation. What are the kinds of biotic selection pressures that might occur on these different organs, i.e., against what kind of damage would the resin in the organ be defending? Regarding both insect and vertebrate herbivores: 1) the leaves must withstand the damage levels that would be lethal to seedlings and cause defoliation of mature trees, 2) the young stem must withstand lethal girdling and in the trunk, the meristematic tissue of the cambial region must be protected, 3) in the fruit, injury to the seed must be prevented, and 4) microbial invasion (especially by pathogenic fungi in the moist tropics) could damage any living tissue or result in decomposition of heartwood.

**Leaf Resin.** As is true of most current studies in chemical ecology of trees, the most extensive investigation of *Hymenaea* resins has been done on leaves. Although these are not the resins most directly concerned with amber studies, they display some of the problems in understanding variability in resin composition and yield. We noticed in systematic studies of *Hymenaea* that leaves of different species had observably different patterns of resin pockets, i.e., the resin secretory structures (78). Leaf resins in all species were comprised principally of about a dozen sesquiterpene hydrocarbons, with a few oxygenated ones (Figure 3) that occurred in repetitive compositional patterns. Chemosystematic studies demonstrated that these patterns were more correlated with populational differences than specific ones (6-7). These compositional types within an individual tree apparently were under genetic control, as had been found for some chemotypes in conifers (e.g., 8).

However, the role of abiotic factors, such as light, temperature, moisture, and nutrients is important to assess because of their possible effects on the qualitative and/or quantitative variation of the resins. Growth chamber and greenhouse experiments on seedlings demonstrated that composition in the mature leaf was not significantly affected by any of the factors, but that light intensity (irradiance) significantly increased yield (Table III) (79-81). Field studies of soil nutrient effects on resins of *Copaifera*, in comparative studies with *Hymenaea*, likewise showed no statistically significant effects on composition, but that increased availability of nitrogen decreased the yield (82-83)—as had been discovered in studies of monoterpenes (e.g., 84). Therefore, tree-to-tree intrapopulational as well as interpopulational compositional variation apparently could be significant in defense against foliovores and pathogens and hence may be due, in part, to biotic selection pressures (4, 85-86), i.e., the trees with resin composition that successfully defends the tree survive whereas those with other composition do not. This represents a part of the co-evolution of the tree and its herbivores and pathogens. The greatest number of compositional types (at least six) occurred in rainforest populations, whereas often only a single type occurred in dry forest populations, a finding that also points to the greater biotic pressures in the former. Laboratory experiments and field studies with various lepidopterans, the primary foliovores (86-90), and with frequent leaf-spotting fungi (91) supported this



**Table III. Effect of abiotic environmental conditions on leaf resin composition and yield as indicated by growth chamber and greenhouse experiments on *Hymenaea* seedlings**

	Composition	Yield (Total Amount)
Light Duration (photoperiod)	No	No
Light Intensity (Irradiance)	No	Yes ↑
Temperature	No	No
Moisture Stress	No	No
*Total Soil Nutrients	No	No
*Nitrogen + Light	No	Yes ↓

\*Field studies done on closely-related genus *Copaifera* in comparison with *Hymenaea*.

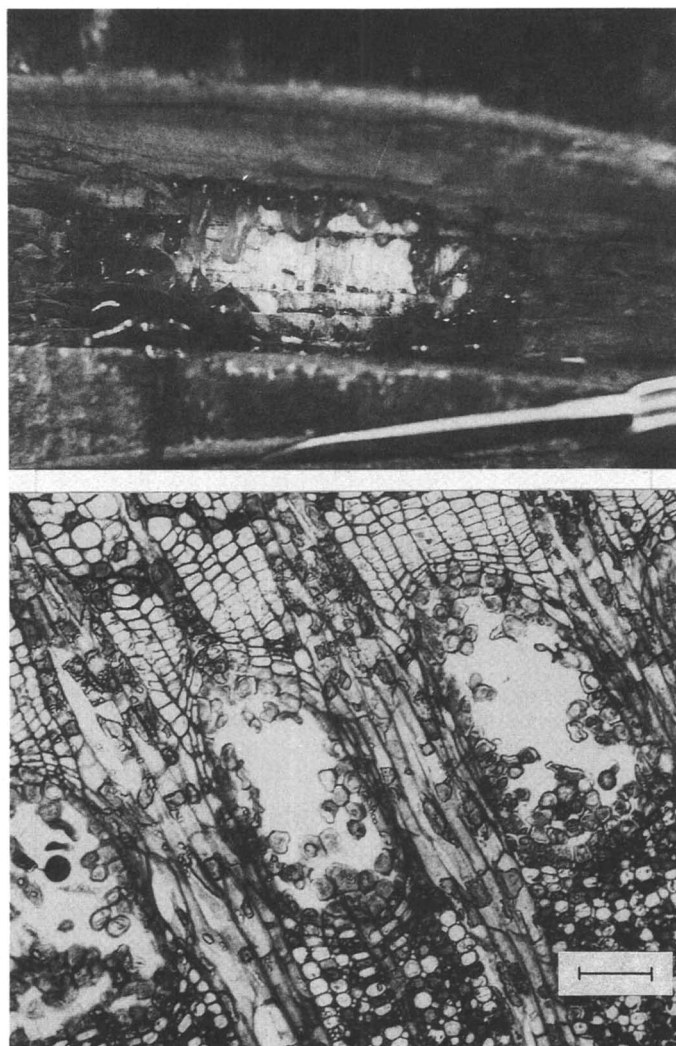
view, with all cases showing dosage-dependent deterrent or inhibitory effects of resin components amid quantitative variability of the other components.

Thus, for amber studies it is important not to draw arbitrary conclusions regarding compositional variation being primarily species related—especially with the small sample size often available for amber analysis. Rather it is important to recognize the potential for tree-to-tree quantitative compositional variation that may well relate to the defensive role of the resin. Furthermore, compositional variation should not automatically be assigned to seasonal changes. This appears to be an obvious interpretation, and hence there is a tendency to state it with no supporting evidence. In fact, even in the chemical ecology literature, changes thought to be seasonal have been confused with changes associated with the development of an organ through the seasons (4; Gough, unpublished manuscript).

**Pod Resin.** Although all *Hymenaea* species contain in the leaves essentially the same suite of sesquiterpenes (which predominate over the diterpenoids), in the pods the diterpenoids (which predominate over the sesquiterpenes) have different skeletons in the New World and Africa (Figure 4). Morphological differences in the pods parallel the structural differences of the diterpenoids(22). The pods are one-seeded in the African species (*H. verrucosa*) with a verrucose surface of small masses of resin and the diterpenoids that have been identified are predominantly either tetra- or pentacyclic acids with the kaurane (I) and trachylobane (II) skeletons (92-94) (Figure 4). A small amount of a bicyclic diterpenoid with a labdane skeleton also has been reported. The resin in these pods occurs in such quantity that it has been expressed for commercial use (69). The pods of New World species, represented by *H. courbaril*, are one-to-many-seeded with relatively smooth surfaces, although resin pockets form a protective continuous barrier within the epidermal layers as does the African species (Figure 4). However, all diterpenoid resin acids in the New World pods are bicyclic labdanes (95). This is also true of all *Hymenaea* trunk resins. The bicyclic diterpenoid resin acids in the New World *H. courbaril* pods, however, are more highly rearranged (III, Figure 4) than those isolated from the trunk (95).

In the New World *Hymenaea* pods, masses of resin are secreted when the epidermis is ruptured by any form of injury, and the amount apparently may be increased by lysigeny of the secretory cells. In fact, young fruits exude such large masses of resin that the accumulation often can encase the pods. Present evidence indicates that *Hymenaea* pods in the New World have resisted attack by bruchid beetles (which often attack legume pods) but can be heavily attacked by curculionid weevils (39, 96-97). Janzen (97) pointed out that the amount of resin in *H. courbaril* pods in Puerto Rico, where the curculionid weevils that attack *Hymenaea* do not occur, is significantly less than in Costa Rica, where these weevils are prevalent. He further suggested that this increased production might be due to selection pressures by the insects in Costa Rica. Since these weevils oviposit by biting out a piece of the outer wall and inserting an egg into it, the insect must circumvent the highly viscous and tacky resin, which could either physically exclude the eggs or have a toxic effect on them. Because the resin polymerizes within the wall of the pod as it is maturing, resulting in a hard impenetrable covering, timing must be accurate for the weevil to oviposit through the pod wall. The striking differences in both diterpenoid chemistry and morphology of the outer wall (in which small masses of resin occur in the African species in contrast to the New World species) suggest the possibility of even greater intensity of herbivore selection pressures on these pods in Africa than those in the New World.

**Branch and Trunk Resin.** In the young stem or branches, the secretory pockets are arranged to form a continuous wall of resin that an insect, such as the bark beetle, must circumvent. Enormous amounts of resin are seen, particularly in rainforest habitats, hanging from branches in long stalactite masses or in large lumps in the crotches of the branches. In the trunk, bicyclic labdane resin acids (Figure 3) are produced in pockets in the xylem (wood) side of the cambium and the amounts are increased by lysigeny of the pockets (Figure 5) (1, 98). Thus far, only bicyclic labdanes have been isolated in the trunk of *Hymenaea* species (39, 94, 99-101), and large quantitative differences in the composition of diterpene resin acids were found in 10 species, and in 13 populations of the widespread *H. courbaril* (103; Langenheim, J. H. and Martin, S. S., 1980, unpublished manuscript). All but one resin acid on which structural data are available to date are bicyclic labdanes that belong to the entanio-labdane skeletal series. However, Amazonian *H. courbaril* has yielded both copalic acid (Figure 3-16) and the antipodal lab-13-en-8-ol-15-oic acid (Figure 3-12) (102). It is not known how different the induced resin may be from that formed constitutively in the pockets. As the resin exudes from the trunk, labdatriene acids provide the predominant monomers, which may undergo a photoinduced polymerization by oxyradicals, such as semiquinones, as the resins issue into the atmosphere and are exposed to UV radiation (104). Cunningham *et al.* (105) have suggested that the primary monomers in *H. verrucosa* are labdatrienes related to the structure of zanzibaric acid, but having a 6- $\beta$ -hydroxyl function in lieu of a 6- $\beta$ -acetoxy group. Mills and White (106) have hypothesized that the primary monomers in these types of resins are ozic acid. Anderson *et al.* (3), however, note the co-occurrence of zanzibaric and ozic (Figure 3, 11, 13, 14) in *H. verrucosa* (94); since an ozic acid isomer has been identified as a major component of the soluble portion of *H. verrucosa* resins (102), it seems that a copolymeric resin polymer could be based on varying proportions of these monomeric compounds. The polymer-forming acids (and probably alcohols) occur with the above mentioned soluble acids (Figure 3) and others that become incorporated into the polymeric structure. Thus the trunk resin has the requisites for production of large deposits of amber. Furthermore, *Hymenaea* resins apparently have properties that enable more consistent preservation of soft tissues of



**Figure 5.** Resin production in trunk of *Hymenaea courbaril*. Upper photo shows cut through bark to cambial region from where the resin exudes. Lower photo is a section taken from cambial region showing pockets on xylem side with epithelial cells beginning to lyse in formation of larger areas of secretory tissue. Line scale is 50  $\mu$ . Taken from plate in (1) with permission from American Association for the Advancement of Sciences.

insects than the Baltic amber (107). The possible reasons for this are under current investigation.

**Evidence for Amber Derived from *Hymenaea*.** Compelling chemical evidence from IR spectra (1, 46, 73), CP/MAS  $^{13}\text{C}$  NMR (29, 105, 108), PY-GC-MS (3) indicates that *Hymenaea* is the source of amber from both the large deposits from Mexico and Dominican Republic. Furthermore, inclusions of plant parts support this source but from species in different taxonomic sections of the genus for the two areas. The Dominican amber has all floral parts included in the amber, with highly characteristic petals of the *H. verrucosa* type being particularly abundant (25, 71-72). Also, DNA from the fossil *H. protera* has been related to that of *H. verrucosa* (109). The Mexican amber, however, has no petals but abundant stamens included in the amber, possibly as a result of bat pollination of the species in the taxonomic section to which *H. courbaril* belongs. Abundant stamens occur on the ground under these trees today following a night of pollination by bats. Leaflets in the Mexican amber have characteristics of both *H. intermedia* and *H. courbaril* (73). Other concomitant evidence for a *Hymenaea* source for the Mexican amber results from study of its depositional conditions. The amber primarily occurs in lignites and associated marine sandstones. Pollen analysis of these sediments indicated that the amber was deposited in a complex of mangrove vegetation, i.e., a fluctuating shallow sea fringed by mangrove trees, or in brackish estuaries or backswamps dominated by these trees (110). *H. courbaril* trees today line mangrove-dominated estuaries and swamps along the Mexican Pacific coast. Here the resin can be washed from the soil directly into mangroves or indirectly via streams that enter the mangroves. The roots of the mangroves offer excellent conditions for trapping resins, and hence a good depositional site for the resin. Thus some species of *Hymenaea* today occur near depositional sites that duplicate those in which the amber was discovered.

### Case Study of *Agathis* (Araucariaceae)

There are several reasons why a discussion of the biology of *Agathis* is emphasized: 1) chemical similarities of ambers from different ages and different geographic locations to *Agathis australis* resin, 2) several *Agathis* species produce copious amounts of diterpenoid resin with diterpenoid labdatrienes that polymerize (similar process to that in *Hymenaea*) into durable material, resulting in massive accumulations and 3) several characteristics of *Agathis* resins combine to suggest the involvement of *Agathis*, or a relative, in the botanical source of the predominant amber, Succinite, from the Baltic Sea region.

**Distribution of *Agathis* and Its Resin Production.** With the most tropical distribution of any conifer, the 13 species of *Agathis* today are restricted to the Southern Hemisphere—extending from the subtropics of New Zealand to the tropics of Malaysia (67). Certain *Agathis* species have been of considerable interest for the commercial utilization of their resins (69, 111). The resin is a mixture of mono-sesqui- and diterpenes; *Agathis* is unusual in that the diterpenoids from the bark have been analyzed chemosystematically (67, 112-114). Each species has a characteristic mixture of diterpenoid acids, but considerable variation may occur between individual trees in a population. Furthermore, some species either lack the labdatrienes that produce polymerization or agathic acid (114). Amber has been reported to have derived from *Agathis* in both New Zealand and Australia, i.e., within its present distribution (114-115). The source of Oligocene and Miocene amber from New Zealand was documented to be *A. australis* by both IR and GC analyses and associated plant remains. However, Thomas (113) thought that Eocene amber from New Zealand,

which gave a similar IR spectrum to *A. australis* but had differences in the volatile fraction, suggested another species. *Agathis*, as the source of Pliocene Australian amber, is supported by leaves embedded in it, associated wood from *Agathis* and the presence of agathic acid. However, some compositional variation again led to questions as to whether the source was a species other than *A. australis*. Anderson *et al.* (3) have suggested that the compositional differences reported by Thomas could be due to differences in the maturation of the samples rather than in botanical origin.

IR and CP/MAS  $^{13}\text{C}$  NMR analysis have suggested that *Agathis*, or a closely related relative, is the source of amber from various ages from the Cretaceous through the Tertiary at widely separated geographic locations in the Northern Hemisphere (1-2, 25, 63, 116). The most extensive early Cretaceous deposits, with the amber referable to an araucarian source, are the Near Eastern ones, i.e., Israel, Lebanon and Jordan (117-118). In Jordan the chemistry was supported by *Agathis*-like remains. As previously discussed, chemical analyses have suggested an araucarian source (closely related to *Agathis*) for most of the late Cretaceous amber associated with mixed conifer plant fossils in 13 lignitic localities along the Atlantic Coastal Plain of the United States (62, 107), as well as some of the amber from the Alaskan-Arctic Coastal Plain (25). The secondarily deposited amber along the beach at Cedar Lake, Manitoba, Canada, thought to be late Cretaceous in age (119), has been analyzed chemically by IR (46, 62), Py-MS (30),  $^{13}\text{C}$  NMR (29). All of these analyses suggest an araucarian origin similar to *A. australis*.

Araucarian plant remains reported to be *Agathis* [e.g., wood, *Araucarioxylon*- (120); pollen, *Araucariacites australis*- (121)] that were associated with the amber from lignitic deposits in North America (62) have been questioned recently by paleobotanists studying the evolution of the Araucariaceae (122). Stockey (122), in fact, attributes all araucarian Northern Hemisphere plant fossils, often poorly preserved, to *Araucaria*, the other genus in the family. She admits, however, that *Agathis* and *Araucaria* wood are indistinguishable (Stockey, R., University of Alberta, personal communication, 1994). Stockey also thinks that the two genera probably evolved from a common ancestor. However, *Araucaria* today does not produce either large quantities of resin or ones that polymerize to form durable masses, as do most species of *Agathis*.

In fact, some *Agathis* species, such as *A. australis*, produce large amounts of resin under natural forest conditions in which the labdatriene communic acid, and possibly communol, readily polymerize (3, 112, 123). The well-documented story of *A. australis* as a commercially used resin-producer (111, 124) is particularly instructive with regard to massive accumulation of resin. Collections of resin from *A. australis*, based upon surface resin deposits (commonly collected to a depth of 5 m), which accumulated presumably over the past several thousand years, supported a major industry in the establishment of New Zealand (124). Thomas (113) has estimated that 25 T/Km<sup>2</sup> were accumulated over the last thousand years just over the distribution of *A. australis*—presumably under natural forest conditions.

***Agathis* Involvement as Source of Baltic Amber (Succinite).** Amber thought to have been derived from *Agathis* has been reported from several localities in Europe, such as Austria (125) and France (25). The most pressing perennial question has surrounded the botanical origin of the predominant amber (Succinite) from the Baltic region, and most recently the role of *Agathis* as the source (1-2, 25, 126-127). Table IV summarizes the pros and cons for the involvement of *Agathis* resin as the botanic source of Baltic Succinite.

**Chemistry and Massive Accumulations.** One of the most compelling arguments for the involvement of *Agathis* is the striking chemical similarities of the resin of *A. australis* to Succinite (126). First, the presence of agathic acid, which is

**Table IV. Dilemma Regarding the Involvement of *Agathis* as the Plant Source of Baltic Succinite**

Pro <i>Agathis</i> Involvement	
1)	Amber chemistry predominantly similar to extant <i>A. australis</i> (Araucariaceae)
2)	Massive accumulation with many large pieces similar to extant <i>A. australis</i>
3)	Much of associated amber flora similar to that in some extant Pacific subtropical-tropical forests where <i>Agathis</i> occurs today
Con <i>Agathis</i> Involvement	
1)	Absence of succinic acid
2)	Only resin-producing plants included in amber or associated with it, from other coniferous sources
3)	Wood with enclosed Succinite considered to be "pinaceous"

characteristic of most species of *Agathis*, occurs in Baltic amber; the soluble and polymeric fractions of Succinite contain 75 to 85% labdanes, 15-21% pimaranes and only 1.5-4% abietanes, a composition of diterpenoid acids closely matched by the modern *A. australis* (127). Secondly, the volatile terpenoids closely resemble those found in amber further documented by *Agathis* fossils within the current distribution of *Agathis* (128). Third, the ether-insoluble fraction of hydrolyzed Succinite yields an infrared spectrum almost superimposable on that of *A. australis* (127). The major difference between them is the lack of succinic acid in *Agathis* resin, which is present in Baltic Succinite. Interestingly, when Gough and Mills (127) succinylated *A. australis* resin, it gave a very similar IR spectrum, essentially identical to Succinite.

The apparently inexhaustible quantities of Baltic Succinite and the occurrence of large-sized pieces have continually raised questions as to how and why trees would have produced so much resin (1-2, 116, 129-130). Because the northern pines, which occur in the Baltic region today, do not exude such massive amounts of resin, Conwentz (129) proposed that the entire forest of amber-producing trees had been infested by some unique pathological condition that he called "succinosis," relating the name to Succinite. So much thought had been directed toward temperate zone conifers that investigators did not give attention to the enormous masses of resin produced and accumulated by a subtropical-tropical conifer, such as *A. australis*. Also they did not know at this time that pine resins do not have constituents that readily polymerize or that pine resins are not induced by pathogens as are other conifers. On the other hand, because the Baltic Succinite has been secondarily deposited, some have thought that the great accumulations could result from the concentration of the amber before it was finally deposited (e.g., 130).

**Plant Remains Associated with Succinite.** Despite the chemical similarities to *A. australis* (excepting the lack of succinic acid) and the massive accumulation of this resin, a dilemma still exists regarding the source of the Baltic Succinite because no obvious araucarian remains are associated with the amber. To the contrary, a variety of coniferous plant parts, apparently representing members of the Pinaceae, Taxodiaceae and Cupressaceae (and possibly the extinct family Cheirolepidiaceae) are enclosed within the amber. As previously indicated, a similar coniferous mixture of macrofossils occurs in Cretaceous lignitic deposits along the U.S. Atlantic Coastal Plain (62), but in these cases, some poorly preserved remains have additionally been considered to be "araucarian." Although Succinite contains

remains of other conifer genera enclosed within it (24-25, 116, 131)—in fact, specimens of *Thuja* (Cupressaceae) exceed those of *Pinus* in abundance of foliage remains—the Pinaceae have classically been considered its source. Several pines, and possibly a spruce, have been included within a single epithet, *Pinus succinifera* (129). Droplets of amber in the resin canals in wood parenchyma of a "pinaceous type" have generally been considered the most convincing evidence for a pinaceous source (1-2, 132). However, Schubert (132) did not consider this wood related to that of modern pines. As numerous workers (e.g., 116, 126, 128) have pointed out, it thus is probably best to return to Goepfert's (133) designation of *Pinites* indicating only that it is a "pine-like fossil wood." In fact, Millar (134) thinks that most modern species of pine did not arise until the Miocene. Furthermore, Paleozoic wood (Cordaitalian *Dadoxylon*), and Triassic-Cretaceous wood (*Araucarioxylon*) are very similar to modern wood of *Araucaria* and *Agathis* which are essentially indistinguishable (135); even distinction of early Tertiary araucarian and pinaceous wood could as well be questionable (Wheeler, E., North Carolina State University, personal communication, 1994). Nonetheless, the question still remains as to why there is no evidence for various plant parts of *Agathis*, such as those that have been traced to other coniferous families. *Agathis* pollen does not preserve well and the cones are relatively large, but at least some evidence of leaves or parts of cones might be expected.

Despite the major caveat of lack of associated plant remains, it is interesting that, floristically, the relationship to an Asian conifer, such as *Agathis* or its relatives, seems possible. Much of the Eocene and Oligocene floras associated with the Baltic Succinite-producing trees were similar to those found in the subtropical regions of Assam, Burma and Yunnan, China today (136). Also some bordering areas were clearly tropical, with a flora similar to some elements in Indo-Malaysia. *Agathis* is an unusual conifer in that it occurs today amid angiosperms in lowland rainforests as well as with subtropical elements in other habitats. A few species of pine (e.g., *P. merkusii*) occur in the Indo Malaysian rainforests today (137), but Millar (134) thinks that most early pines and their relatives (although probably not all) disappeared from the middle latitude of the Northern Hemisphere during the Eocene because they were poorly adapted to the subtropical-tropical conditions that occurred in a broad zone there (to 70°N).

**Hypothesis to Resolve Dilemma.** Thus, although numerous characteristics of Baltic Succinite seem to be closely related to those of *Agathis* (and particularly *A. australis*), the lack of succinic acid in the resin and any clear evidence of supporting plant remains continue to perpetuate an enigma. A suggested hypothesis for the resolution of this puzzle is that pinaceous-appearing plants associated with the Succinite were derived from either an araucarian ancestor or one in common to both with some of the chemical similarities of the resin carried along with other characters as pinaceous plants evolved. In fact, Miller (138), discussing the origin of modern conifer families, states that present evidence suggests that the families Araucariaceae and Pinaceae are polyphyletic, and that the older Araucariaceae and younger Pinaceae are "sister groups" with a possible common ancestor. Furthermore, it should be pointed out that although much amber has been produced by extinct, but closely related, species within modern resin-producing genera, it is possible that resin may even have been produced by extinct genera, which once were copious resin producers but are not today. A more detailed hypothesis regarding coniferous relationships and the source of Succinite is presented by Anderson and LePage in a later chapter in this volume.

## Summary and Conclusions

Knowledge regarding the biology of resin-producing trees can be useful in amber studies for various reasons. First, is the recognition that the different fractions (volatile

and nonvolatile) of terpenoid resins vary with different organs of the tree, a variation that probably is related to the defensive role of the resins. Resins provide a versatile defense in that they may act either chemically or physically, the latter often depending on the ratio of volatile to nonvolatile fractions. Hence, the probable defensive role provides a context for thinking about the evolution of resin characteristics, and consequently about amber.

Second, the volatile fractions of leaves have been primarily utilized in chemosystematic and ecologic studies of extant plants, whereas nonvolatile fractions that polymerize from the tree trunk have been emphasized in most amber studies. Using examples from detailed chemosystematic and ecologic studies of the leguminous genus *Hymenaea*, compositional variation was shown generally not to be due to either intraspecific or seasonal differences. Intraplant, intra- and interpopulational qualitative and quantitative compositional variation of constitutive resins in *Hymenaea* are much greater than that among species. Additionally, compositional variation of constitutive resin in the *Hymenaea* leaf appears to be under strong genetic control in individual trees and biotic selection pressures to be significant in influencing compositional patterns, whereas abiotic factors, such as light and nutrients, primarily influence the total quantity of both constitutive and induced resins. In *Hymenaea* leaves, seasonal variation is related to the development of the organ; little variation occurs once maturity is attained. Therefore, speculations that compositional variations in amber are due primarily to interspecific differences or seasonal changes probably should be qualified. On the other hand, it is important to ask whether in all cases it is possible to transfer information gained from the study of resins in one organ to another organ, particularly since resin composition and total quantity are under selection pressures from different organisms in different organs. Additionally, abiotic factors may affect one organ and not another; for example, moisture availability did not affect *Hymenaea* resin yield in experimental studies of the leaves but it appears to increase the resin yield in the trunk, as observed for trees that occur in rainforests or along streams in dry forests. These are important concerns in comparing studies of resin from extant trees to those of amber.

Third, the type of resin secretory tissue apparently can sometimes be correlated with the quantity and variation of constitutive and induced resins, as well as to the general stage of forest development in which the tree occurs. Thus, in comparing ambers with resins, some indication of the type of the tree's secretory tissue and whether the resin is constitutive or induced are factors that may assist with deductions regarding the nature of the forest and whether the trees may have been producing their resin primarily in response to attack by herbivores or pathogens.

Fourth, both the greatest number of taxa of resin-producing trees and the most copious production of resin occur today in tropical-subtropical forests. This production of large quantities of resin may partially be related to, essentially, continuously favorable abiotic conditions in these forests. The favorable living conditions in tropical environments for plant metabolism also support the great diversity and activity of phytophagous insects and pathogenic fungi that probably have exerted selection pressure on trees for defensive properties of resins, such as compositional variation and copious production. Additionally, resins that readily polymerize tend to occur in tropical and subtropical trees. The resulting hardened coating of wounds may have been selected for, since fungal infections are particularly lethal to trees in tropical forests. Thus, keeping this tropical perspective in mind may be valuable in amber studies for determining not only the plant source of the amber but the environment in which the tree may have existed as well.

Fifth, the sole use of common names for resins in reference to their botanical source can possibly produce two kinds of confusion. In some cases the names perpetuated by long commercial usage have created semantic confusion in the scientific literature and hence should be carefully and thoughtfully used—especially in drawing



botanical conclusions. Furthermore, it is important to recognize the possibility that the common name could lead to confusion regarding the botanical source as well as in obtaining mixtures of resins when purchasing samples from commercial dealers for comparative studies with amber. It is always best to make an effort to obtain a resin specimen from botanists who can document the authenticity of the sample and provide an identification (optimally with a voucher plant specimen) for its source.

Sixth, despite numerous resin-producing tree families, only a few (Leguminosae, Araucariaceae or Pinaceae and possibly Dipterocarpaceae) have definitely been demonstrated in current amber studies to have contributed substantially to amber production. The leguminous *Hymenaea* seems conclusively to be the botanical source of large deposits of mid-Tertiary Mexican and Dominican amber based on corroborative evidence from resin chemistry and inclusions of plant parts in the amber. Furthermore, this evidence supports a close relationship of the Dominican amber to the rainforest African species of *H. verrucosa* and the Mexican amber to New World rainforest species *H. courbaril*-*H. intermedia*.

The araucarian genus *Agathis* appears to have been a significant source of amber from early Cretaceous through the Tertiary in various geographic locations throughout the world, based upon chemical evidence, which is corroborated in a few cases with associated plant parts of *Agathis*. Although the botanical source of Succinite, the predominant Baltic amber, is still an enigma, the possible involvement of *Agathis*, or an *Agathis* relative, is supported by the considerable chemical similarity of Succinite and resin from extant *A. australis*, the massive accumulation of *A. australis* resin, and Asian subtropical and tropical affinities of numerous floral inclusions in the amber. However, the lack of succinic acid in extant *Agathis* and *Agathis* plant remains in the amber make this source *as such* questionable. The many pinaceous remains in the amber leads to an hypothesis that they could have been derived from an araucarian-type ancestor or common progenitor and that the araucarian chemical properties of the resin are conservative and were carried along with other characters as pinaceous plants evolved.

Although amber in a large North American coal deposit has been related to the Dipterocarpaceae, this source now may be in question. Despite chemical similarities of amber to resin from extant trees, doubt has existed for both members in the Dipterocarpaceae and *Agathis* having occurred in the Northern Hemisphere in the past because a lack of their fossil plant parts to substantiate the sources for these trees, which now are restricted to the Southern Hemisphere. Additionally, in the case of the Northern Hemisphere "dipterocarpaceous amber," the resin polymer thought to be distinctive for dipterocarps has recently been found to occur in a totally different plant family (Cornaceae). This family, however, is also not represented with amber in North America. Some evidence exists for small amounts of taxodiaceous amber but data are more limited for the Cupressaceae. The common factor for survival of all the above resins appears to be their containing either polylabdanes or polycadinenes. Resins from trees with such a capacity for polymerization retain sufficient structural character, even at high levels of maturity, to establish important characteristics regarding the origin of amber.

A unique chemistry that involves the preservation of styrene supports a Hammelidaceous source for this relatively uncommon Cretaceous and early Tertiary amber. If indeed the evidence holds for the presence of  $\alpha$ -amyrin in some Tertiary amber, a burseraceous source could possibly be supported (although other tropical families contain  $\alpha$ -amyrin). Evidence for sources from the Anacardiaceae and Styraceae have not been subject to recent evaluation. It seems possible that other botanical sources will be found in the future, as numerous small deposits of amber have not been analyzed chemically as yet. Study of these, as well as more extensive deposits, could benefit from resin analyses of many living taxa, particularly by recently available powerful chemical techniques. Furthermore, more detailed physiological and

ecological studies of modern resin-producing trees, especially those living in the tropics, may provide answers to centuries-old enigmas regarding amber.

In sum, biological knowledge regarding resin-producing trees is not only useful for determining the botanical source of amber, but in evaluating its compositional variation, factors determining the quantity produced, and the environments in which the trees lived and their relationship to depositional sites. Some caution is necessary, as always, in extrapolating data from the present to the past—and in the case of ambers from different fractions of the resin and from different organs of the tree.

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## Chapter 2

# Stable Isotope Composition of Amber

Arie Nissenbaum and Dan Yakir

Weizmann Institute of Science, Rehovot 76100, Israel

Amber, a fossil tree resin, is known in the geological record from at least the Triassic period, 220-230 million years ago, to the sub-Recent. GC/MS analyses have shown that amber is composed mainly of macromolecular structures retaining the chemical fingerprints of the original resin. Diagenetic reactions seem to have relatively minor effects on the resin, suggesting that the amber can preserve most of the isotopic signature of the original resin. Recent pine (*P. halepensis*) and araucaria resins from Israel gave stable isotope ratio values of:  $\delta^{13}\text{C} = -24$  to  $-25.7\text{‰}$ ,  $\delta\text{D} = -172$  to  $-188\text{‰}$ , and  $\delta^{18}\text{O}$  of  $+14.5$  to  $+16\text{‰}$ . Philippine copal, 400 years old, gave values of  $\delta^{13}\text{C} = -24\text{‰}$ ,  $\delta\text{D} = -236\text{‰}$ , and  $\delta^{18}\text{O}$  of  $+14.5\text{‰}$ . Fossil amber, mostly belonging to diterpenoids based polymers (Anderson's class I amber), gave  $\delta^{13}\text{C}$  values ranging from  $-19$  to  $-25\text{‰}$ ,  $\delta\text{D}$  from  $-160$  to  $-270\text{‰}$ , and  $\delta^{18}\text{O}$  of  $+16$  to  $+19\text{‰}$ . Because the isotopic composition of the original resin is influenced by the metabolic pathway of carbon fixation (for carbon) and the composition of environmental water (for oxygen and hydrogen) and presumably it is probably not strongly modified by diagenetic effects it is probable that paleobotanical, paleoclimatological and paleoenvironmental information can be potentially obtained from the stable isotope composition of the amber. In addition, the relatively small spread of the isotopic values of amber within a defined deposit, relative to the variance among isotopic values of amber from different sources, indicate the potential of amber as a stratigraphic marker as well as in identifying sources of amber in archaeological artifacts and gemology.

Amber, a fossil tree resin, has been found in the geological record of all continents, barring Antarctica, and ranges in age from at least the Triassic, 220-230 million years ago, to the Holocene, although it is particularly abundant in the Neogene (1,2). In the recent environment, resins are produced from a wide variety of plants. According to (2), about 10% of the recent plant families produce resins. Two-thirds of the resin-producing plant species are tropical, and most are angiosperms (2). Of the temperate plants, the major resin producers are conifers, particularly those belonging to the Araucariaceae and Pinaceae families.

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Humans have been interested in amber since pre-historical times. Amber is one of the oldest gems to be used (3). As a consequence of its appeal, chemical investigations of amber begun in the early years of the 19th century (4). Most of those studies used elemental analysis and physical properties such as specific gravity, hardness, and so forth, to characterize amber from different localities and to investigate its chemistry. Application of modern analytical techniques to amber is of relatively recent vintage. Because amber is largely insoluble in organic solvents, much of the work on the chemistry of amber performed in the 1980s was done by techniques that can be applied to the total solid, such as infra-red spectroscopy (5), C-13 NMR (6,7) and PC/MAS C-13 NMR (8). Those techniques provided information on the carbon functionalities of the amber. Molecular analysis by GC-MS was done on the solvent-soluble fraction (8,9,10). More recently, detailed molecular studies by several techniques led to the development of a comprehensive classification scheme for amber and fossil resins (11) which is a reflection of the family of plants which produced the resins. van Aarsen et al. (12,13) used pyrolysis-GC-MS to elucidate the structure of polycadinene diterpenoid polymers in fossil gymnosperm resins. Those studies showed that a complex mixture of diterpenoids and sesquiterpenoids is a major component of amber or, at least, of its solvent soluble fraction. The chemical composition of amber is thus controlled by two major factors, the botanical source of the resin and possible diagenetic and catagenetic alteration of the amber since its burial. Although the geological history of amber deposits is not always accurately known, the indications are that amber is found in deposits which had only relatively low thermal history. Under more severe thermal regime the amber will probably chemically crack and would not be recognized as such anymore.

The study of fractionation of the stable isotopes of carbon and hydrogen by terrestrial and aquatic plants has become an important tool for the study of their physiology and ecology (14-18). This information, coupled with isotopic studies of organic matter in soils and sediments, provides paleoclimatic information and sheds light on the source of organic matter in sediments, its diagenesis, and its conversion into commercially viable deposits such as coal, oil and gas, as well as for deciphering paleoclimates (e.g. 18,19). Recently, work on oxygen isotope fractionation in plant biomass and in oxygen-containing sedimentary organic matter, such as peat and in cellulose separated from sub-recent lacustrine sediments (20,21), as well as in cellulose from modern plants (17) indicated the potential of this isotope, in addition to carbon and hydrogen isotopes, for paleoclimatic analysis.

Very little information is available on the carbon, oxygen, and hydrogen stable isotope composition of amber. Previous work on carbon isotopes was reported on Levantine Amber (22) and a few carbon isotope analyses reported by (23) as part of a study on the C-14 ages of amber. On conceptual grounds, amber could have a range of isotopic values of carbon because the resins are produced by a variety of plant families that may differ somewhat in their chemistry and metabolic pathways and also perhaps to stress during growth (2). The deuterium and  $^{18}\text{O}$  content of amber, which may also vary according to the regional climate that controls the isotopic composition of environmental water, is also expected to show a range of values depending on the habitat of the resin-producing tree. The present study seeks to provide some baseline information on the stable isotope composition of amber from several localities and to make a comparison of amber with several recent resins.

## Materials and Methods.

Amber samples were collected in the field or were received from the British Museum collection through the courtesy of Dr. P. Whalley. Recent resin samples were collected in Rehovot, Israel. Peach gum was collected in Jerusalem, Israel. Based on the Anderson classification scheme (11) all the amber samples belong to class I with the possible exception of the Burmese amber which may belong to class II and had been produced from a Dipterocarpacea origin.



The amber and resin samples were not treated chemically prior to preparation for isotope analysis. To make the comparison between recent resins and ancient amber more valid, the resin samples were mildly heated (50 °C) under continuous pumping to remove the volatile terpenes which are largely absent in ancient amber. Carbon and hydrogen isotopic analyses were made on CO<sub>2</sub> and H<sub>2</sub>O produced by combustion of amber and resins with CuO in 900 °C in closed quartz ampoules. δ<sup>18</sup>O was determined by pyrolyzing the samples to CO by a Carlo Erba Elemental Analysis instrument model 1108 and converting the CO to CO<sub>2</sub> with I<sub>2</sub>O<sub>5</sub>. Mass spectrometric measurements were made on a Finnigan MAT-250 instrument.

The results are reported in the δ notation:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000 \text{ [‰]}$$

where R is the ratio of <sup>13</sup>C/<sup>12</sup>C, D/H or <sup>18</sup>O/<sup>16</sup>O. The standards were the PDB (Pee Dee Belemnite) standard for carbon and the SMOW (Standard Mean Ocean Water) standard for deuterium and oxygen. Samples were run in duplicates or triplicates and the precision was ± 0.2‰ for δ<sup>13</sup>C, ± 0.4‰ for δ<sup>18</sup>O, and ± 5‰ for δD.

## Results

Data on the localities from which the amber and resins were collected and their isotopic values are given in Table I. The botanical source for the amber is taken from the publications of Langenheim (2) and Nissenbaum and Horowitz (24) and references therein. The carbon isotope values ranged between -18.7 and -25.6‰ and do not show any correlation with age. In comparison with modern resins, some of the amber samples are depleted in <sup>13</sup>C although basically they are in the same range. A possible exception is the Burmese amber which, as noted before, is the only Anderson class II sample (11) and therefore represents a different biosynthetic pathway.

Deuterium values for resins show a broader range of between -21 ‰ and -270‰. Noteworthy, however, the heavy deuterium value was found only in Peach gum. The reason for the difference between this sample and the others probably relates to the difference in biosynthetic pathways between the terpene-based amber and resins and the carbohydrate-based gum. Unpublished results on deuterium in other recent gums also showed highly enriched deuterium values, supporting the evidence for metabolic enrichment of deuterium in carbohydrates (Nissenbaum, Yakir and Langenheim, in preparation). The isotopic values for the amber do not show any correlation between the different isotopes or between the isotopic values and the H/C ratio. Because, at present, the botanical source of many amber samples is either uncertain or unknown, no clear correlation between the isotopic values and the source plants could be established.

To test the isotopic homogeneity of amber in a single deposit, five different samples were analyzed from two occurrences: Mt. Hermon, Israel, and Cedar Lake, Manitoba, Canada. The deposits differ greatly in their spatial extent, ranging from 20 meters for the Hermon deposit to almost 2 kilometers in the Canadian deposit. The samples were taken as to represent different occurrences, rather than being pieces of the same specimen, and represented a complete spectrum of colors and opacities, ranging from pale yellow, completely transparent amber to almost black opaque amber. The isotope data for carbon and oxygen isotopes is given in Table II. It is clear that within these two occurrences the distribution of carbon isotopes is highly homogenous while the spread of δ<sup>18</sup>O values being somewhat larger. Another indication of carbon isotopic homogeneity in amber of the same source and age over large distances is in the report by (22), who showed that Early Cretaceous amber from four localities in Israel range in δ<sup>13</sup>C between -19.1 and -19.8‰ over a distance of

Table I. Isotopic and Geographical Data on Amber and Resin Samples Used for the Present Study  
(data is given in ‰ against PDB standard for carbon and SMOW standard for hydrogen and oxygen )

Sample	Location	Age	$\delta^{13}\text{C}$ (‰)	$\delta\text{D}$ (‰)	$\delta^{18}\text{O}$ (‰)	Assumed Botanical Source
Pine resin	Israel	Recent	-25.7	-188	+14.3	Pinaceae
Araucaria resin	Israel	Recent	-23	-172	+16.8	Araucariaceae
Peach gum	Israel	Recent	-23.9	-21		
Copal	Philippines	400 years	-24.1	-236	+14.5	Araucariaceae (Agathis)
Amber	Burma	Miocene	-18.9	-191		?
Amber (Simitite)	Sicily	Miocene	-23.5	-190		Araucariaceae
Amber (Rumanite)	Romania	Miocene	-23.3	-158		?
Amber	Dominican Republic	Eo-oligocene			+19.2	Leguminosae (Hymenaea)
Amber	NW Territories, Canada	Tertiary			+15.8	?
Amber	Mexico	Oligocene	-25.1	-175	+16.9	Leguminosae (Hymenaea)
Amber	Baltic Sea coast	Eo - oligocene	-24.6	-243	+19.5	Araucariaceae? Pinaceae?
Amber (Gedanite)	Baltic coast of Poland	Eo - oligocene	-25.6	-217		Araucariaceae? Pinaceae?
Amber	Spain	(?)	-18.7	-167		?
Amber	Southwestern Poland	U. Cretaceous	-22.9	-234		?
Amber (Chemawinite)	Manitoba, Canada	U. Cretaceous	-23.9	-272	+16.5	Araucariaceae
Amber	Hermon, Israel	Early Cretaceous	-20.2	-210	+19.7	Araucariaceae

Table II. Spread of Carbon and Oxygen Isotope Values in Two Amber Deposits

	<b>Mt. Hermon</b>		<b>Canada</b>	
	$\delta^{13}\text{C}(\text{‰})$	$\delta^{18}\text{O}(\text{‰})$	$\delta^{13}\text{C}(\text{‰})$	$\delta^{18}\text{O}(\text{‰})$
	-19.9	+15.5	-22.8	+18.9
	-19.8	+16.9	-23.4	+18.9
	-20.1	+18.7	-23.4	+16.5
	+19.1	+17.5	-21.4	+21.5
	-19.9	+19.1	-22.3	+18.8
<b>Average</b>	$-19.9 \pm 0.1$	$+17.7 \pm 1.6$	$-22.5 \pm 0.7$	$+18.9 \pm 2.0$
<b>Areal Spread</b>	<b>20 m</b>		<b>2 km</b>	

Table III. Comparison of Chemical and Isotopic Data for Fresh and Weathered Amber from Hermon, Israel

<b>Amber Sample</b>	<b>C/H</b>	$\delta^{13}\text{C}(\text{‰})$	$\delta\text{D}(\text{‰})$
Fresh	1.42	-20.2	-210
Altered Patina	1.39	-20.3	-191

150 kilometers. Also, Burleigh and Whalley (24) reported a value of  $\delta^{13}\text{C} = -24.3\text{‰}$  for Baltic amber, which is consistent with a value of  $-24.6\text{‰}$  obtained in the present study for Baltic amber (probably from a different locality on the Baltic Coast).

The effect of weathering and surficial alteration was checked by analyzing an amber sample from Mt. Hermon, Israel, that was covered with a very thin, white alteration patina which was produced by reaction with sulfuric acid, formed *in-situ*, by oxidation under surface, or near surface conditions, of reduced sulfur species in this organic-rich deposit. This patina was carefully separated and analyzed for carbon and hydrogen isotopes. The data is given in Table III. It can be seen that alteration has very little influence on the carbon isotopic values and the C/H atomic ratios. Even the deuterium, which is expected to be more amenable to isotopic exchange with water during weathering, is not affected very strongly compared with the range of values observed for the worldwide amber samples.

The isotopic values for carbon fall in the range expected for the bulk of organic matter in C3 plants (14), although they tend, on the average, to concentrate in the  $^{13}\text{C}$  enriched segment of the carbon isotope distribution histogram (Fig.1). This corresponds to the finding (25) that sesquiterpenoids and diterpenoids isolated from tertiary brown coal from the Baise Basin, China, are enriched in  $^{13}\text{C}$  by 1-2‰ as compared with the bulk coal. Other terpenoids, occurring in leaf waxes of plants, are markedly depleted in  $^{13}\text{C}$  (26), probably indicating a difference in the biosynthetic pathways, as pointed out (25). The range of measured carbon isotope values exceeds the 2 to 3‰ differences observed by (26) for intrasite variability of tree rings, which considered by those authors as the natural "noise" in evaluating the isotopic signal of  $^{13}\text{C}$  in tree-derived materials.

The observed values for deuterium correspond to the values of  $-255\text{‰}$  for the lipids and resins fraction for Bristlecone Pine from California (27), which should be compared with  $-156\text{‰}$  for whole wood, and to (28) of  $-170\text{‰}$  reported for the saponifiable lipids in California Redwood. Those values are highly depleted in deuterium as compared with the cellulose and lignin components, with the average difference being around  $-150\text{‰}$ , which reflects the general phenomena of the depletion of lipids in the heavier isotopes as compared with carbohydrates and proteins. A similar biochemical effect was observed in the present study for recent resins where the lipidic, terpene-based resins of pine and araucaria gave values of around  $-180\text{‰}$  as compared with the  $-21\text{‰}$  value for the carbohydrate-based peach gum (Table I).

The values for  $\delta^{18}\text{O}$  in the amber range from  $+14.3$  to  $+19.7\text{‰}$ . Information on the distribution of oxygen isotopes in plant organic matter is very scant and is practically limited to cellulose (15,17,20). Values for cellulose in recent plants are on the order of magnitude of  $+29\text{‰}$ , as compared with  $\delta^{18}\text{O}$  of  $+0.6\text{‰}$  in the water in the plant, suggesting a fractionation of 27-28‰. Amber is a product of a different biosynthetic pathway than that of carbohydrates, but the lack of oxygen isotope data on lipids, terpene acids or other oxygen-containing terpene-based components precludes any further evaluation, except to point out that the resin-bound oxygen is strongly depleted in  $^{18}\text{O}$  as compared with plant cellulose (17).

Comparison of the isotopic composition of amber and resins with that of recent plants and in particular, plant cellulose - the plant component on which the isotope information is the most comprehensive - is graphically presented in Fig.1. Summarizing the information shows:

1.  $\delta^{13}\text{C}$  values of amber are in the range of plant cellulose.
2.  $\delta\text{D}$  values of amber are about 100 ‰ more negative than plant cellulose. The values are in the range reported for lipids, alcohols and "non-exchangeable" hydrogen in plants. Since exchange with environmental water will result in enrichment of the amber in deuterium, the low observed values supports the

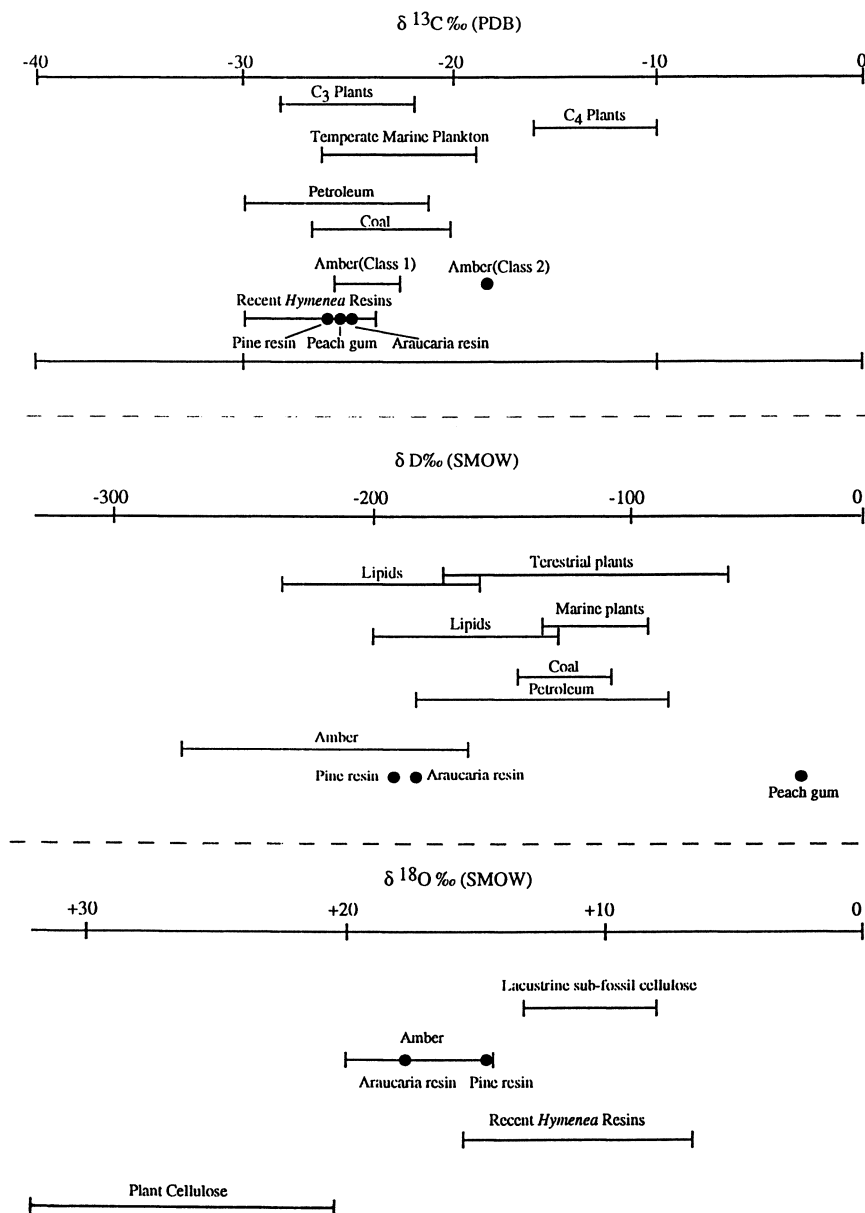


Figure 1. Distribution of Stable Isotopes of Carbon, Hydrogen, and Oxygen in Various Types of Organic Matter, Including Resins and Amber

hypothesis that diagenetic reactions do not modify much, or at all, the original deuterium signature.

3.  $\delta^{18}\text{O}$  values are about 10 ‰ more negative than plant cellulose. This possibly indicate a complete exchange during formation of the resin, with the source water for the plant and not with leaf water which are about 10 ‰ heavier as compared with stem water.
4. The biosynthetic pathways involved in resin production, and presumably in metabolic production of other lipids, produces very different hydrogen and oxygen isotopic fractionation pattern than that of carbohydrates.

## Discussion

The usefulness of the application of stable isotopes in studies of amber depends to a large degree on the preservation of the isotopic signature of the source materials without any major modification by diagenetic processes. A detailed organic geochemical study of amber and resins ranging in age from the Early Cretaceous to the present (8), reached the following conclusions with respect to the age effect on resins which has not been subjected to strong thermal stress:

1. A progressive loss of unsaturated bonds with age,
2. A decrease in functionalized groups with increasing age, and
3. An increasing proportion of aromatized groups.

Those effects, if of a general nature, indicate that diagenetic effects are relatively minor and would result mostly in second-order modification of the deuterium to hydrogen values. Moreover, the amount of hydrogen added or abstracted during diagenesis is likely to affect only in a minor way the quantity of hydrogen present in the amber and, hence, the deuterium to hydrogen ratio of the amber. Support for the small effect of diagenesis on isotopic composition can be cited from the relative constancy of the C/H atomic values in the amber (Table IV) and from the small spread in carbon and, to a lesser degree, oxygen isotopic values in the deposits from Canada and Israel (Table II). Those conclusions (8) refer to diagenetic changes only, and imply that the samples they investigated were not subjected to considerable thermal stress. This is probably true for most amber deposits where the geological evidence does not point to past deep burial which could result in catagenetic cracking and possible modification of the original isotopic signature. There is a definite need to establish the geological history of the amber samples prior to making any comparative studies.

If indeed, the isotopic signature of hydrogen and oxygen in the amber fairly faithfully conserves the original isotopic composition of the resin-forming plant, then it opens up the possibility of obtaining information on the isotopic composition of the environmental water utilized by the tree and, hence, on paleoclimatology. Although the factors that influence the isotopic composition of environmental water that are used by plants are highly complex and depend on several factors (such as the isotopic composition of the rainwater and groundwater, evaporation and relative humidity, temperature, etc.), the tree resins that give rise to amber are mostly tropical or subtropical species, where the variations in several of those parameters are minimized. In a way, this might limit somewhat the usefulness of amber as an isotopic recorder of paleoclimate in tropical areas which do not demonstrate wide variation in environmental conditions. However, much of the amber is probably produced in semi-tropical and temperate climatic zones, where environmental conditions are much more varied. Thus, it might be possible to obtain information on the paleoclimate during which the resin was formed. Clearly, to make this hypothesis more tenable, studies should be made on the isotopic composition of recent resins and their relationship with the measured isotope distribution in the water used by the tree. A hint about such a possibility is the observation that the deuterium values for the recent

Table IV. C/H Atomic Ratios of Amber and Resins

Sample	Location	Age	H/C (Atomic)
Pine resin	Israel	Recent	1.30
Araucaria resin	Israel	Recent	1.35
Peach gum	Israel	Recent	1.55
Copal	Philippines	400 years	1.34
Amber	Burma	Miocene	1.45
Amber (Simetite)	Sicily	Miocene	1.41
Amber (Rumanite)	Romania	Miocene	1.37
Amber	Mexico	Oligocene	1.35
Amber	Baltic Sea coast	Eo-oligocene	1.35
Amber	Dominican Rep.	Eo-oligocene	1.53
Amber (Gedanite)	Baltic Coast of Poland	Eo-oligocene	1.35
Amber	Spain	(?)	1.35
Amber	Southwestern Poland	U. Cretaceous	1.30
Amber (Chemawinite)	Manitoba, Canada	U. Cretaceous	1.35
Amber	Hermon, Israel	Early Cretaceous	1.41

resins from Israel are fairly similar at  $-180\%$ . Because the irrigation water at Rehovot, Israel, where the resins were collected, is around  $-10\%$ , it implies a fractionation of around  $-170\%$  between the resin and environmental waters. Recent copal from the Philippines gave  $\delta D$  value of  $-236\%$  as compared with an average  $\delta D$  in rainwater from Manila of  $-44\%$  (29), resulting in a fractionation of about  $-192\%$ . Thus, considering the range of deuterium values in nature and the difference in environment between the two locations, the two fractionation values are quite similar. In fact, a study on isotopic composition of lipids led to the claim that this class of compounds may be a better recorder of past climate than the much more widely used cellulose fraction (30).

The observed constancy of carbon isotope values in a single deposit, if it can be confirmed for other occurrences in addition to the two described in this report, can be a useful marker in stratigraphic correlation. Amber frequently occurs in the geological record as "amber belts" extending over hundreds of kilometers. Those belts are formed in a particular environment, such as tropical and semitropical areas, and the resins are carried into near-shore water bodies (from which they can be reworked). If the isotopic signature of each belt is shown to have specific values, these fingerprints could be used as a stratigraphic marker. This may be particularly important in sandstone sequences where paleontological markers are not abundant or not indicative.

Isotope data might also prove to be of importance in determining the provenance of amber in archaeological finds and in the geological column. Amber occurs widely in archaeological excavations from the Neolithic period onward. The amber in Europe was traded from the Baltic area to Central Europe, Italy, and perhaps even farther, by land, river, and sea (31). Analysis of archaeological amber artifacts might

thus be used in tracing ancient trade routes. Most of the recent work in this field was done by infra-red analysis (32), and it can be complemented by stable isotope data. The isotopic information may also have bearing in gemology, where often the origin the amber is not known or not given.

The number of data points obtained in the present study does not allow an in-depth analysis of the geochemistry of the stable isotope composition of amber, and only preliminary deductions can be made. However, there seems to be much promise in continuing this type of work, especially when combined with studies on recent resins, where the relationship between the environmental parameters and the isotopic composition of the resin might be easier to interpret.

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## Chapter 3

# Resin-Derived Hydrocarbons in Fresh and Fossil Dammar Resins and Miocene Rocks and Oils in the Mahakam Delta, Indonesia

Scott A. Stout

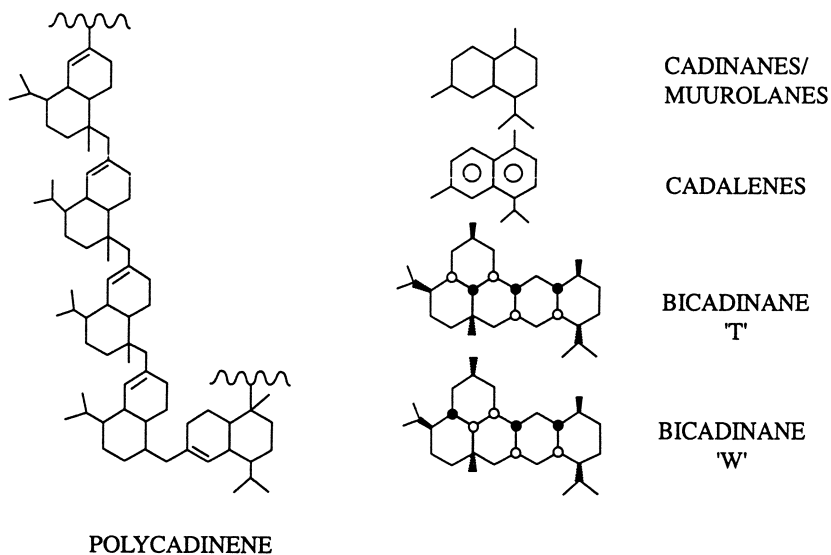
Unocal Corporation, P.O. Box 76, Brea, CA 92621

The hydrocarbons derived from fresh dammar resin are compared to those in Miocene fossil resins, resin-rich rocks and Miocene-sourced oils in the Mahakam Delta. It is shown that dammar resins undergo few chemical changes during early diagenesis. They contain 'primary' hydrocarbons (monomeric and dimeric sesquiterpanoids with a cadalene carbon skeleton), up to 16 wt. %, which can contribute directly to oil formation without passing through a polymeric kerogen intermediate. Evidence, mostly isotopic, suggests variation among bled dammar resins from different Dipterocarpaceae species exists.

Bicadinanes are absent in fresh and immature ( $R_o < 0.30\%$ ) fossil resins but form upon heating in the laboratory or subsurface. Bicadinanes first form in the laboratory over a narrow temperature range of 300-325°C (72h) and in the subsurface over a narrow  $R_o$  range of 0.30-0.35%. In the subsurface the distribution of bicadinane isomers formed varies with maturity. As such the T/(T1+R) ratio increases systematically over the  $R_o$  range 0.35 to 0.72% making it a useful molecular maturity parameter through early catagenesis. The W/T ratio increases from ca. 0.2 to 0.6 from 0.35-0.50%  $R_o$  before rapidly decreasing. An argument is made that this decrease is due to the preferential expulsion of the *cis-cis-trans* isomer (W) upon reaching the 'top of the oil window' at  $R_o$  0.50%.

Recognition by geochemists of the contribution which plant resins can make to crude oil has led to a re-assessment of the oil potential of terrestrial source rocks, including coals (*J*). Nowhere does resin's role appear to be more prominent nor widespread than in the Tertiary basins in Southeast Asia where dammar (or damar) resin, the natural resin exuded from trees of the Dipterocarpaceae family, is a common constituent of the region's petroleum source rocks.

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**Figure 1.** Proposed structure of polycadinene (left) and some of its breakdown products (right) commonly found in oils derived from source rocks containing dammar resin. (Modified from 2).

Recent studies on fresh and fossil dammar resin showed they are composed of a non-polymeric fraction containing predominantly functionalized triterpenoids and a polycadinene polymeric fraction (Figure 1; 2,3). Upon heating polycadinene disassociates yielding monomeric and dimeric cadinenes, the latter of which are thought to internally cyclize and become saturated to form characteristic C<sub>30</sub> pentacyclic triterpenoids, known as bicadinanes (Figure 1; 2,3).

The subsurface conditions under which polycadinene disassociates and subsequently forms bicadinanes is poorly constrained. In the laboratory, bicadinanes have not been formed below 300°C, and at this temperature, only fossilized resins have produced bicadinanes; fresh resins have yielded bicadinanes only after heating at 350°C (3-5). In nature, bicadinanes occur in rock extracts with 20S/(20S+20R) C<sub>29</sub> sterane ratios as low as 0.07 (6) and 0.15 (7). Lower maturity rocks were not studied therefore, the threshold for bicadinane formation is unknown.

As evidenced by the presence of bicadinanes, dammar resin is thought to have made a marked contribution to oils in SE Asia. Bicadinanes have been reported in oils from the South Sumatera, West Irian Jaya, Tarakan, and Arjuna Basins (Indonesia; 4,6,8,9), South China Sea (4,9,10), Malay Basin (9), Lower Assam Basin (India; 11), Surma Basin (Bangladesh; 7,12), Central Myanmar Basin (Myanmar; 13), SE Luzon Basin (Philippines) and Taranaki Basin (New Zealand; 6). The occurrence of bicadinanes in oils has, in some cases, expanded beyond the known geographic and stratigraphic distribution of Dipterocarpaceae as determined from paleobotanical evidence (14-16); e.g., the late Cretaceous-sourced oils in the Taranaki Basin (6). Such occurrences however, will probably become more widely reported since polycadinene-type resins have been recently reported in families outside the Dipterocarpaceae (17). It seems reasonable to assume however, that in the SE Asian oils which contain significant amounts of bicadinanes that the Dipterocarpaceae resin is present in significant amounts in the source strata.

The most commonly recognized bicadinanes in oils are trivially-referred to as compounds T and W (4), which have been identified as *trans-trans-trans* and *cis-cis-trans* isomers (Figure 1; 18,19). Numerous isomers of T and W are also often present, as well as C<sub>31</sub> homobicadinanes, two of which may be ring-methylated analogues of T and W (6). The typical distribution of bicadinane isomers in oils differs markedly from what is observed in rock extracts (6,9) and resin pyrolyzates (3-5). For example, W is almost always more enriched (relative to T) in oils than it is in rock extracts or pyrolyzates. This and other isomeric variation among the bicadinanes has been suggested to arise due to thermal maturity and/or preferential migration fractionation (9,10).

In this paper, several items mentioned above in regard to hydrocarbons (HCs) derived from dammar resins are addressed through a study of fresh and fossil resins, Miocene source rocks and Miocene-sourced oils from the Mahakam Delta area, East Kalimantan (Indonesia). First, the indigenous HCs present in six fresh and three fossil resins are described. Inter-species variation among dammar resins is cursorily-investigated and the non-polymeric fractions of the resins are described. Second, the breakdown of the polycadinene polymer and the formation of polymer-derived HCs (bicadinanes *et al.*) in laboratory pyrolyzates and in a maturation series of Miocene rocks is investigated with emphasis on the timing of bicadinane formation and changes

in their isomeric distribution. Third, the presence and character of resin-derived HCs in Miocene-sourced oils in the Mahakam Delta is demonstrated and discussed. The observed trends in the region's rock extracts and oils are described in relation to source rock maturity and inferred expulsion/migration histories.

### Samples and Methods

**Samples.** In May 1994 bled resin samples were collected from the bark of six mature trees in Wanariset Research Forest south of the modern Mahakam Delta. These represented three different genera and six different species within the Dipterocarpaceae family (Table I). All of the trees occurred within a small area (< 3 hectares). The resins were exuded from 1.5 year old wounds in the tree's bark. Fresh resin continues to exude from these wounds so that the resins collected are estimated to be approximately 3 months old. The resins were solids at room temperature and exhibited a brittle fracture which suggested that most or all of the water had been lost and that the polymerization of the resins was, if not complete, well along. These resins had a distinct resinous smell indicating that some volatiles were still present. The resins exhibited a wide range of colors (translucent to dark brown; Table I).

Large lumps of fossil resin (up to 380g) were hand-picked from three mid-Miocene lignites which outcrop south of the modern Mahakam Delta near Balikpapan. Vitrinite reflectance ( $R_o$ ) of the outcropping lignites were determined to range from 0.25 to 0.30% (Table I) indicating that the resins could be considered thermally 'immature'. The fossil resins were also brittle solids at room temperature but exhibited no resinous odor. In each instance only a single, large 'lump' of resin was selected and analyzed.

Forty-four oil and 13 rock samples were collected from boreholes in the Attaka Field region, Northeast of the present Mahakam Delta. The rock series consisted of increasingly mature Miocene coals and shales, collected from conventional and sidewall cores. These ranged in maturity from 0.26% to 0.72%  $R_o$  (Table II).

**Extraction/Separation.** Resin and rock samples were crushed and homogenized using a mortar and pestle. Any megascopically-visible plant fragments were removed from the resins prior to extraction. Resins and rocks were extracted with a boiling (38°C)  $\text{CH}_2\text{Cl}_2$ :MeOH aziotrope (93:7) for 2 hours using a SOXTEC 1045 extraction unit. The soluble fraction was deasphalted in excess (100:1) hot n-heptane forming a cloudy, white asphaltene precipitate. The n-pentane soluble portion of the extractable organic matter (EOM) was recovered and fractionated into aliphatic HC, aromatic HC and polar compounds (non-HC) using conventional normal bonded-phase HPLC procedures. Fractions were taken to dryness for quantification using a TurboVap apparatus where  $n\text{C}_{12}^+$  was maintained. Stable carbon isotopic analysis was determined on the  $n\text{C}_{12}^+$  HC fractions using conventional methods and are reported relative to PDB.

**Gas Chromatography and Gas Chromatography-Mass Spectrometry.** GC-FID analysis of the resins' and rocks' non-HC, aliphatic HC and aromatic HC fractions were obtained on HP 5890 gas chromatographs using 0.1mm x 60m DB-1 (0.1 $\mu$ ),

Table I: Description and Geochemical Data for Fresh and Fossil Resins Studied

Sample	Origin of Sample	Resin Type	Color	$P_{He}$ (unground)	$CH_2Cl_2$ : MeOH soluble wt %	$CH_2Cl_2$ : MeOH insoluble wt %	Asphalt- ene wt %	Aliph. HC wt. %	Arom. HC wt. %	Non-HC (NSO) wt. %	$\delta^{13}C$ Whole Resin	$\delta^{13}C$ Aliph. HC	$\delta^{13}C$ Arom. HC
IH03033	<i>Corylelobium</i> sp.	fresh	orange-red		86.5	13.5	21.3	2.2	5.5	57.6	-32.27	-29.93	-31.92
IH03034	<i>Shorea lamellata</i>	fresh	clear		95.1	4.9	9.9	5.4	3.6	76.3	-26.60	-27.45	-28.32
IH03035	<i>Shorea leprosula</i>	fresh	brown		46.7	53.3	27.6	1.0	1.0	17.0	-30.83	-29.23	-30.83
IH03036	<i>Shorea smithiana</i>	fresh	dark brown	1.09	13.2	86.8	5.9	0.9	0.4	6.1	-30.57	-26.72	-29.16
IH03037	<i>Shorea parvifolia</i>	fresh	tan		77.9	22.1	44.3	5.5	1.3	26.7	-31.10	-31.12	-32.43
IH03038	<i>Vatica umbonata</i>	fresh	yellow-orange		94.8	5.2	36.5	3.5	3.8	51.0	-32.26	-30.75	-32.20
IG00401	mid-Mio. coal core, $R_o$ 0.30%	fossil	yellow-orange		97.5	2.5	63.9	4.3	11.8	17.5		-27.05	-27.71
IG00477	mid-Mio. coal otcp., $R_o$ 0.25%	fossil	orange-red	1.03	95.7	4.3	61.2	2.0	4.7	27.7		-24.07	-25.73
IH02172	mid-Mio. coal otcp., $R_o$ 0.28%	fossil	tan		44.3	55.7	17.7	1.3	5.6	19.7	-26.57	-25.53	-25.76

otcp=outcrop

0.25mm x 60m DB-5 (0.1 $\mu$ ) and 0.25mm x 60m SPB-35 (0.25  $\mu$ ) fused-silica capillary columns respectively, using H<sub>2</sub> as a carrier gas. GCMS data (selected ion monitoring and full scan, i.e., 60-600 amu) were obtained using an HP 5890 GC interfaced to a VG 70-250SE MS according to previously described methods (20). Ions specific to resin-derived pentacyclics monitored during the SIM experiments included m/z 151, 191, 207, 217, 218, 367, 369, 383, 410, 412, and 426. Identification of compounds were based on comparison to published mass spectra, spectral interpretation and/or retention time comparisons. The ratios reported among compounds are based on measured peak heights on the described GC or GCMS trace.

**Microscale Pyrolysis.** Microscale sealed vessel (MSSV) pyrolysis of whole resin samples (~0.5 mg) were carried out in sealed glass tubes following previously described methods (21). Pyrolysis was performed off-line under isothermal conditions at 325°C for 72 hours. Glass tubes were subsequently cracked on-line using an MSSV-1 injector interfaced to a HP 5890 gas chromatograph. The interface temperature was 325°C. Pyrolyzates were separated using a 0.25mm x 60m DB-1 (0.1 $\mu$ ) fused-silica capillary column with H<sub>2</sub> carrier gas. Detection and identification of pyrolysis products was achieved using an FID or HP 5970 mass selective detector (MSD) operated under either full scan (50-600 amu) or SIM modes.

## Results and Discussion

A survey of fresh and fossil dammar resin chemistry is warranted since the studies to date (2-5) have focused exclusively on two commercially-available fresh dammar resins and fossil dammar resins from a single deposit in Brunei. The commercially-available fresh dammars were composites obtained from a variety of Dipterocarpaceae species so that any inter-species variation in resin chemistry would not have been observed. The variety of physical properties (e.g., color or solubility) and isotopic properties (Table I) exhibited by the fresh dammars collected for this study impressed upon me the potential for such variation. Similarly, extending the range and number of fossilized resins investigated beyond those from the Brunei deposit is appropriate.

**Solubility and Bulk Composition of Resins.** The solubility of the fresh and fossil dammar resins studied varied widely and no relationship between 'fresh' versus 'fossil' was apparent. Between 13 and 95 wt% of the fresh and 44 to 96 wt% of the fossil resins were soluble in CH<sub>2</sub>Cl<sub>2</sub>:MeOH (Table I). The insoluble fraction of the resins (by definition, kerogen) is considered to be a high molecular weight (HMW) polymer, e.g., polycadinene (Figure 1). A considerable fraction of the soluble materials was asphaltenic and therefore, also considered to be polymeric. Together the two polymeric fractions made up between 15 wt% (*S. lamellata*) and 93 wt% (*S. smithana*) of the fresh resins while the range among the fossil resins studied was smaller (66-73 wt%). The ranges observed suggests that the degree of polymerization and/or cross-linking can vary widely among fresh dammar resins and that neither necessarily increase upon fossilization.

The largest fraction of the lower molecular weight (LMW) material extractable from fresh and fossil resins were non-HCs (Table I). These comprised 6 to 76 wt%

and 18 to 28 wt% of the fresh and fossil resins respectively. Again, there is no clear distinction that can be made between the fresh and fossil resins. The GC-amenable non-HC components for all of the resins were comprised of an assortment of functionalized bicyclic sesquiterpenoids and triterpenoids including triterpen-ones, -oic acids and -ols with dammarane, oleanane and ursane skeletons. This is consistent with the previous study by Van Aarssen et al. (2) of the  $\text{CH}_2\text{Cl}_2$  and MeOH soluble fraction of a fresh dammar resin composite. A significant loss of oxygen-containing functional groups from triterpenoids in the fossil resins has not occurred at these low maturities (0.25-0.30%  $R_o$ ; Table I). No further work was performed on the non-HC components in this study although it should be stated that upon further diagenesis these compounds could be important precursors to higher plant (non-hopanoid) triterpanes in oils derived from resinous source rocks.

LMW HCs comprised 1-9 wt% and 7-16 wt% of the fresh and fossil resins respectively. Thus, a significant proportion of indigenous or 'primary' HC (i.e., oil) can exist in dammar before the resin ever enters the sediment and this can further increase upon fossilization (Table I). At a maturity of only 0.30%  $R_o$  up to 16 wt% of a resin body is comprised of 'oil'. That is to say that, even prior to the thermal disassociation of the polymeric fraction of the resin there already exists a significant proportion of HCs which can contribute directly to oil formation (i.e., no kerogen/polymeric intermediate). This would seem to be a volumetrically-important source of HCs from strata containing significant amounts of dammar resins. It also opens the possibility of generation and expulsion of these HCs at less than conventional maturities.

### **Characterization of Hydrocarbons Indigenous to Dammar Resins.**

Characterization of the 'primary' HCs present in resins is important since these compounds can contribute directly to oil formation. Previous investigations on dammar resin chemistry (2-5) have not distinguished between indigenous HC and those produced upon heating.

Representative GC traces for the aliphatic and aromatic HC distributions for a fresh and fossil resin are shown in Figures 2 and 3 respectively. The dominant aliphatic HCs in both the fresh and fossil resins are  $\text{C}_{15}$  bicyclic sesquiterpanoids and their  $\text{C}_{30}$  dimers (Figure 2). The sesquiterpenoids present ( $M^+$  208, 206, 204) contained zero to two double bonds and isopropyl groups (strong  $M^+$ -43 peaks) suggesting a cadalene carbon skeleton. Sesquiterpenes were the predominant monomers in both fresh and fossil resins studied. However, up to eight sesquiterpanes, presumably cadinane/muurolane isomers (2), were present in the fossil resins but were absent from the fresh resins. This difference indicates that saturation of some double bonds occurs during fossilization which is consistent with other studies on fossil resins (22). Homocadinene(s) ( $\text{C}_{16}\text{H}_{28}$ ) and homocadinane(s) ( $\text{C}_{16}\text{H}_{30}$ ) were not observed in either the fresh or fossil resins.

The dimers ( $M^+$  406, 408, 410) consist predominantly of  $\text{C}_{30}\text{H}_{48}$  compounds which, with strong  $m/z$  201-205,  $m/z$  159-163 and  $m/z$  365-367 fragments, are probably dimeric cadinenes (Figure 2). (Whether bicadinenes are among these compounds cannot be confirmed nor refuted on the basis of the mass spectra). These dimers have been observed in resin pyrolyzates but not in solvent extracts (2,3). The



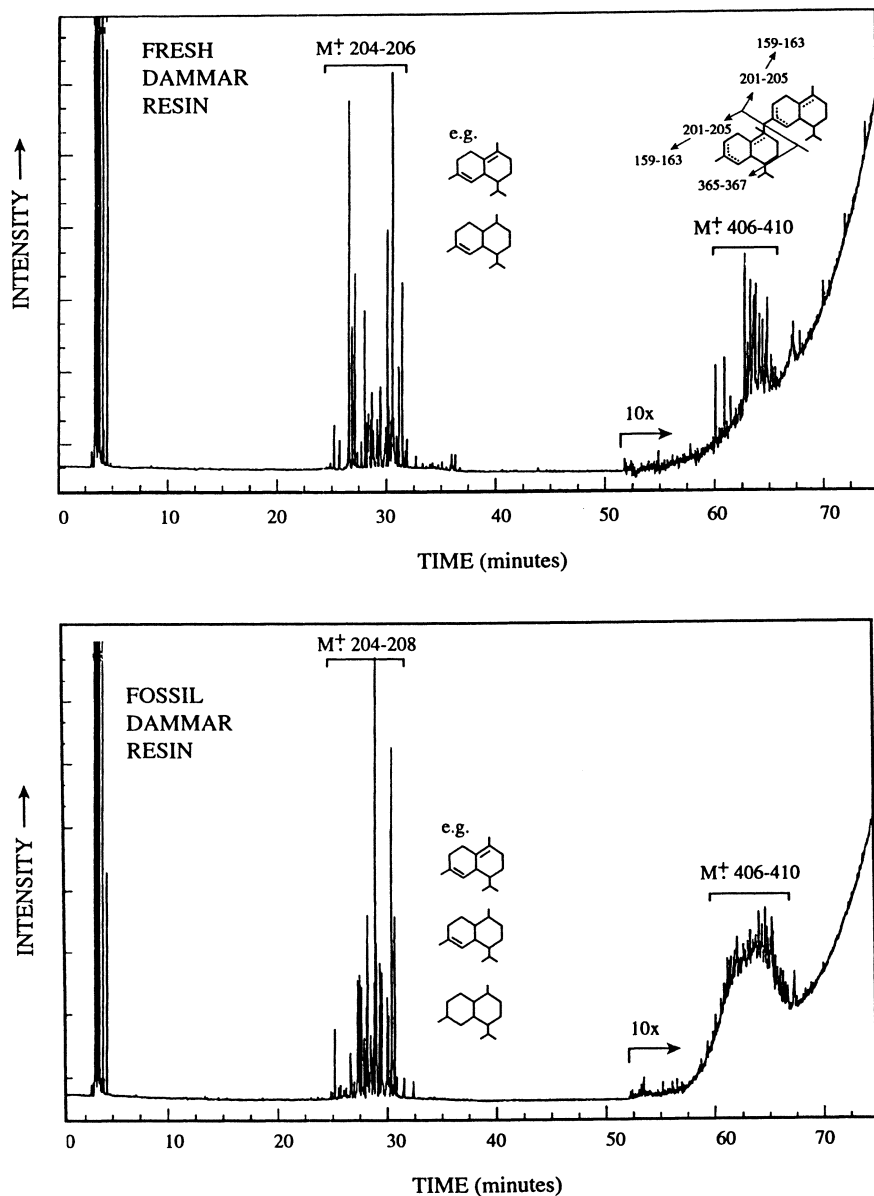


Figure 2. Gas chromatograms showing 'primary' aliphatic hydrocarbons extracted from fresh (1H03033) and fossil (1H02172) dammar resins.

saturated equivalents of these dimers (i.e., seco-bicadinanes) have been observed in some SE Asian oils (9).

Upon cyclization and saturation these dimeric cadinenes could form bicadinanes. However, no bicadinanes were present in the extracts of the fresh nor fossil resins. The absence of bicadinanes in these 'immature' resins, in spite of the presence of the dimeric cadinene precursors, indicates that the conditions for cyclization and saturation have not yet been reached in the fossil resins studied ( $R_o$  0.25-0.30%). The cadinene dimers' presence in both the fresh and fossil resins extracts suggests that some polycadinene "building blocks" may survive fossilization without being incorporated into a polycadinene-type polymer. Furthermore, their presence suggests that some bicadinanes (and seco-bicadinanes) may form directly from these non-polymeric LMW compounds and not only from polycadinene as previously suggested (2).

The aromatic HCs extracted from the fresh and fossil resins consist predominantly of compounds structurally-related to cadalene (Figure 3). Calamanene (1,2,3,4-tetrahydrocadalene) was consistently present in greater abundance than 5,6,7,8-tetrahydrocadalene supporting preferential loss of hydrogen from the B-ring (over A-ring) as suggested previously by Van Aarssen et al. (2). Abundant and ubiquitous aromatic compounds in the fresh resins included numerous  $C_{15}H_{24}$  compounds ( $M^+$  204) which based on their mass spectra (strong  $m/z$  161 and 119 fragment ions) are interpreted to be 4-alkyl-toluenes (i.e., 1-methyl-4-(1,5-dimethyl)-hexanes; see Figure 3). Several  $C_{18}H_{32}$  ( $M^+$  248) and  $C_{21}H_{36}$  ( $M^+$  288) compounds, also suspected to be alkyl-toluenes, are sometimes present in the fresh resins studied. Smaller relative concentrations and fewer isomers of the alkyl-toluenes are present in the fossil resins (e.g., starred compounds in Figure 3). The scarcity of these compounds in the fossil resins suggests that during fossilization most of the alkyl-toluenes have cyclized (forming calamanene and 5,6,7,8-tetrahydrocadalene) and further dehydrogenated (forming cadalene). These two-ring aromatics are predominant components in many oils (see below) and their presence in the fossil resin extracts suggests they can also form at low maturities directly (and not only upon the disassociation of polycadinene).

Various aromatic dimers are present in both the fresh and fossil resin extracts. The fresh resin contains a series of monoaromatic dimers with  $M^+$  408 and major fragments ions of  $m/z$  207, 202, 161 and 151 (e.g., Figure 3). Other unknown compounds, presumed to be dimers, have  $M^+$  368, 396 and 424 amu's. The predominant dimers present in the fossil resins are diaromatics with  $M^+$  376 and major fragments of  $m/z$  145 and 158 indicating the presence of two linked monoaromatic bicyclics (e.g., Figure 3). A greater degree of aromatization among the fossil resin extracts is to be expected since dehydrogenation of some compounds typically accompanies diagenesis.

In spite of an overall similarity in the types of indigenous HCs in the fresh resins from different species their relative distributions did vary. Figure 4 shows the distribution of aliphatic monomers ( $m/z$  204+206), aliphatic dimers ( $m/z$  406+408+410) and aromatic monomers ( $m/z$  198+200+202+204) for three of the fresh resins studied. The distribution of each of these compound types varies among the resin obtained from different species. The implication of these differences is

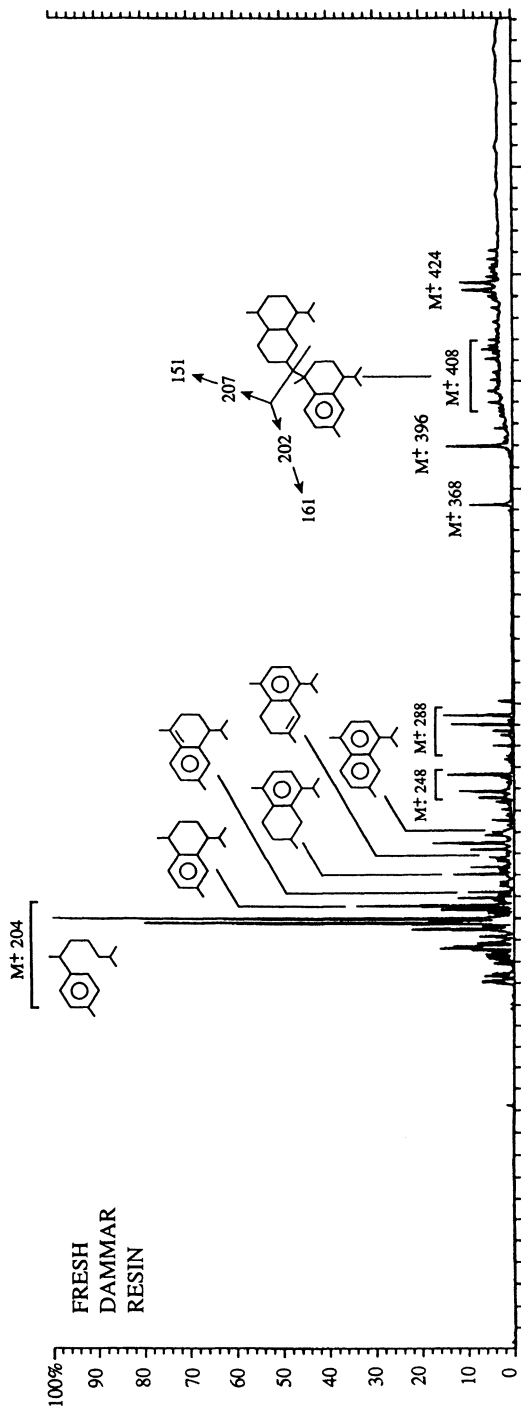
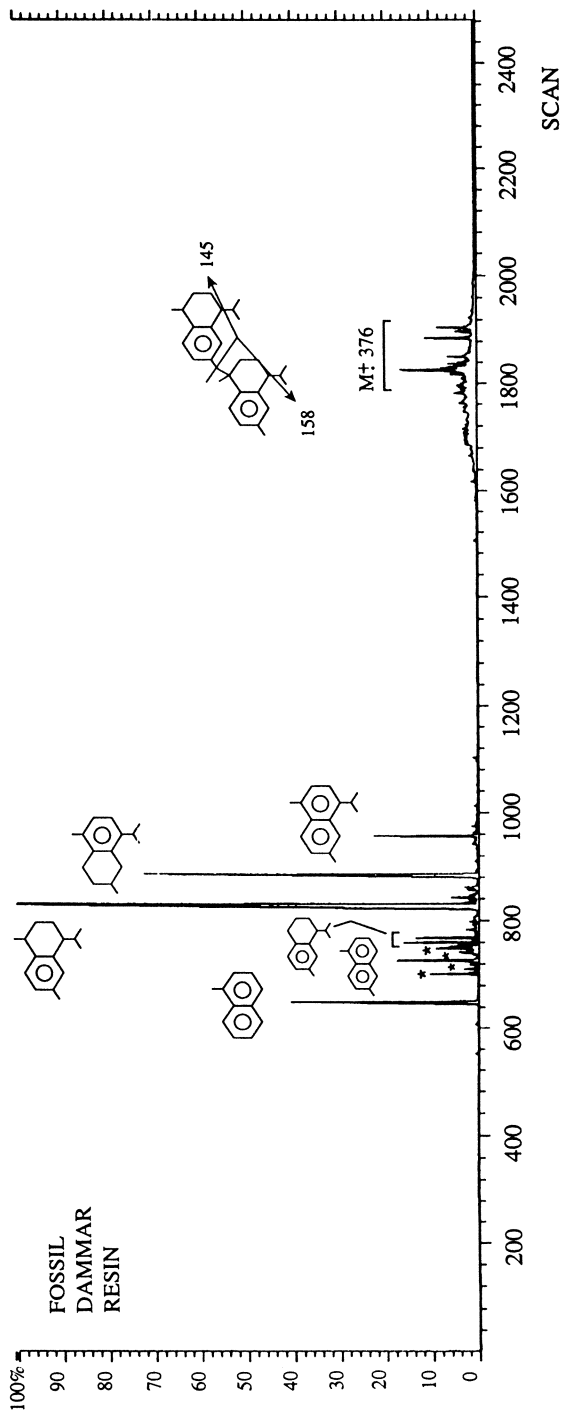


Figure 3. Total ion chromatograms showing 'primary' aromatic hydrocarbons extracted from fresh (IH03033) and fossil (IH02172) dammar resins.

Figure 3. *Continued*

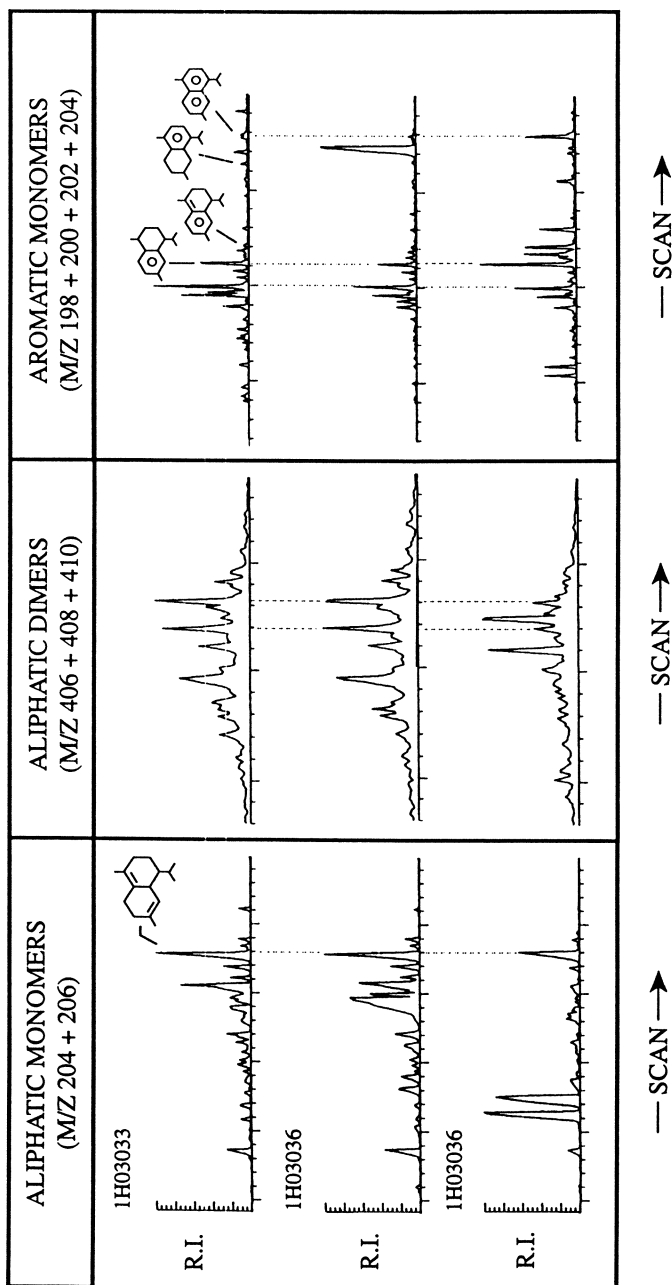


Figure 4. Partial mass chromatograms showing the distributions of aliphatic monomer ( $m/z$  204+206), aliphatic dimers ( $m/z$  406+408+410) and aromatic monomers ( $m/z$  198+200+202+204) extracted from three fresh dammar resins from different Dipterocarpaceae species.

currently unknown but the potential for some genetic control on the biosynthesis of these polycadinane “building blocks” is a possibility. This possibility may be supported by the  $\delta^{13}\text{C}$  ratios for the bulk resins, aliphatic HC and aromatic HC fractions for the fresh resins which varied up to 5.66, 4.40, and 4.11 ‰ respectively (Table I). The carbon isotopic ratio of the bulk *S. lamellata* resin was particularly unique among those studied but it is comparable to the only other  $\delta^{13}\text{C}$  ratio reported for fresh dammar resins (-26.5‰;5). Such a wide range is very surprising (among genetically-related trees growing in the same area) and suggests that (1) different biosynthetic processes which fractionate carbon may exist in the different species or (2) different carbon sources (e.g.,  $\text{CO}_2$  absorbed through leaves vs.  $\text{HCO}^-$  absorbed through roots) may precede the synthesis of the dammar resin by different Dipterocarpaceae species. Notably,  $\delta^{13}\text{C}$  ratios of HCs in the fossil resins can also vary significantly (up to 2.98 ‰; Table I). These were generally isotopically heavier than the fresh resins which could reflect differences in the Recent and Miocene carbon pool. A Miocene Brunei fossil resin was also quite heavy (-26.2 ‰;5). However, the potential for species control on isotopic variation cannot be ruled out.

The geologic significance of the differences in HC distributions (Figure 4) and carbon isotopic composition (Table I) among fresh resins is probably small. Much more work is required before conclusions can be drawn on the specificity of different resin types and oils derived from them. Nonetheless, such differences testify to the variability which potentially exists among different ‘types’ of dammar resins.

**Characterization of Hydrocarbons Generated from Dammar Resins.** In this section the HCs formed from dammar resins upon laboratory and subsurface heating are described and compared. Special consideration is given to the timing of formation of bicadinanes and their isomeric distribution.

**Heating in the Laboratory.** GC-FID traces of representative fresh and fossil dammar resins pyrolyzed at 325°C for 72 hours are shown in Figure 5. In each case, the major pyrolyzates formed were compounds related to the  $\text{C}_{15}$  cadalene-type monomers observed in the extracts (Figures 2 and 3). Among these were cadinane(s), cadinenes with one degree of unsaturation, alkyl-toluenes, mono- and diaromatic cadinanes (i.e., cadalene) and the *des*-isopropyl equivalents of all of these compounds (Figure 5). Among the latter, 1,6-dimethylnaphthalene was typically the most abundant pyrolysis product indicating that the loss of the isopropyl group is thermally-controlled. There was a great similarity among the type of cadalene-type compounds in the pyrolyzates formed from all of the resins although, like the extracts, their distributions varied slightly. The similarity between the fresh and fossil pyrolyzates testifies to the minimal changes which resin typically experiences during fossilization (early diagenesis).

Most prominent among the HMW compounds formed during pyrolysis of both fresh and fossil resins were  $\text{C}_{30}$  bicadinanes. Bicadinanes W, T and R were identified on the basis of their mass spectral characteristics, relative retention times and by co-injection of oil containing bicadinanes. Mass chromatography revealed the presence of additional compounds also suspected to be bicadinanes (Figure 5, *inset*). The most abundant of these have been labeled W1 and T1 in accordance with Murray et al. (6;

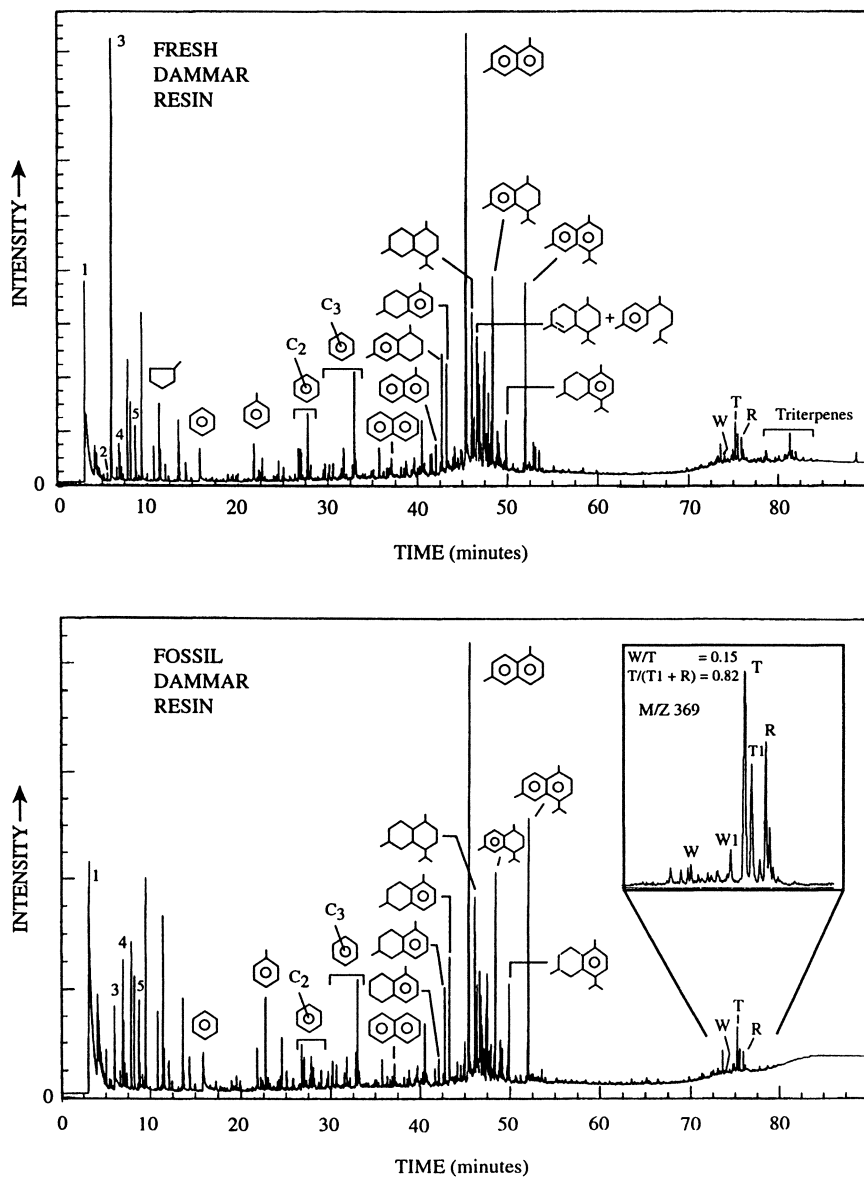


Figure 5. Gas chromatograms for pyrolyzates (325°C/72h) from fresh (1H03033) and fossil (1H02172) dammar resins. Inset shows partial mass chromatogram ( $m/z$  369) showing the bicadinanes produced upon pyrolysis.

who believe them to be *cis-cis-trans* and *trans-trans-trans* isomers similar to W and T, respectively). Homobicadinanes were not observed in any of the pyrolyzates.

Other HMW compounds observed in the pyrolyzates include numerous C<sub>30</sub> triterpenes with one degree of unsaturation (Figure 5). Most of these compounds are thought to have oleanoid and ursanoid skeletons and to be produced upon defunctionalization of the pentacyclic triterpen-ones, -ols, and -oic acids which dominated the extractable, non-HC fraction of the resins (see above). The occurrence of bicadinanes among these triterpenes could not be confirmed or refuted on the basis of mass spectra. In several resin pyrolyzates earlier eluting compounds with spectra characteristic of sesquiterpane dimers (as were observed in the extracts; Figures 2 and 3) were also abundant (see Figure 6 below).

The formation of bicadinanes from fresh dammar resin at 325°C (72h) in the laboratory is notable. Previous pyrolysis experiments on fresh dammar resin at 300°C (72h) did not produce bicadinanes while pyrolysis at 350°C (72h) did (3,5). Thus, the lowest temperature at which bicadinanes are produced from fresh resin under these laboratory conditions lies somewhere between 300 and 325°C. As with most chemical reactions temperature is not the only factor. Duration of heating is also important and evidence for this was obtained when no bicadinanes were produced when the fresh resins were heated to 325°C for only 10 minutes. The narrow range of temperature at which bicadinanes form in the laboratory suggests that they form over a similarly narrow range of subsurface conditions. This is investigated below.

The distribution of bicadinanes formed during pyrolysis of the fresh resins at 325°C is also of relevance. Figure 6 reveals, as had been previously observed upon pyrolysis at 350°C (3,5), that compound T was consistently the most abundant bicadinane formed during pyrolysis of fresh resin. Compounds W, W1, T1, and R were formed in near equal proportions relative to each other and to T. This overall consistency among the different resin pyrolyzates suggests that the formation of the different bicadinane isomers is not random. Their distribution is probably a function of the thermal stability of the various isomers and is likely to be kinetically-controlled. Heating to 325°C for 72 h favors formation of the *trans-trans-trans* (T) isomer over the T1 and R isomers, and T1 and R over W1, and W1 over *cis-cis-trans* (W).

Some bicadinane-related ratios that recently have been suggested to vary with increasing thermal stress (6) are very consistent among both the fresh and fossil resin pyrolyzates (Figures 5 and 6). For example, the T/(T1+R) ratio for the fresh and fossil resins ranges from 0.77-0.99. The similarity between the fresh and fossil resins is somewhat surprising if one assumes that the fossil resins have experienced at least some level of thermal stress. However, as indicated by the extract data above, the level of stress experienced by these fossil resins has not initiated the bicadinane formation process.

**Heating in the Subsurface.** To investigate the conditions for the formation of bicadinanes in nature and any changes in their isomeric distributions with maturity a series of 13 rock extracts from increasingly mature rocks which ranged from R<sub>0</sub> 0.26 to 0.72% in the offshore Mahakam Delta region were examined (Table II). Mass chromatograms (m/z 369) for the aliphatic HC fraction of four of these rock extracts are shown in Figure 7. Bicadinanes were first observed in a rock with a R<sub>0</sub> of 0.35%.



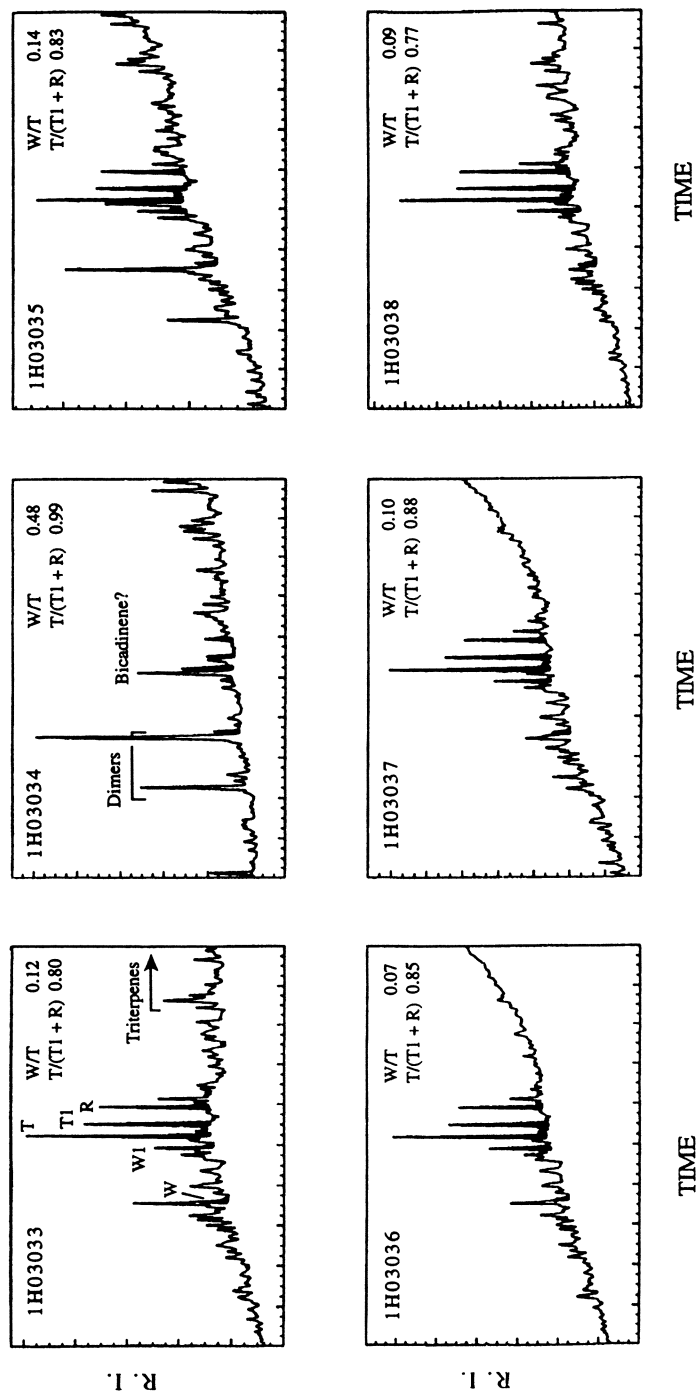


Figure 6. Partial gas chromatograms for pyrolyzates (325°C/72h) of fresh dammar resins from different species showing bicadinane distributions.

Table II: Geochemical Data for a Maturation Series of Rocks from the Offshore Mahakam Delta

LIMS	Lithology	TOC	EOM/ TOC	% Aliph. HC	% Arom. HC	% Non- HC	HI	R <sub>o</sub> (%)	C <sub>31</sub> hopane 22S/(22S+22R) <sup>1</sup>	C <sub>29</sub> sterane 20S/(20S+20R) <sup>2</sup>	W/T <sup>3</sup>	T/TH+R <sup>2</sup>
1H02940	coal	21.87	891	8.5	14.6	76.9	169	0.26	0.11	ndp	ndp	ndp
1H02175	coal	59.24	1707	9.3	21.4	69.3	231	0.30	0.14	0.05	ndp	ndp
1G00460	coal	53.4	240	21.8	18.2	60.0	166	0.35	0.09	ndp	ndp	0.61
1H02937	coal	52.08	870	14.4	22.9	62.7	261	0.36	0.13	0.05	0.22	1.35
1H02942	coal	33.12	1185	15.7	23.0	61.3	187	0.38	0.16	0.05	0.19	1.40
1G00372	coal	59.2	801	42.8	17.7	38.9	162	0.42	0.12	0.09	0.40	1.54
1H00124	shale	nd	nd	nd	nd	nd	nd	0.43	0.27	0.09	0.32	2.43
1G00398	shale	1.09	2569	55.5	16.3	28.2	121	0.46	0.27	0.13	0.45	2.15
1G00405	coal	54.9	1342	41.6	27.4	26.5	202	0.48	0.25	0.17	0.58	2.75
1H00332	shale	nd	nd	nd	nd	nd	nd	0.52	0.47	0.21	0.35	2.95
1G00361	shale	1.19	1286	27.4	43.3	29.3	173	0.59	0.55	0.34	0.32	3.05
1H02170	coal	79.87	103	23.0	46.7	30.3	327	0.63	0.59	0.38	0.29	3.55
1G00412	shale	5.38	1946	35.8	32.9	31.3	247	0.72	0.56	0.48	0.31	4.40

nd = not determined

ndp = no determination possible

<sup>1</sup>Based on m/z 191 peak heights of 17 $\alpha$ (H),21 $\alpha$ (H) C<sub>31</sub> hopane isomers.

<sup>2</sup>Based on m/z 217 peak heights of 14 $\alpha$ (H),17 $\alpha$ (H) C<sub>29</sub> sterane isomers.

<sup>3</sup>Based on m/z 369 peak heights of bicadinane isomers.

This is well below the  $R_o$  0.44% at which they were observed in rocks from the Surma Basin, Bangladesh (7). The  $C_{31}$  22S/(22S+22R) hopane ratio in this extract was only 0.09, also well below the value at which bicadinanes were observed in extracts from Visayan Basin, Philippines (*ca.* 0.20; 6).

The appearance of bicadinanes at *ca.*  $R_o$  0.35%, and their absence at slightly lower maturities (including the hand-picked resins;  $R_o$  0.25-0.30%; Table I), indicates that bicadinanes first form in the subsurface between  $R_o$  values of 0.30 and 0.35%. This is well below what is conventionally thought of as necessary maturities for oil generation. Assuming most bicadinanes form from the breakdown of the polycadinene polymer (2) implies it is far less stable than the polymers comprising most kerogens. However, it is important to recall that LMW bicadinane precursors occur in resins throughout early diagenesis, e.g., the dimeric cadinenes in Figure 2. Therefore, it is possible that some of these earliest-formed bicadinanes are not necessarily derived from breakdown of polycadinene but simply from the cyclization and saturation of these dimers.

At their first appearance the most abundant bicadinane formed is the R isomer while only a trace amount of the T isomer and no T1, W1 or W isomers are formed (Figure 7). This distribution contrasts the pyrolysis results (Figures 5 and 6) suggesting the laboratory heating experiments were too severe to observe  $R > T$ . The R isomer apparently is less thermally-stable than T at all but the lowest maturities. The T1 and W isomers first appear, and the proportion of T increases markedly and rapidly, at *ca.*  $R_o$  0.42%. Above this level of maturity compound T is consistently the most stable bicadinane isomer.

Compound T1 is not nearly as (relative) abundant in the rock extracts as it was in the pyrolyzates (Figures 5 and 6). Furthermore, compound W1 was not observed in any of the rock extracts whereas it was common in the pyrolyzates (Figures 6 and 7). These differences indicate that the formation of bicadinane isomers in the laboratory does not wholly mimic their formation in the subsurface (where catalytic reactions may occur).

With further increases in maturity (*ca.*  $R_o$  0.48%) the proportion of W increases and the proportion of R decreases, both relative to T (Figure 7). At higher maturities (*ca.*  $R_o > 0.50\%$ ) there is a decrease in the proportion of W relative to T while the proportion of R continues to decrease. The implications of the changes observed in the isomeric distributions with increasing maturity are discussed following a brief review of the region's oil chemistry.

### **Bicadinanes and Other Resin-Derived Hydrocarbons in Mahakam Delta Oils.**

Previous descriptions of the molecular characteristics of oils from the region have demonstrated that there is a remarkable similarity among the organic facies in the region's source rocks (23). The oils studied from Attaka Field bear this out and testify to a predominance of terrestrial organic matter derived, in large part, from flowering plants, including Dipterocarpaceae in the region's source rocks. In this paper, the character of the resin-derived HCs only are emphasized.

Figure 8 shows the whole oil GC of a typical Attaka Field oil. These oils are dominated by n-alkanes which range up to  $nC_{36}$ . Pr/Ph ratios of the 44 oils studied averaged 4.2 (range: 2.1 to 6.1) and Pr/ $nC_{17}$  ratios averaged 1.15 (range: 0.62-1.52).

Numerous cadalene-type sesquiterpanoids and bicadinanes can be observed even among the n-alkanes (Figure 8). Among the sesquiterpanoids are several aliphatic and aromatic monomers such as those found in the extracts of immature resins (Figures 2-4). This serves to emphasize the potential for 'primary' HCs, indigenous to dammar resins, to contribute directly to oil formation. Among these compounds, saturated cadinane/muurolane isomers, cadalene and 1,6-dimethylnaphthalene are typically most abundant. Mass chromatography has tentatively identified at least four cadinane/muurolane isomers ( $m/z$  208 and 165) and two homocadinane isomers ( $m/z$  222 and 179) in Attaka oils and similar compounds have been observed in other SE Asian crude oils (9). Notably, cadalene and 1,6-DMN consistently dominate the  $nC_{12}^+$  aromatic HC fractions of these oils.

Typical triterpane and sterane distributions for the Attaka oils are shown in Figure 9. Both traces reveal the occurrence of numerous bicadinanes. Compound T is always the most abundant bicadinane in the Attaka oils. The ratio of W/T (as determined from the  $m/z$  217 trace) averages 0.55 but ranges from 0.38 to 0.73 (see below). Unfortunately, the relative abundance of the other bicadinanes (e.g., T1 or R) in the  $m/z$  217 trace is difficult to assess due to co-eluting steranes. Mass chromatographic data specific for bicadinanes ( $m/z$  369) was not typically available, therefore, the T/(T1+R) ratio could not be determined for the oils. Oleanane and several unidentified (non-hopanoid) pentacyclic triterpanes, all associated with an angiospermous higher plant origin, are also prominent in the Attaka oils (Figure 9). As shown above, functionalized precursors to these tripterpenoids have been shown to occur in the non-HC fractions of the resins. Thus, at least some of these unidentified triterpanes may also have a dammar resin origin. However, the ratios of oleanane/(T+W) or  $(X_1+X_2)/(T+W)$  (as determined in the  $m/z$  191 trace) ranged widely among the oils of comparable maturity suggesting that the triterpenoids and bicadinanes are not derived from a single precursor. It is suggested therefore that oleanane and the unidentified triterpanes (X in Fig. 9) are derived from multiple Angiosperm sources of which the Dipterocarpaceae is only a subset.

The typical distribution of bicadinane, homobicadinane and dimeric cadinane isomers (i.e., seco-bicadinanes) in Attaka Field oils are shown in Figure 10. As noted above, compound T, followed by compound W, are always the most abundant bicadinanes in the oils. However, compounds T1, R and at least eight other bicadinanes are typically present but in much smaller proportions. The homobicadinanes are always dominated by the compound eluting immediately before bicadinane R. This homobicadinane is visible in the  $m/z$  191 mass chromatogram and, based on its mass spectra, is thought to be a ring-methylated analogue of compound T (6). Seco-bicadinanes are present in much smaller relative concentrations than bicadinanes. The origin of these compounds would seem to be saturation of unsaturated cadinane dimers as were present in the resin extracts (Figure 2).

**Possible Implications of Bicadinane Distributions on Maturity and Migration.** The changes in isomeric distribution of bicadinanes in rock extracts with increasing maturity described above (Figure 7) are consistent with data recently published for a series of rocks from the Visayan Basin, Philippines (6). Murray et al. (6) showed that the T/(T1+R) and  $C_{29}$  sterane 20S/(20S+20R) ratios increased proportionately with

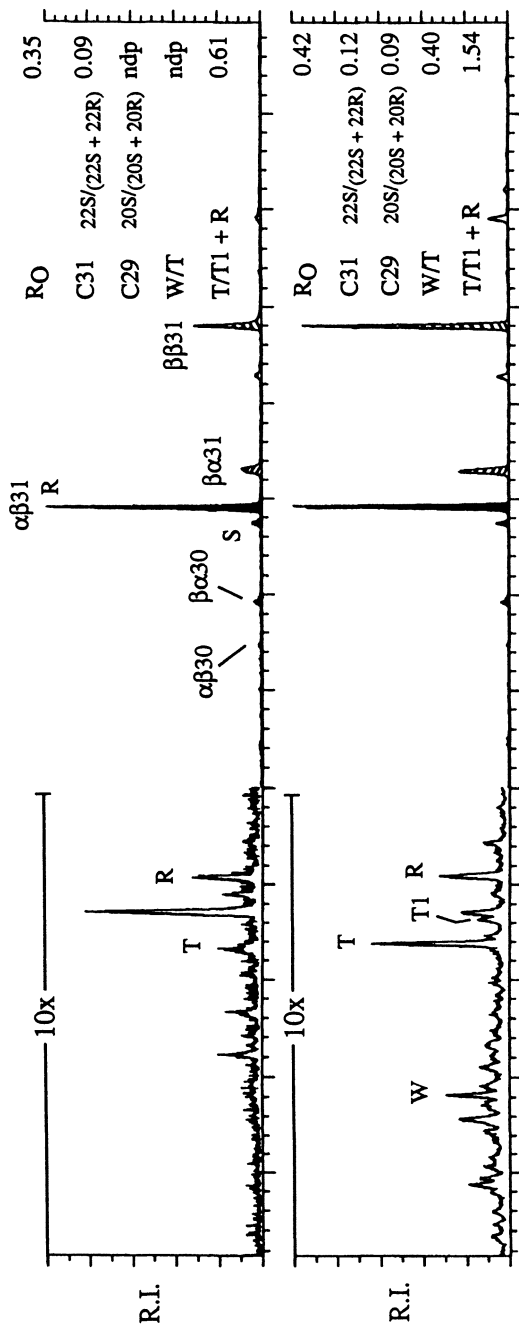
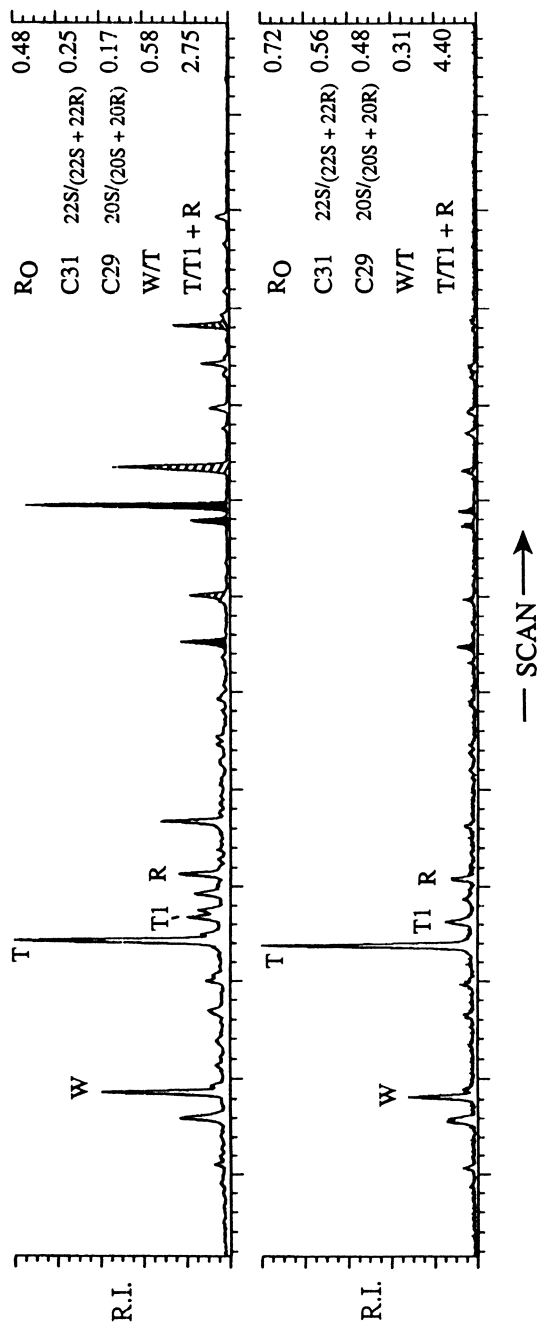


Figure 7. Partial mass chromatograms ( $m/z$  369) for four rock extracts from increasingly mature rocks showing changes in the distribution of bicadinane isomers. Samples are: 1G00460, 1G00372, 1G00405 and 1G00412 (top to bottom).

Figure 7. *Continued*

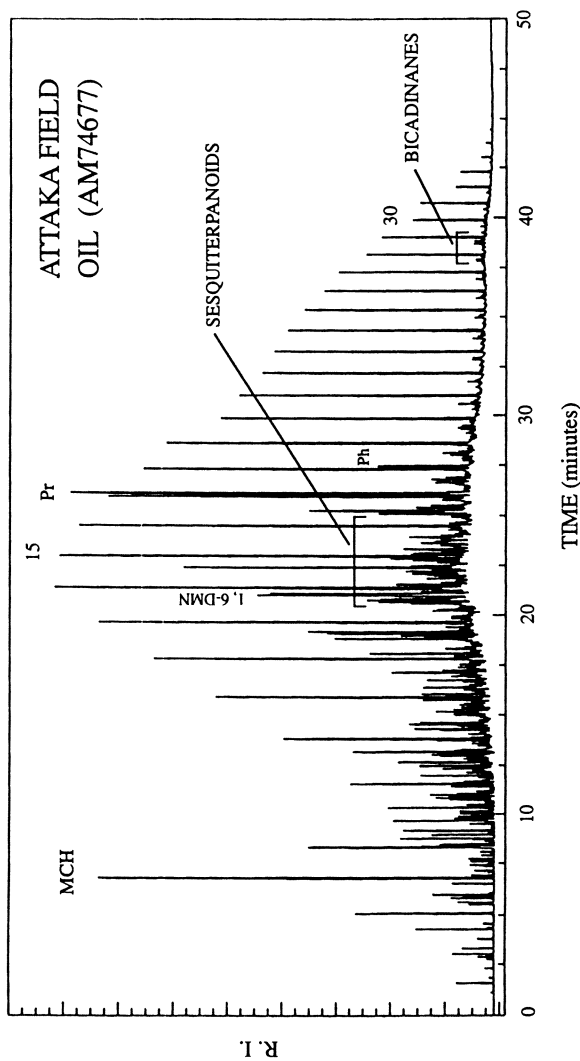


Figure 8. Whole oil gas chromatogram for an Attaka Field oil.  
MCH=methylcyclohexane, 1,6-DMN=1,6-dimethylnaphthalene.

increasing maturity implying that T1 and R are less stable than T. The increase in the T/(T1+R) ratio for the Mahakam Delta rocks studied is consistent with this interpretation (Table II). For these rocks T/(T1+R) is shown to increase systematically with vitrinite reflectance ( $R_o$ ),  $C_{31}$  hopane S/S+R and  $C_{29}$  sterane S/S+R values (Figure 11A-C). As best as can be determined, the correlation between T/(T1+R) and  $C_{29}$  S/S+R is quite consistent for the Mahakam Delta and Philippine datasets. However, whereas T/(T1+R) is shown to increase over the entire range of maturity for the Mahakam dataset (reaching values as high as 4.4 at  $R_o$  0.72%; Figure 11), the Philippine rock series indicated that T/(T1+R) ratio reached 'equilibrium' at values *ca.* 2.7-3.0 (see Figure 8 in Ref. 6). The cause for this discrepancy is unclear, nonetheless, the Mahakam Delta dataset suggests that the T/(T1+R) ratio does not reach 'equilibrium' and is useful molecular maturity parameter over a wider range of maturities than previously thought.

Van Aarssen et al. (2,10) and Murray et al. (6) have also suggested that the ratio of W/T may also increase with maturity since oils were observed to have significantly higher W/T ratios than resin pyrolyzates (4,5) or immature rock extracts (7). This possibility was supported by the Philippine rock dataset described above which showed the *cis-cis-trans* isomers (W and W1) increase relative to the *trans-trans-trans* isomers with increasing maturity (T, T1 and R;6). Van Aarssen et al. (2,10) also suggested an alternative explanation for the higher W/T ratios observed in oils was the preferential migration of a *cis-cis-trans* isomer (W) over a *trans-trans-trans* isomer (T) leading to the enrichment of W in oils over rocks. (Preferential migration of the 'less flat' *cis-cis-trans* isomer would be predicted due to its lower potential for van der Waals' bonding to clays). The Mahakam Delta rock series may exhibit evidence that both maturity and preferential migration effect the relative proportion of the *cis-cis-trans* and *trans-trans-trans* bicadinane isomers.

Figure 11D-F show that the W/T ratio increases from *ca.* 0.2 to 0.6 over the maturity range of  $R_o$  0.35 to 0.50% (or  $C_{29}$  S/(S+R) between 0.05-0.20). If only thermal processes were at work one would expect W/T to continue to increase with maturity. However, above  $R_o$  of 0.50% (or  $C_{29}$  S/(S+R) of 0.20) there is a rapid decrease in the W/T ratio of the rock extracts to values *ca.* 0.35. The cause for the observed trend is unknown but one possibility is that the thermal process(es) (which drives the increase in W/T below  $R_o$  0.50%) are rapidly 'out competed' by the preferential expulsion of W from the rocks above  $R_o$  0.50%. Supporting this argument is the fact that whatever is driving the rapid decrease in W/T *ca.*  $R_o$  0.50% has no effect on the T/(T1+R) ratios (Figure 11A-C). The principal difference among these four bicadinanes is that W has a *cis-cis-trans* molecular configuration whereas the T, T1 and R isomers are all thought to be *trans-trans-trans* (6). Therefore, since the T/(T1+R) ratio is monitoring changes in isomers which all have *trans-trans-trans* configurations it would not be effected by preferential migration whereas the W/T ratio could be.

The initiation of the expulsion of oil from source rocks beginning at this maturity is supported by the fact that the most 'immature' Attaka oils have  $C_{29}$  S/(S+R) epimer ratios of *ca.* 0.20. That is to say that the maturity at which the W/T ratio maximizes ( $C_{29}$  S/(S+R) 0.20 or  $R_o$  0.50%) corresponds to the 'top of the oil window' in the Attaka area. Preferential expulsion of W because of its *cis-cis-trans*



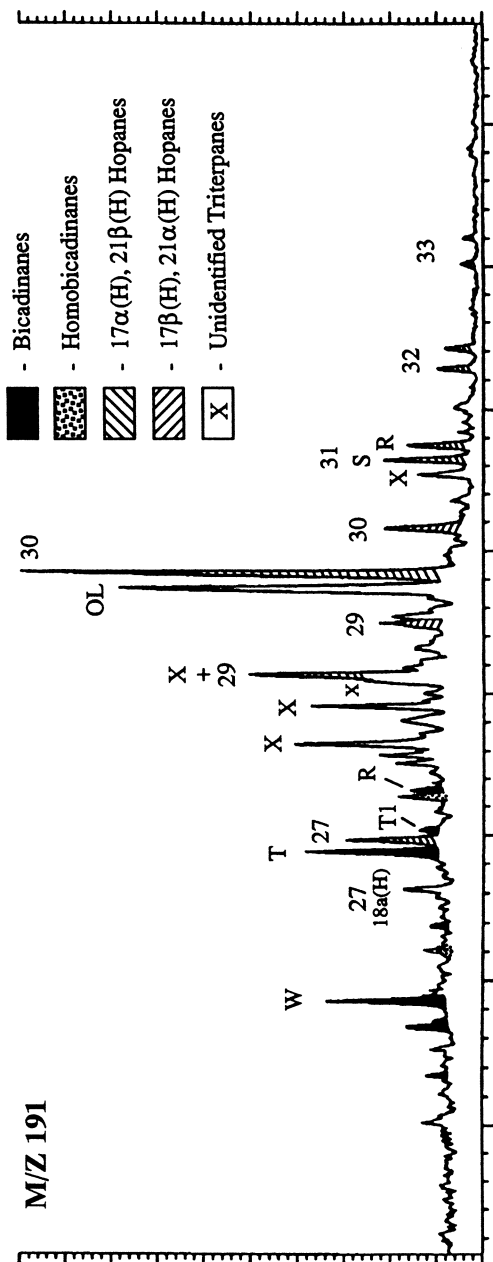


Figure 9. Partial mass chromatograms showing the distribution of triterpanes ( $m/z$  191) and steranes ( $m/z$  217) in an Attaka Field oil.

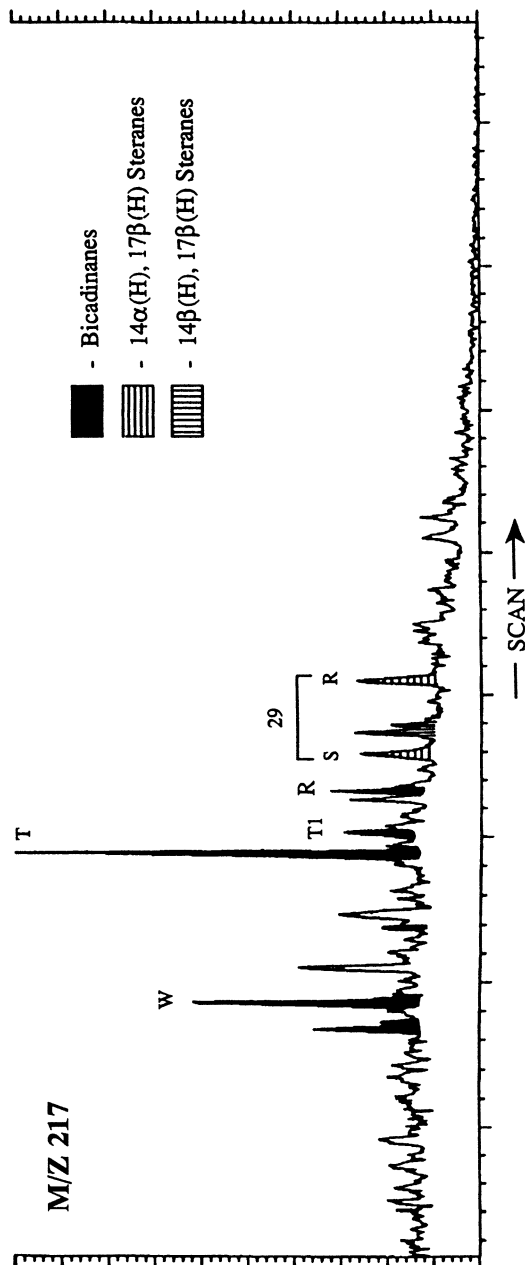


Figure 9. *Continued*

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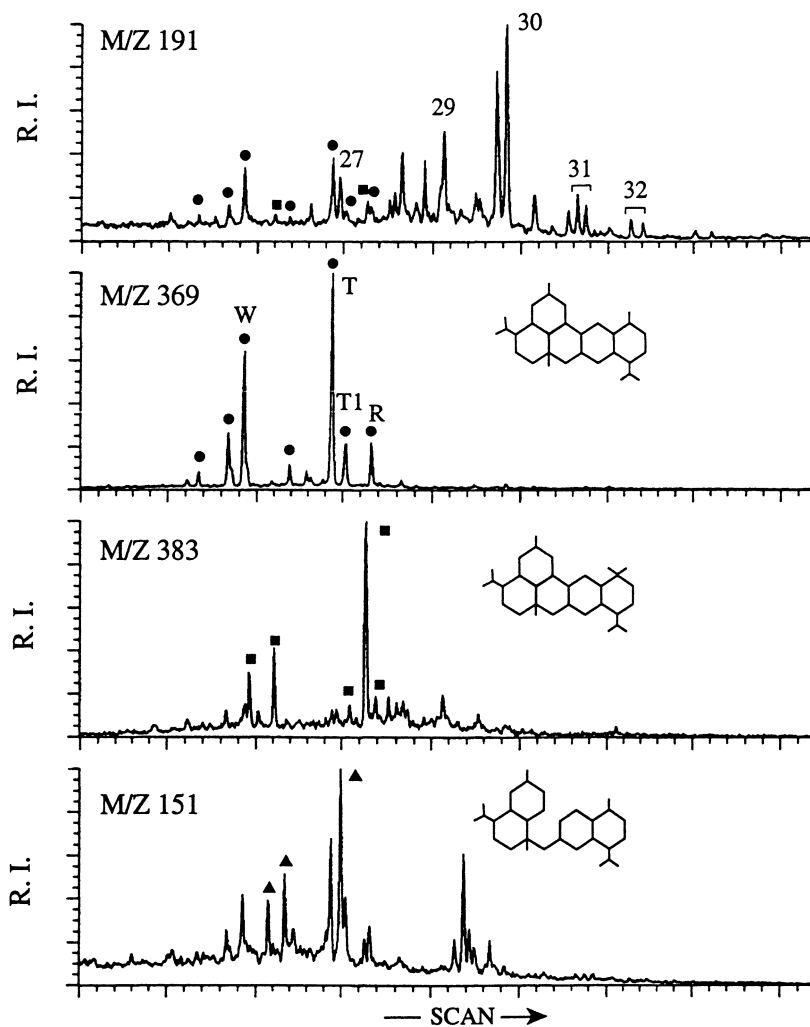


Figure 10. Partial mass chromatograms showing the distribution of bicadinanes ( $m/z$  369), homobicadinanes ( $m/z$  383) and seco-bicadinanes ( $m/z$  151) in an Attaka Field oil.

molecular configuration could explain the rapid drop in W/T observed above this maturity (Figure 11D-F). If preferential expulsion of W were to occur, one would predict that the most 'immature' oils in the region would have W/T ratios higher than the 0.6 values observed in the least mature source rocks (ca. 0.50%  $R_o$ ). This is indeed the case.

Figure 12A shows the rock extract data (as in Figure 11F) along with the data for the 44 Attaka Field oils studied. The  $C_{29}$  sterane 20S/(20S+20R) values for the Attaka oils range from 0.23 to 0.56 and show a poor relationship with W/T. However, generally speaking it is clear that the 'immature' oils, i.e. those with the lowest S/(S+R) ratios do have W/T ratios that are above 0.6 (as would be expected if W was preferentially expelled from the least mature source rocks). On the other hand, the 'mature' oils (with S/(S+R) ratios ca. 0.5) generally have lower W/T values than 'immature' oils, yet the 'mature' oils have W/T ratios which are still higher than those in 'mature' source rocks. Again, preferential expulsion of the W isomer at higher maturities could explain this.

Figure 12B attempts to graphically depict the processes giving rise to the rock and oil data shown in Figure 12A. At lower maturities the W/T ratio systematically increases in rock extracts as thermal stress increases. Assuming a first-order kinetic control on W/T, one would expect this increase to continue at higher maturities along the same near-linear trend observed. However, the rapid drop in W/T around  $C_{29}$  S/(S+R) of 0.20 (i.e., the top of the oil window) suggests that some other process (likely a non-kinetic process) causes a rapid drop in W/T. Preferential expulsion of W from source rocks upon reaching the maturity where the threshold of expulsion is surpassed and HCs are expelled could account for the rapid drop. 'Immature' oils are thereby preferentially enriched in W. Supporting evidence for this is found in the HC/Non-HC ratio for the rock extracts which shows that HCs increase in abundance up to this 'threshold' maturity, thereafter co-eval generation and expulsion result in a constant HC/Non-HC ratio (Figure 12C). The near constant W/T ratio observed in mature source rock extracts ( $R_o > 0.50\%$  and  $C_{29}$  S/S+R  $> 0.20$ ) suggests that there is a balance between the thermal (kinetic) process(es) which tend to increase W/T and the preferential expulsion of W which tends to decrease W/T. 'Mature' oils are depleted in W relative to 'immature' oils yet are still enriched in W relative to mature source rocks. The scatter among the W/T in oils could reflect preferential migration of W during secondary migration (rather than during expulsion).

## Conclusions

This study of fresh and fossil dammar resins derived from various Dipterocarpaceae trees, a maturation series of Miocene resin-rich rocks and Miocene-sourced oils in the Mahakam Delta has demonstrated several important aspects in regard to the role of dammar resin in oil formation.

The results have shown that dammar resins undergo few chemical changes from the time following their exudation/polymerization through their fossilization (i.e., early diagenesis). Functionalized triterpenoids with oleanane, ursane and dammarane skeletons dominate the solvent extracts of both fresh and fossil resins. Though these triterpenoids are derived from Angiosperms other than the Dipterocarpaceae, dammar

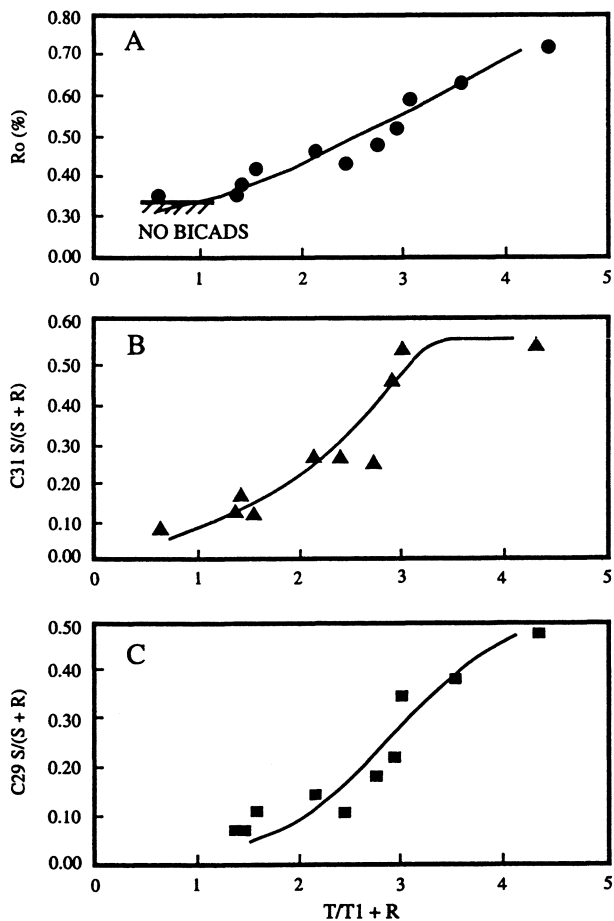
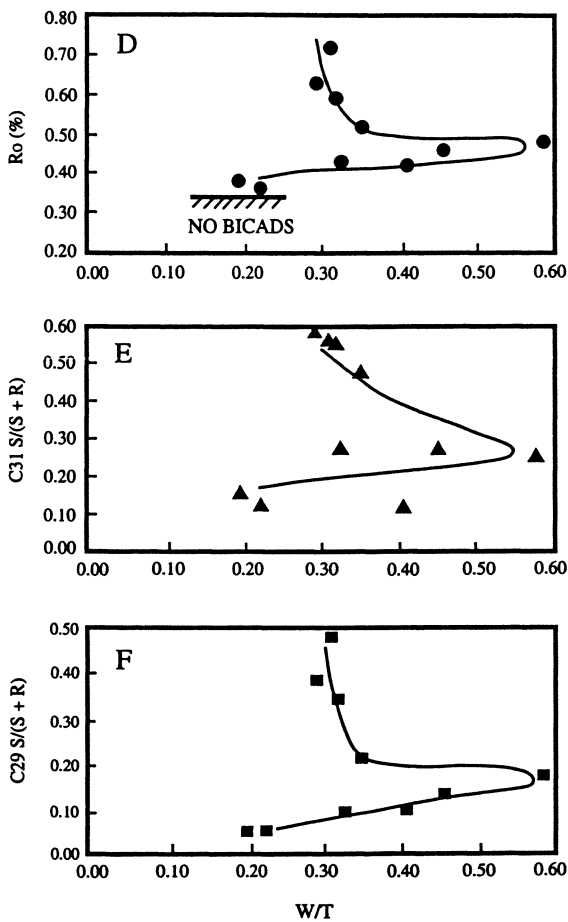


Figure 11. Plots of various maturity parameters *versus* bicadinane ratios for a series of increasingly mature rocks. (See Table II).

Figure 11. *Continued*

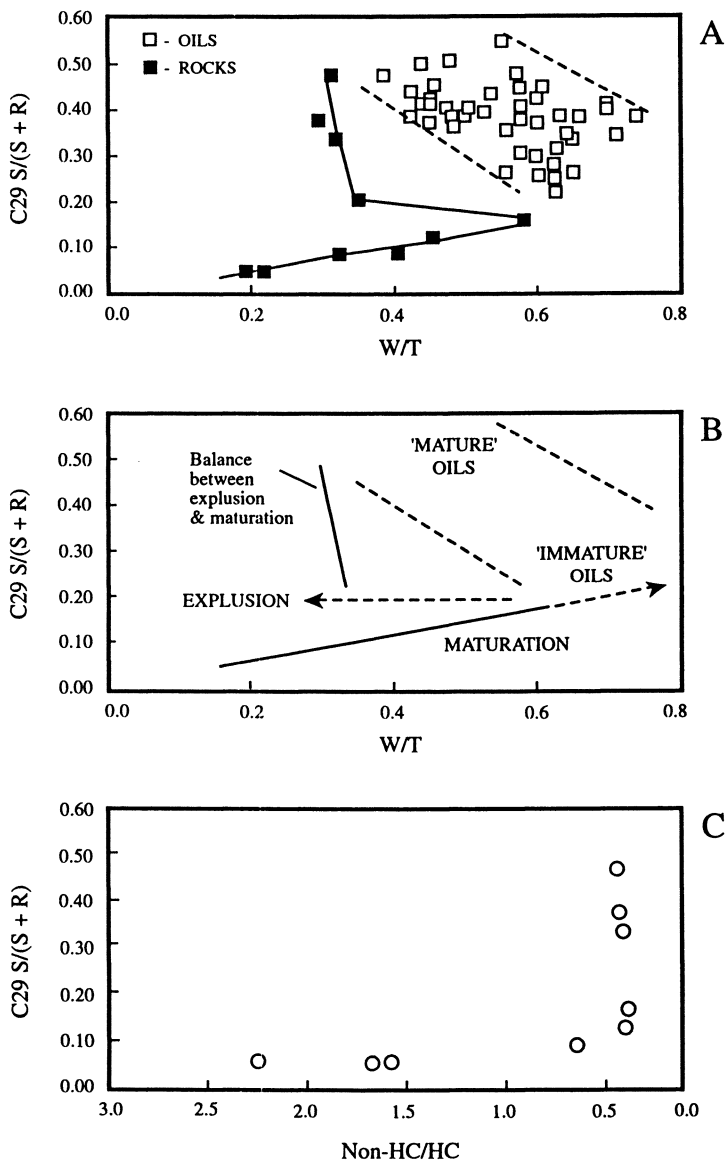


Figure 12. (A) Ethylcholestane 20S/(20S+20R) ratio *versus* W/T for a series of increasingly mature rocks and 44 Attaka Field oils; (B) Graphical depiction of the processes responsible for the data in (A); (C) Ethylcholestane 20S/(20S+20R) ratio *versus* non-hydrocarbon/hydrocarbon ratio in the rock extracts studied.

resin must be considered an important source of higher plant triterpanes in the region's oils.

Dammar resins contain varying proportions of solvent-extractable 'primary' HCs, up to 16 wt. %, which can contribute directly to oil formation without necessarily passing through a polymeric kerogen (polycadinene-type) intermediate. The most abundant 'primary' HCs are monomeric and dimeric sesquiterpanoids with a cadalene carbon skeleton and various degrees of saturation. No bicadinanes are present in fresh or immature fossil resins although cadinene dimer precursors are present. These dimers, seemingly leftover "building blocks" of a polycadinene polymer, could form bicadinanes upon cyclization and saturation. Similarly, cadinanes and cadalene could form upon saturation and dehydrogenation, respectively, of the monomeric sesquiterpanoids. Thus, at least some of the cadinanes, bicadinane and cadalene-type compounds in oils may be derived from these 'primary' HCs.

Inter-species variation among bled dammar resins from different Dipterocarpaceae species is suggested by the differences in physical properties (e.g., color), the distributions of indigenous HCs and carbon isotopic ratios in which the latter show variations of up to several per mil. More work needs to be done to determine the geologic implications of these differences.

The HCs generated from the whole dammar resins upon heating in the laboratory or in the subsurface are structurally-related to the monomeric 'primary' HCs and support the contention of a polycadinene polymer as previously described by Van Aarssen et al. (2). Bicadinanes are first formed in the laboratory over a narrow temperature range of 300-325°C (72h). Under laboratory conditions, the distribution of bicadinane isomers formed is consistent for both fresh and immature fossil resins indicating the formation of the different isomers is not random but temperature dependent. In the subsurface, bicadinanes are first formed over a narrow  $R_o$  range of 0.30-0.35%. Their formation in the subsurface at these low levels of maturity *versus* their formation only above 300°C in the laboratory emphasizes the important role which time plays in their formation. The isomer known as Compound R is most stable at this low maturity however, the *trans-trans-trans* isomer (Compound T) is most stable at higher maturities. The formation of bicadinanes below what is conventionally thought of as necessary for oil generation implies the polycadinene in dammar resins is much more labile than other kerogen types.

The distribution of certain bicadinane isomers varies with increasing maturity. The ratio among three *trans-trans-trans* isomers (T/(T1+R)) in rock extracts increases systematically over the  $R_o$  range 0.35 to 0.72% (or  $C_{29} 20S/(20S+20R)$  range 0.05-0.48). This extends the utility of this molecular maturity ratio beyond the range recently reported by Murray et al. (6). In rock extracts the ratio of *cis-cis-trans/trans-trans-trans* bicadinanes (W/T) increases from *ca.* 0.2 to 0.6 over the maturity range of 0.35-0.50%  $R_o$  (or  $20S/(20S+20R)$  0.05-0.20) before it rapidly decreases. It is suggested that this decrease is driven by the preferential expulsion of the *cis-cis-trans* isomer (W) upon reaching the 'top of the oil window' at  $R_o$  0.50%. Above this maturity the W/T ratio remains fairly constant (*ca.* 0.30) due to a balance between thermal (kinetic) processes, which favors formation of W over T, and the preferential expulsion of W. The W/T trend observed in the maturation series is consistent with the W/T ratios of oils in the region. That is, 'immature' oils are enriched in W



compared to their source rocks and to more 'mature' oils. Bicadinane ratios involving the *trans-trans-trans* molecular configurations (e.g., T/(T+R)) are therefore more reliable molecular maturity parameters. Ratios involving different molecular configurations (e.g., W/T) are subject to both maturity and migration-related influences.

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## Chapter 4

# Pyrolytic and Spectroscopic Studies of the Diagenetic Alteration of Resinites

Tatsushi Murae, Shuji Shimokawa, and A. Aihara

Department of Earth and Planetary Science, Faculty of Science,  
Kyushu University, Hakozaki, Fukuoka 812, Japan

Alteration of the chemical structure of resinites has been investigated by spectroscopic methods, due to the potential value of resinites as geochemical indicators of sedimentary environments. FT-IR and pyrolysis GC-MS data correlate with the reflectance values of coexisting vitrinites for the specimens of resinite collected from different coal fields of various degrees of coalification. All specimens are included in Class I (Anderson's classification). FT-IR studies of structural changes in resinites on heating indicated decarboxylation to be the major reaction path for thermal structural alteration. The data obtained indicate that spectroscopic analysis of resinites may afford useful maturation indicators for immature samples.

Coal macerals, of which resinite is an example, are petrological components of coals derived from the preserved remains of plant material (*I*). Most macerals are derived from the remains of various types of plant tissues. Therefore, the molecular compositions of macerals are usually very complicated. However, maturation processes modify the original plant tissues, and diminish differences between the molecular structures of each component in the maceral. The degree of modification of the original structures corresponds to the maturity of the maceral. Therefore, we can use the alteration of physical properties of the macerals as an indicator for maturation degree of the samples. Vitrinite reflectance values are the most widely applied indicator for the determination of maturity of sediment samples containing coaly fragments.

Determination of the maturity of sediment samples obtained from environments less mature than the so called "oil window" is very important to understanding of various geological phenomena. However, for low maturity samples, vitrinite

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reflectance values are very irregular and very difficult to use as a maturation indicator due to the remaining molecular complexity reflecting the source plant tissues. Therefore, the development of maturation indicators for low-maturity samples has been desired.

Resinite is derived from plant resins and secretions such as waxes, oils, and fats. Since resins are derived from a number of different sources, they can consist of a variety of substances including terpenes, phenols, alcohols, and acids. Their exact composition and mode of occurrence depend upon the type of plant from which they are derived and their mode of deposition. Resinites are yellowish orange to amber color in transmitted light and gray in reflected light. Due to their diverse origins, they exhibit a variety of fluorescence colors an alteration property (2,3). Consequently, it has been claimed that the optical properties of resinites are not a good indicator of thermal maturity (4).

However, the chemical components of resinites are remarkably simple compared with those of other macerals. Resinites contain only the compounds exuding naturally from plant tissues. Other macerals are formed from the tissues themselves, and hence contain a great variety of chemical components. This fact enables us to classify the resinites on the basis of structural characteristics. Anderson et al. classified them into five classes (Classes I ~ V) (5,6,7). They classified Class I resinites further into three sub groups (Classes Ia ~ Ic) on the basis of details of their composition. Resinites belonging to Class Ia are derived from/based on polymers and copolymers of labdanoid diterpenes, having the regular [1*S*,4*aR*,5*S*,8*aR*] configuration, including communic acids and communol and incorporating significant amounts of succinic acid. Those belonging to Class Ib are also derived from resins based primarily on polymers and copolymers of labdanoid diterpenes having the regular [1*S*, 4*aR*, 5*S*, 8*aR*] configuration, including but not limited to communic acid, communol and biformene. Class Ic resinites are derived from resins based primarily on polymers and copolymers of labdanoid diterpenes having the enantio [1*S*, 4*aS*, 5*R*, 8*aS*] configuration, including not limited to ozic acid, ozol, and *enantio* biformenes.

Thermal maturation probably modifies the chemical structure of the polymer in resinites. The differences of chemical structure among resinite samples belonging to the same class possibly indicate differences in the degree of maturation. The chemical reactions that alter the structure of the polymer in resinites probably proceed at lower temperature than aromatization reactions. Therefore, it may be possible to use the alteration of the chemical structure of resinites as a diagenesis indicator for the samples whose maturity is too low to be determined by vitrinite reflectance.

In order to determine the alteration of the chemical structure of the polymer in resinites, it is necessary to examine resinite samples from different source having different thermal histories. Analyses by elemental analysis, FT-IR (Fourier transform infrared spectroscopy), pyrolysis GC-MS (gas chromatography-mass spectrometry) and CP-MAS <sup>13</sup>C NMR (cross polarization-magic-angle spinning solid-state <sup>13</sup>C nuclear magnetic resonance) are usually effective for characterization of organic polymers. Comparisons between the data obtained using the above methods on resinites, and the reflectance values of vitrinites coexisting with the resinite samples may provide information about the correlation between the structural alteration of

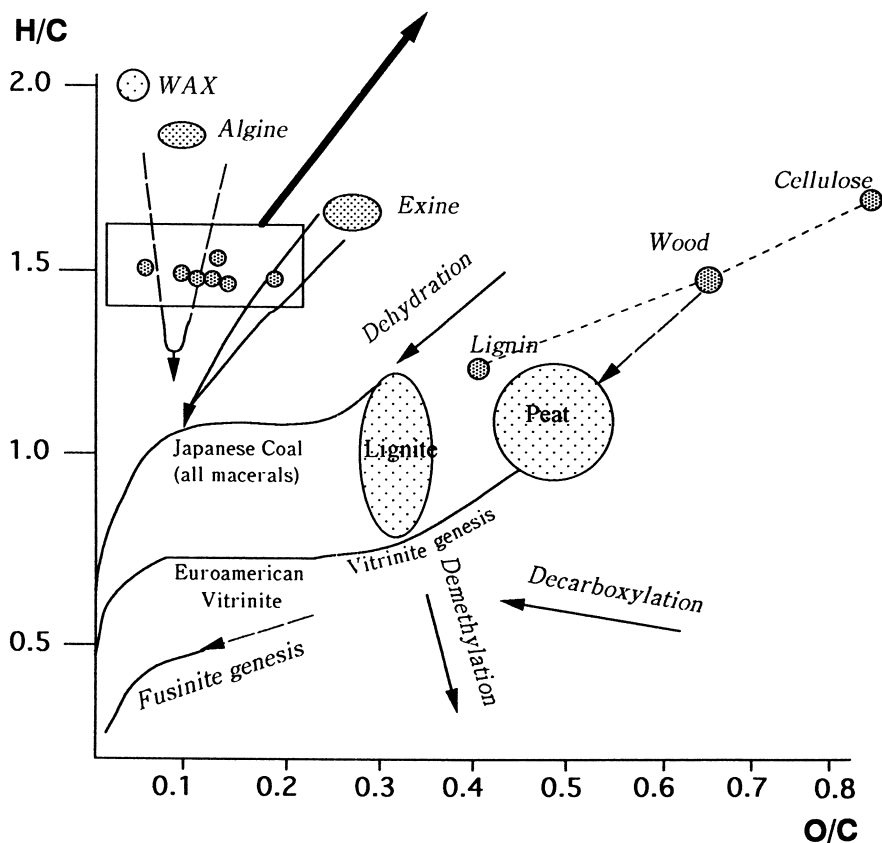
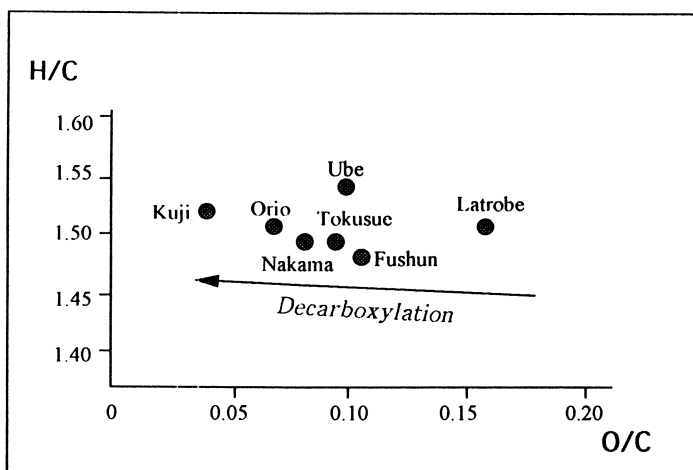


Figure 1. Plot of the resinites in a van Krevelen diagram.

resinite and its thermal maturity. Laboratory experiments of the structural alteration of resinite samples by heating also afford a useful information on their thermal maturity.

### Resinite Samples

Resinites usually coexist with other macerals, although some low maturity resinite samples exist as soft lumps. High maturity resinites are hard, and relatively large pieces are referred to by the name "amber". Table I lists the characteristics of the resinite samples used in this work along with reflectance values of coexisting vitrinites, type of coal from which the resinite was picked out, and the geologic age of the strata containing the coal sample. Most of the samples listed in table I were collected at coal fields in Japan except the Latrobe (Australia) and Fushun (China) resinites. All of the resinites was collected by hand picking from coal samples.

Thermal maturity is the multiplied result of maximum temperature with effective heating period. Geological heating mechanisms are very complicated in Japan. Therefore, the geologic age is not always directly proportional with the thermal maturation of the samples.

### Experimental

IR spectra were recorded from KBr pellets using a Perkin Elmer 1600 FT-IR spectrometer. IR spectroscopic examinations of thermal alteration of Latrobe resinite were carried out for a KBr pellet cooled down to room temperature after keeping at each heating temperature for 10 minutes under nitrogen.

Pyrolysis GC-MS experiments were carried out using a Hitachi G-5000 gas chromatograph coupled to a JEOL D-300 mass spectrometer and a JAI JHP-2 Curie point pyrolyser (for analysis of low boiling point pyrolysates) or JAI JHP-3S Curie point pyrolyser (for analysis of high boiling point pyrolysates). For analysis of high boiling point pyrolysates, simultaneous pyrolysis methylation procedures were employed in order to methylate acidic products (8). The GC oven was operated from 60 °C to 260 °C with temperature ramp rate of 4 °C/min. An OV-1 chemically bonded fused silica capillary column (25m x 0.25mm I.D., 1.5µm dp) was used under helium carrier gas. Each sample was pyrolysed at 445 °C and 740 °C.

### Results and Discussion

**Elemental Composition of Resinites.** The elemental composition of macerals changes according to their degree of maturation. This alteration is well represented using a van Krevelen diagram (9), in which H/C vs. O/C atomic ratios are plotted. Figure 1 shows the plot of resinite samples in a van Krevelen diagram. The H/C atomic ratios of the resinites are much higher than those of vitrinites indicating the presence of highly saturated structures in the resinites. Although the difference of O/C values between the samples is remarkable, the H/C values are almost the same for all samples. Similar tendency of the alteration of elemental composition has been reported for amber and resinite samples from other sources (10). This fact probably

Table I. Data for Resinite Samples and Coexisting Macerals

<i>Coal field</i>	<i>Color</i>	<i>Size (mm)</i>	<i>Vitrinite reflectance(%)</i>	<i>Coal type</i>	<i>Geologic Age</i>
Latrobe	Opaque white yellow	100	0.40	Brown	Late Oligocene
Ube	Clear yellow ~yellow brown	~10	0.51	Sub bituminous	Middle Eocene
Fushun	Clear yellow ~ yellow brown	~5	0.59	Sub bituminous	Eocene
Orio	Clear brown	~10	0.60	High volatile bituminous	Early Oligocene
Tokusue	Clear yellow ~ brown	~3	0.61	High volatile bituminous	Early Oligocene
Nakama	Clear yellow brown	~5	0.67	High volatile bituminous	Early Oligocene
Kuji	Clear yellow	~10	0.69	High volatile bituminous	Late Cretaceous

indicates that decarboxylation is the major chemical reaction during the maturation of the resinite samples provided all of these resinites belongs to Class I of Anderson's classification (5-7).

**IR Spectra of the Resinites.** IR spectra reflect the type of functional groups present in organic molecules very well. The IR spectra of the resinites in Table I are shown in Figure 2. Gross features of the IR spectra of the resinites in Figure 2 resemble each other. This fact indicates the presence of similar functional groups in all samples. This suggests that these resinites have similar chemical structures and are consistent with the spectra of other Class I resinites (5, 11, 12, 13, 14). Closely similar IR spectra have been reported for other amber and resinite samples by Grimalt et al. (10). The absorptions at ca. 3000  $\text{cm}^{-1}$  and ca. 1450  $\text{cm}^{-1}$  are assigned to aliphatic C-H bonds, and these at ca. 3400  $\text{cm}^{-1}$  and ca. 1700  $\text{cm}^{-1}$  to carboxyl groups. The broad absorption band at ca. 3400  $\text{cm}^{-1}$  may contain the signal due to water also. The absorption at ca. 880  $\text{cm}^{-1}$  in the spectrum of Latrobe resinite can be assigned to exomethylene groups.

The absorption intensities of the signals due to the carboxyl and exomethylene groups differ between the samples of differing maturation. Absorption due to exomethylene disappeared in the spectra of the samples of vitrinite reflectance greater than ~ 0.4% (Latrobe resinite). Grimalt et al. classified ambers and resinites into two major age groups, irrespective of origin, using the exomethylene IR bands. In the samples of lower maturity the bands are easily recognizable, but are absent in more mature samples (10). Our results indicate that the exomethylene group is modified during the early stages of maturation. This fact is consistent with the NMR observations by Lambert et al. (15).

The intensities of absorptions at ca. 1700  $\text{cm}^{-1}$  due to carboxyl groups decrease according to the incremental increase of the reflectance value of coexisting vitrinites. The decrease of the intensity of carboxyl band probably indicates the occurrence of decarboxylation reactions as suggested by the results of elemental analyses. The relative intensities of the IR absorption at ca. 1700  $\text{cm}^{-1}$  to the absorption at ca. 1450  $\text{cm}^{-1}$  are plotted against the reflectance value of coexisting vitrinite in Figure 3. These relative intensities indicate the relative abundance of carboxyl group in the molecules. Figure 3 shows almost linear correlation between the relative intensities of the IR bands and the vitrinite reflectance values. This fact indicates that decarboxylation reaction proceeds mainly during relatively early stages of maturation processes.

**Thermal Alteration of IR Spectra.** Heating causes structural changes in the organic components of sedimentary samples during maturation processes. The maximum temperature and the effective heating period are the factors that determine the degree of the maturation. In order to estimate the thermal history of a sample from chemical analysis, it is very important to know what kind of structural alteration occurs at what temperature for specific compounds incorporated in the sample. Evaluation of the maturity of relatively immature samples using vitrinite reflectance suffers from a number of difficulties (16). The main factors determining alterations of vitrinite reflectance values are aromatization reactions that proceed at relatively high



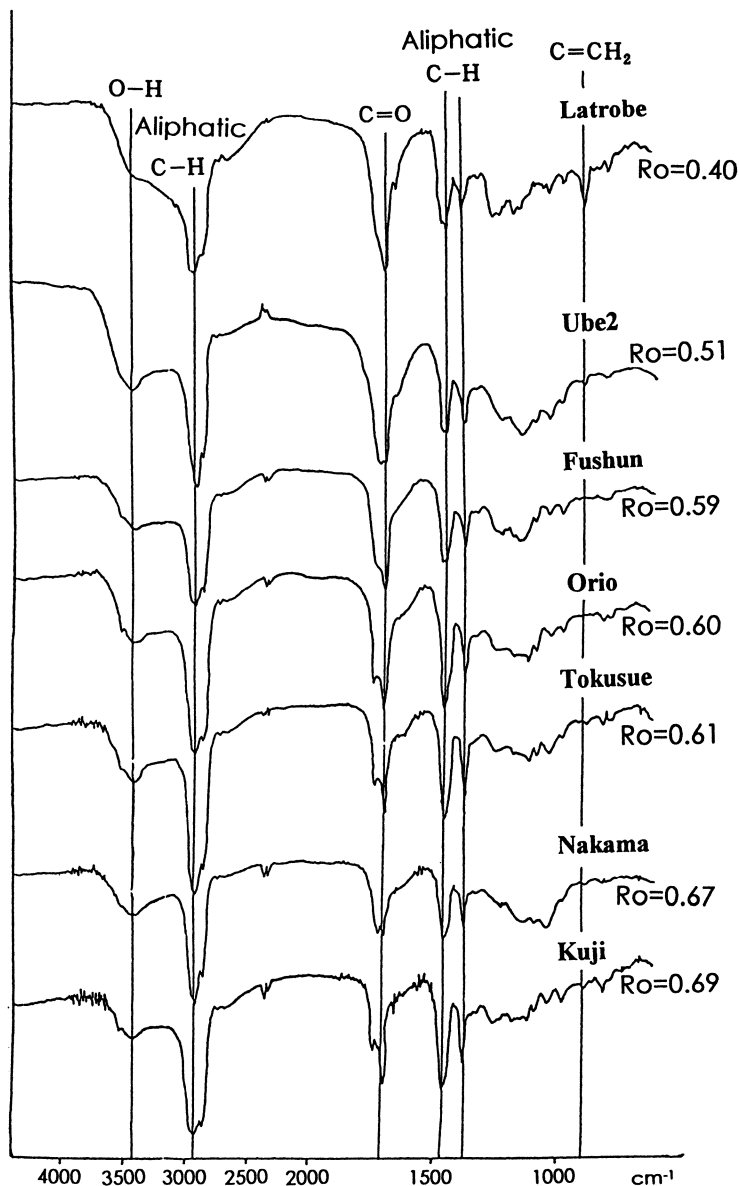


Figure 2. IR spectra of the resinites (vitrinite reflectance value of coexisting vitrinites are indicated).

temperature. Hence it is difficult to use vitrinite reflectance values as thermal indicators for immature samples which have never experienced prolonged high temperatures.

Based on the data described in this report, decarboxylation reactions cause the major structural alteration of resinites. These reactions usually proceed at lower temperatures than aromatization reactions. Therefore, the structural alteration of resinites has a potential as a thermal maturity indicator for immature samples. This was confirmed by observation of the alteration of IR spectra of Latrobe resinite in response to laboratory heating experiments as shown in Figure 4. Vassallo et al. have carried out similar experiments using Fushun and Yallourn resinites (17). Hwang and Teerman measured thermal alteration of IR spectra of resinites during pyrolysis for hydrocarbon characterizations (18). Latrobe resinite is the most immature sample among those in Table I. Therefore, that is the most suitable sample for heating experiment. The results of this experiment indicate that isomerization of exomethylene structures occurs at ca. 250 °C and is complete by ca. 300 °C. Decarboxylation reactions begin at ca. 300 °C and are almost complete at ca. 400 °C. Aromatization reaction follows the decarboxylation reaction. The relative intensity of the absorption at 1700 cm<sup>-1</sup> to that of at 1450 cm<sup>-1</sup> decreases linearly following the elevation of heating temperature between 300 °C and 400 °C. This observation may allow us to estimate the highest temperature limit of the thermal history of immature samples containing resinites on the basis of IR spectra.

**Pyrolysis GC-MS of Resinites.** Analyses of pyrolysis products give us very useful information about the structure of organic polymers. On pyrolysis using a direct inlet probe of a mass spectrometer, most of the resinites in the Table I afforded a characteristic ion peak at m/z 302 whose exact mass 302.2246 indicates the elemental composition of the ion to be C<sub>20</sub>H<sub>30</sub>O<sub>2</sub>. This elemental composition is compatible with that of communic acid (I) which is a major component of Class Ia and Class Ib resinites (5,6,7). The relative intensity of the ion decreased in the spectra of the more matured samples. This may indicate that the modification of the structure unit of the resinite polymers proceeds according to the incremental increase of thermal maturation. Anderson suggested that loss of monomeric diterpene acids such as abietic acid, which is a common component in recent and immature resinites, as maturation increase is the main possible reason of the above observation (19). Structure elucidation of the products yielded on vacuum pyrolysis experiments of Latrobe resinite is under way in order to confirm that possibility.

Distribution pattern of pyrolysis products from a polymer reflects the chemical structure of the polymer. The major components of low boiling pyrolysis products obtained by heating at 740 °C are aromatic hydrocarbons and the distribution patterns of the components resemble each other for all samples (Figure 5). That suggests the presence of similar basic skeletal structure for all samples (consistent with the results obtained by IR spectra). Pyrolysis at such high temperature as 740 °C is accompanied by dehydration reactions to yield aromatic compounds. However, on heating at 445 °C using the same pyrolysis system as above, the resinite samples gave pyrograms that differed significantly. Figure 6 shows the distribution patterns of

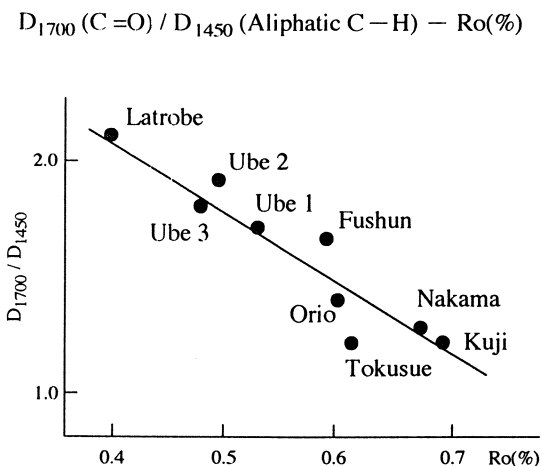


Figure 3. Relative intensity of IR absorption at  $1700\text{ cm}^{-1}$  to that at  $1450\text{ cm}^{-1}$  of the resinites vs. the reflectance value of coexisting vitrinites.

the pyrolyzates. The major components of low boiling pyrolysis products obtained by heating at  $445\text{ }^{\circ}\text{C}$  are aliphatic hydrocarbons and the gross features of the pyrograms divided the samples into three groups. The first group contains the resinite from Latrobe, the second contains the resinite from Ube and Fushun, and the third contains the resinite from Orio, Tokusue, Nakama, and Kuji. The resinites within each group show similar relative intensity of IR absorption at  $1700\text{ cm}^{-1}$  to that at  $1450\text{ cm}^{-1}$ . Therefore, this grouping seems to reflect the differences in diagenetic processes and burial metamorphism.

Only pyrolysis products having low boiling point were analyzed in the experiments described in Figures 5 and 6. According to the report by Anderson et al. (5), some of the major pyrolysis products from Class I resinites contain carboxyl group, and the amount of three characteristic bicyclic acids esters (II, III and IV) derived from the A/B ring system of polycommunic acid (V) is maturity dependent. Pyrolysis of the resinites in Table I at  $445\text{ }^{\circ}\text{C}$  with tetramethylammonium hydroxide as a methylation reagent gave the methyl ester of the acids II and III (and probably IV) along with other various components as shown in Figures 7a and 7b. Examinations of the pyrolysis products by mass chromatograms indicated that the compounds II and III are the major acid ester products. The mass chromatograms also suggested that the peak corresponding to the compound IV may overlap with that of III as a minor component. Therefore, we evaluated the intensity of the peak assigned to III in Figures 7a and 7b as the accumulated amount of III and IV. Figure 8 shows the plot of the ratio of esters II/(III+IV) against the reflectance value of vitrinite coexisting with the resinite. Although, as a general trend, the ratios of the esters correlate with vitrinite reflectance values, much more examples are necessary in order to use the ratio of the acids as an effective maturity indicator.

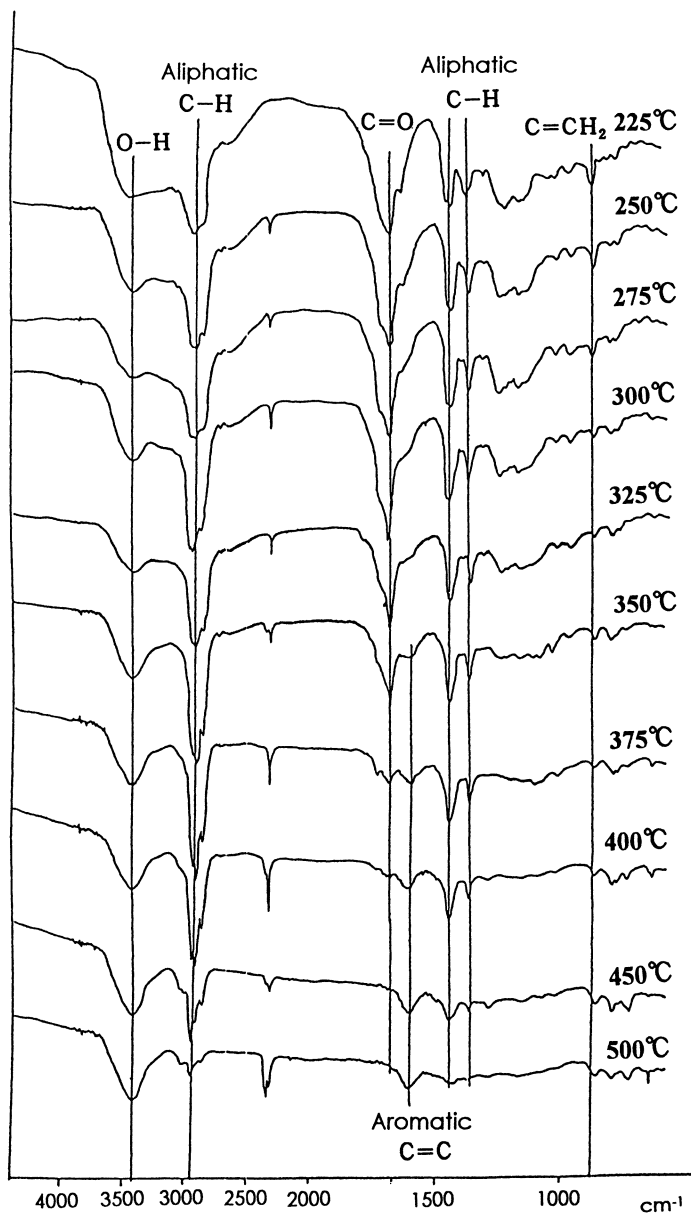


Figure 4. IR spectra of resinites samples heated to various temperatures. Temperatures and assignments of the major peaks are indicated.

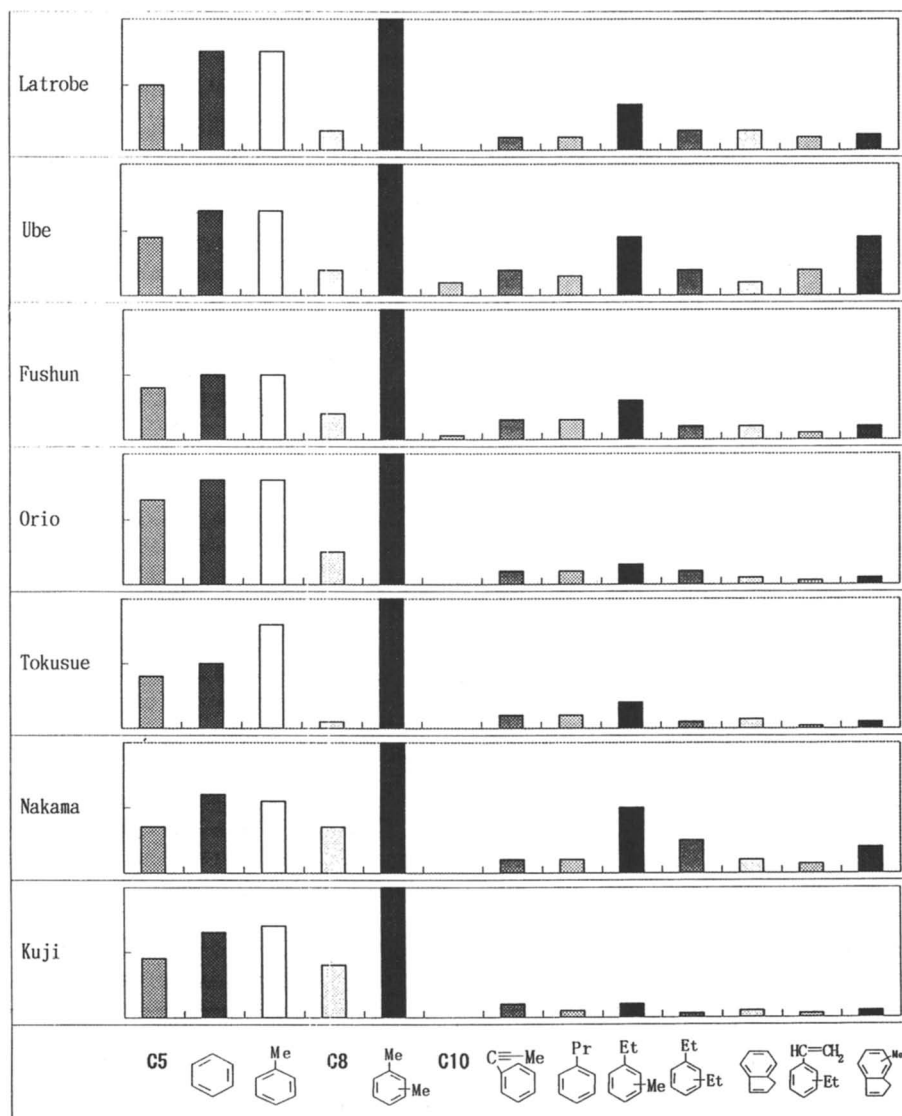


Figure 5. Distribution of components yielded on pyrolysis of the resinites at 740 °C. Amounts of structural isomers are summed. The relative amount of each sum is normalized to the highest amount.

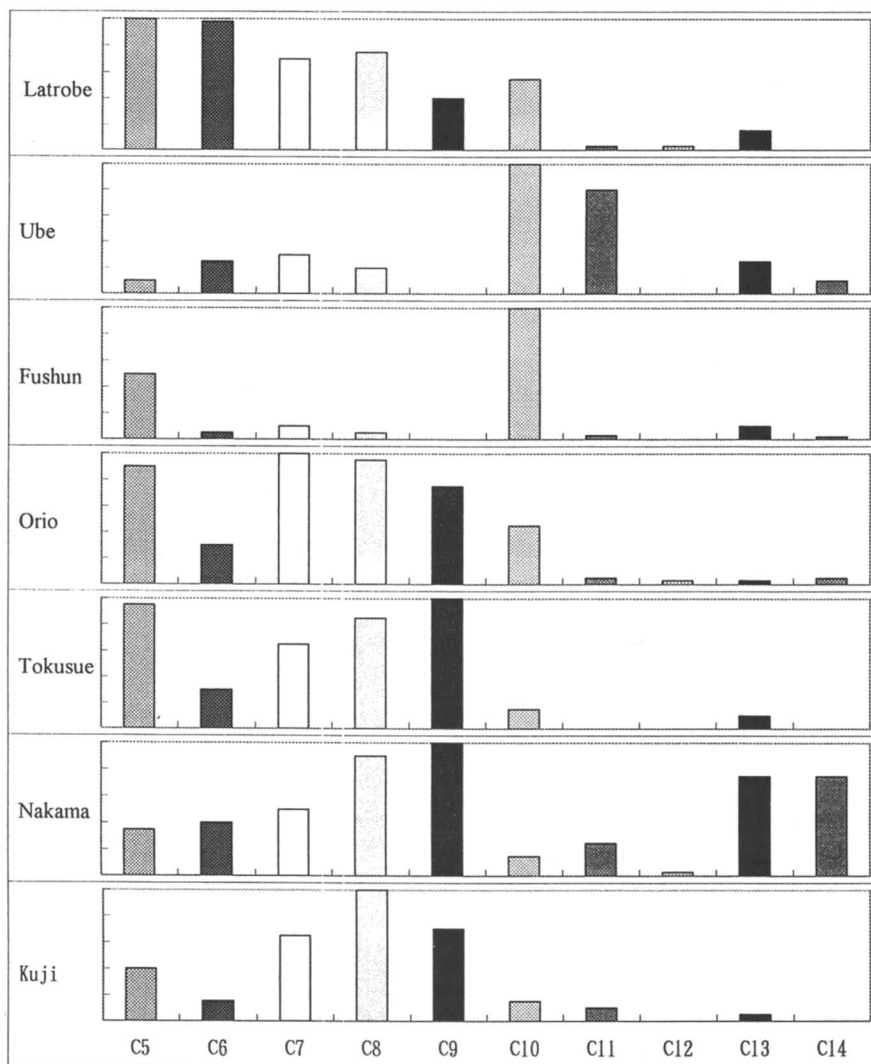


Figure 6. Distribution of components yielded on pyrolysis of the resinites at 445 °C. Amounts of the compounds bearing same carbon numbers are summed. The relative amount of each sum is normalized to the highest amount.

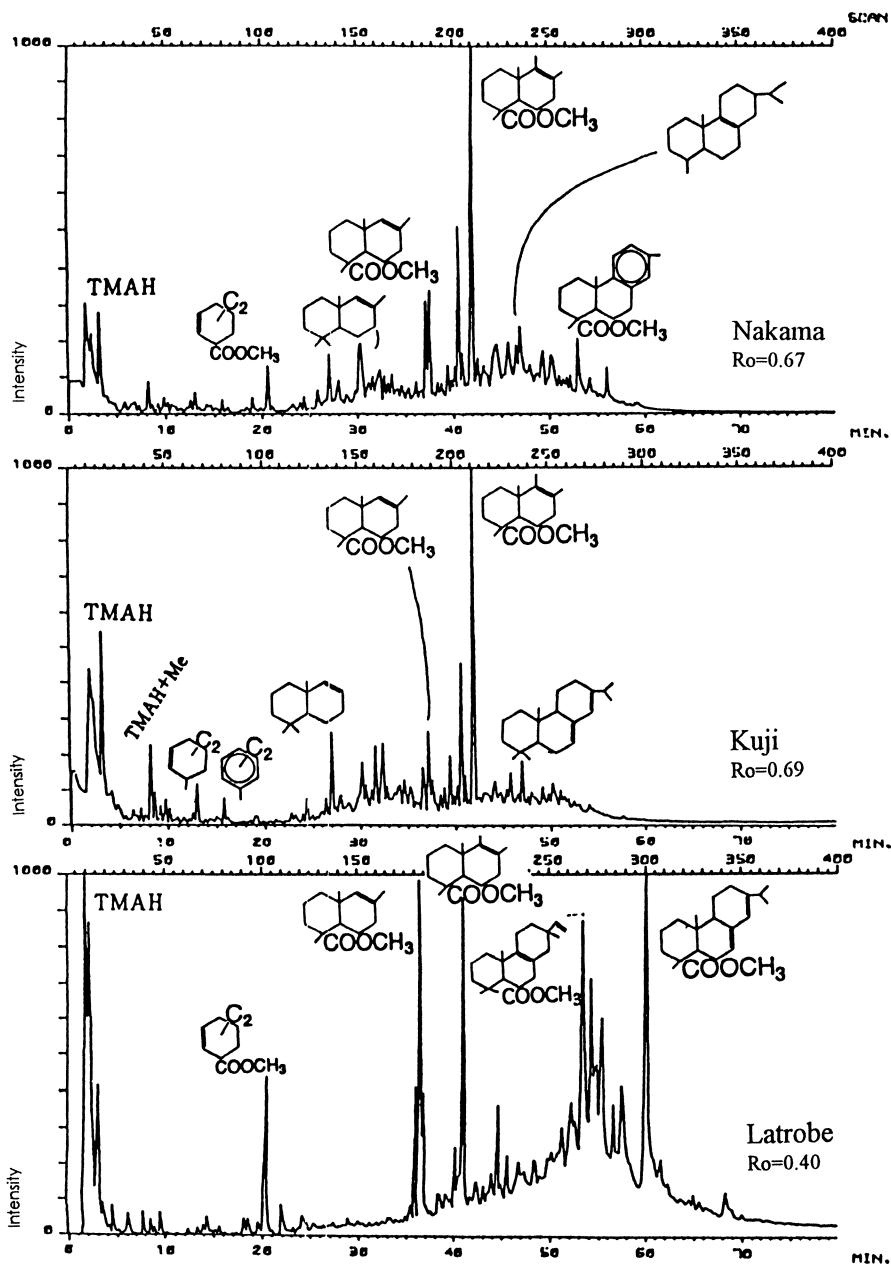


Figure 7a Py-GC-MS total ion chromatograms of Nakama, Kuji, and Latrobe resinites.

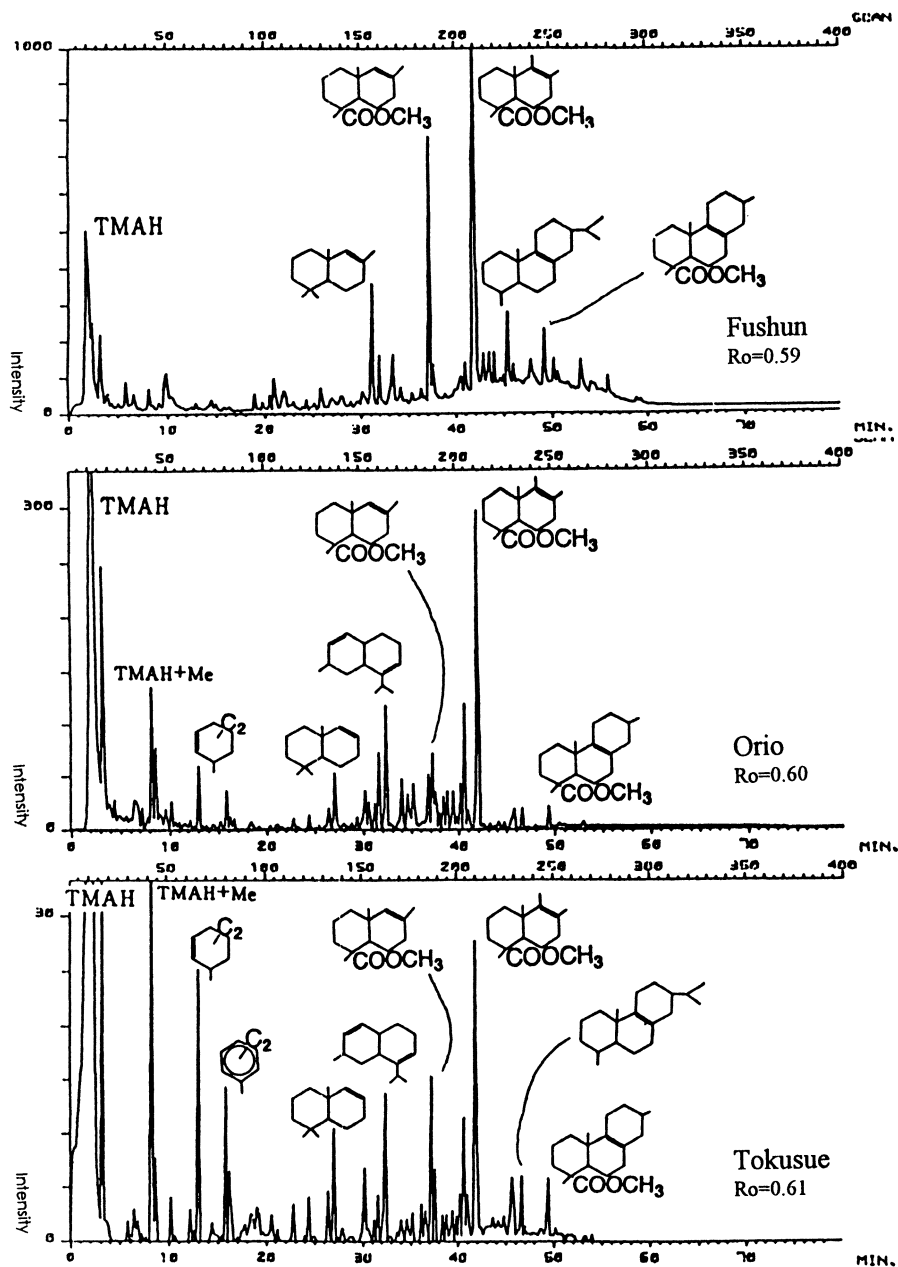


Figure 7b Py-GC-MS total ion chromatograms of Fushun, Orio, and Tokusue resinites.



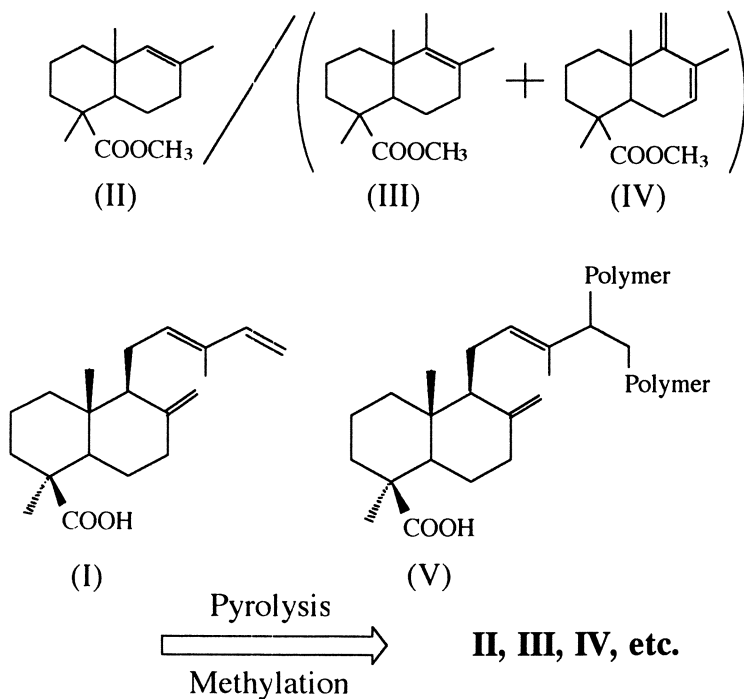
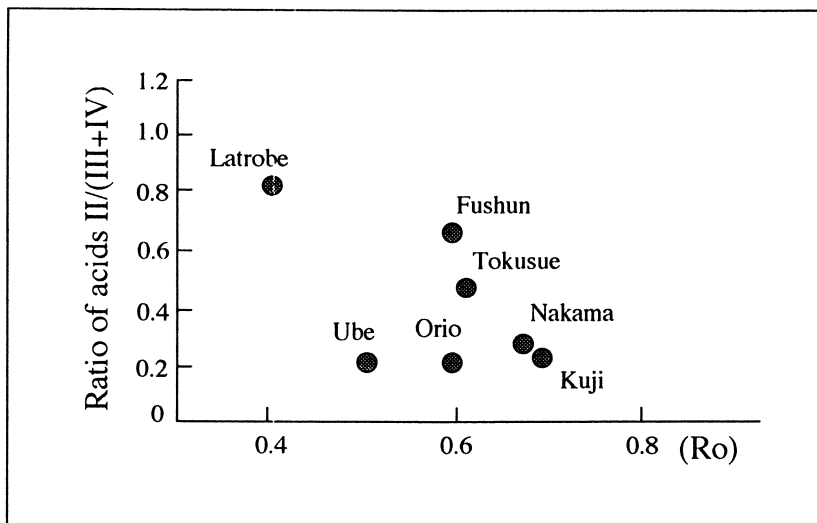


Figure 8. Ratio of characteristic bicyclic acids methyl esters [II/(III+IV)] yielded on pyrolysis of the resinites at 445 °C (with simultaneous methylation) vs. reflectance value of coexisting vitrinites.

## Conclusions

All of the resinites investigated in this work are included in the Classes I of the Anderson's classification (5,6,7). The relative intensity of IR absorption at  $1740\text{ cm}^{-1}$  to that at  $1450\text{ cm}^{-1}$  indicates maturity of the sample. The presence of the IR absorption due to exomethylene indicates that the thermal maturity of the sample is too low to be evaluated by the intensity of carboxyl bands. The ratio of specific components of pyrolysis products of the resinites also works as a maturity indicator for immature samples. Molecular structures of resinites were easily modified irreversibly by heating in short period. Therefore, provided a sample contains resinite, spectroscopic examination of the resinite may be useful for determining the upper temperature limit in thermal history of the sample.

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## Chapter 5

# Maturation of Class Ib (Polylabdanoid) Resinites

David J. Clifford and Patrick G. Hatcher

Fuel Science Department, Pennsylvania State University,  
University Park, PA 16802-2303

Three polylabdanoid (Class Ib) resinites of increasing thermal maturity, which originated from the Goodwins, Giles Creek and Heaphy coal seams of New Zealand, were analyzed by solid-state nuclear magnetic resonance spectroscopy and pyrolysis-gas chromatography-mass spectrometry. The samples were analyzed as received and after Soxhlet extraction with dichloromethane and methanol. Observed maturation trends included depletion of exomethylenes (CPMAS  $^{13}\text{C}$  NMR) and increased abundance of alkylnaphthalenes and alkyhydronaphthalenes relative to compounds indicative of labdatriene precursors (py-GC-MS). Maturation pathways consistent with observed trends have been proposed. Considered reactions include exomethylene isomerization, further polymerization, cyclization and defunctionalization of the resinite polymer.

Polylabdanoid (Class Ib) resinites are the semi-fossilized or fossilized remains of plant resins. Anderson et al. (4) define Class Ib resinites as "derived from/based on polymers and copolymers of labdanoid diterpenes having the *regular* (1S, 4aR, 5S, 8aR) configuration" citing as examples resinites from New Zealand, Australia, and Siberia. Class Ib resinites are thought to derive from polymerization of labdatriene precursors such as communic acid (Fig. 1a), communol (Fig. 1b) and biformene (Fig. 1c) (1-3). They are formed via polymerization of the labdatriene precursors following contact with light and air as they are exuded from trees.

Polymerization is thought to occur across the terminal side chain olefin yielding 14,15-polylabdatrienes (Fig. 1d) (5). Entrapped within the polymeric network are non-polymerizing components of the source resin, which predominantly include abietanes and pimaranes such as dehydroabietic acid and sandaracopimaric acid, for example (6-9). Non-polymeric resinite components are readily characterized by conventional gas chromatography due to their intrinsic solubilities and volatilities.

Following burial and subsequent maturation, polylabdanoid resinites undergo a very specific reaction resulting in depletion of the C8-C17 (Fig. 1) exomethylene, as determined by  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy ( $^{13}\text{C}$  NMR) (1-3) and fourier transform infrared (FTIR) spectroscopy (10-11). Recently, the exomethylene diminution was directly related to the polymeric fraction of Class Ib resinites by  $^{13}\text{C}$

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NMR analyses of extracted samples (12-13). As a result of their study, Clifford and Hatcher (13) established maturation trends based on pyrolysis-gas chromatography-mass spectrometry (py-GC-MS) and  $^{13}\text{C}$  NMR data for a suite of resinites thought to have derived primarily from communic acid. The samples originated from the Yallourn and Morwell coal seams in Victoria, Australia and the Brunner coal measure of Nelson, New Zealand.  $^{13}\text{C}$  NMR results demonstrated a "loss" of one double bond per diterpenoid monomer with the persisting olefin containing a protonated and a nonprotonated carbon, as shown by Grimalt et al. (8). Analyses of extracted samples by py-GC-MS displayed an increasing abundance of alkyl(hydro)naphthalenes relative to compounds directly related to polycommunic acid pyrolysis (i.e. hydronaphthenic acids). From these trends, the authors demonstrated inconsistencies in maturation schemes proposed in the literature to date (2-3, 14). The schemes included isomerization reactions (Fig. 1e) (2-3), exomethylene bonding (Fig. 1f) (14), and intramolecular polymerization reactions (Fig. 1g) (14).

It is the objective of this study to analyze a suite of Class Ib resinites consisting primarily of communic acid/communol copolymers. The samples originate from the Goodwins, Giles Creek and Heaphy coal seams of New Zealand. They are thought to have derived primarily from the polymerization of communol and communic acid based on results of pyrolysis of the unextracted resinites (3, 15). For this study, py-GC-MS and  $^{13}\text{C}$  NMR analyses are conducted on the resinites as received and after Soxhlet extraction. Observed trends are compared with previously reported trends (12-13).

### Samples and Methods

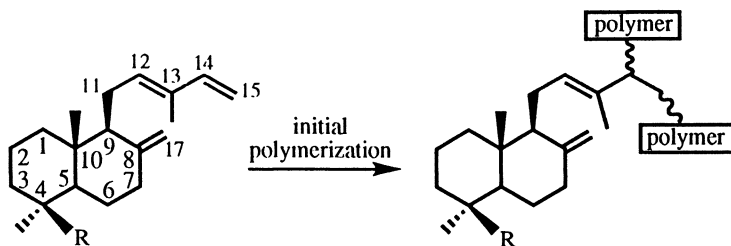
For the purpose of this investigation, three Class Ib resinites of diverse age and maturity were selected for characterization. Listed in Table I, in order of increasing maturity (rank of parent coal), are the sample origins, ranks of coal each can be associated with and elemental compositions. The resinites originate from the Goodwins, Giles Creek and Heaphy coal seams of New Zealand. Samples and elemental compositions were supplied by Dr. Ken B. Anderson (Amoco Oil Company, Naperville, IL) (16) and correspond to the samples described in Anderson et al. (3).

Table I. Sample origins, ranks (parent coal) and elemental compositions

Sample	Origin	Rank (parent coal)	Elemental Data				
			%C	%H	%N	%S	%O <sup>a</sup>
Goodwins <sup>b</sup>	Goodwins, New Zealand	lignite B	79.6	10.2	<0.3	0.04	10.2
Giles Creek <sup>b</sup>	Giles Creek, New Zealand	lignite A	80.1	10.4	<0.3	2.34	9.5
Heaphy <sup>b</sup>	Heaphy, New Zealand	subbituminous	81.9	10.5	<0.3	0.14	7.5

a=by difference; b=values and samples supplied by K. B. Anderson (16).

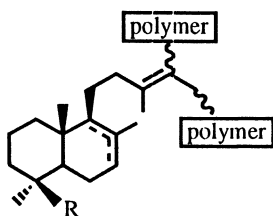
**CPMAS  $^{13}\text{C}$  NMR.** Solid-state CPMAS  $^{13}\text{C}$  NMR experiments were performed using methods described previously (17). The samples were placed in the spinner of a Chemagnetics M-100 NMR spectrometer operating at a field strength of 2.35 T and spun at approximately 3.5 kHz. Data was collected in 0.5 K and zero-filled to 4 K with a pulse delay of 1 s and a contact time of 1 ms over a spectral width of 10 Hz. 50 Hz of proton decoupling was applied as well as an exponential apodization of 10



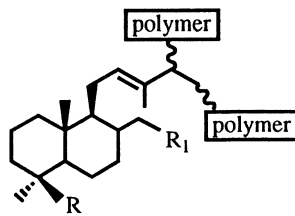
- (1a) Communic acid ( $R = \text{CO}_2\text{H}$ )  
 (1b) Communol ( $R = \text{CH}_2\text{OH}$ )  
 (1c) Biformenc ( $R = \text{CH}_3$ )

(1d) 14,15-polylabdatriene

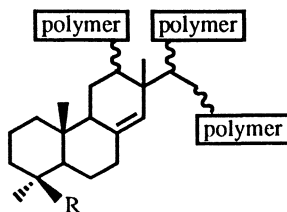
Maturation pathways proposed in the literature to date



(1e) isomerization (2-3)



(1f) exomethylene bonding (14)



(1g) Intramolecular cyclization (14)

Figure 1. Polymerization of labdatriene precursors (1a-c) yielding a 14,15-polylabdatriene polymer (1d). Shown below are maturation schemes occurring in the literature date, which include: 1e) isomerization reactions, 2) exomethylene bonding and 3) intramolecular cyclization reactions.

Hz prior to Fourier transformation. Peak areas were measured using the Galactica Industries Lab Calc curve fitting software package and Silk Scientific's automated digitizing system, Un-Plot-It (Version 3.1 Plus). The samples were analyzed as received and after Soxhlet extraction with a 50:50 v/v mixture of dichloromethane and methanol (30 h).

**Py-GC-MS.** Py-GC-MS was performed using a Chemical Data System Pyroprobe 1000, which can be directly inserted into the injection port of a Varian 2700 gas chromatograph. Directly coupled to the gas chromatograph is a Dupont 21-490B mass spectrometer fitted with a Teknivent Vector/One data system for detection and compound identification. Approximately 1-3 mg of sample was loaded into a quartz pyrolysis boat, placed in the pyroprobe and inserted into the injector port (temperature maintained at 280°C) of the gas chromatograph. Following pyrolysis at 540°C, volatiles were swept into a cryotrapped, 25m x 0.25mm i.d. J&W DB-17 capillary column to be chromatographed from an initial temperature of 30°C to 280°C. To cryofocus the volatiles at the head of the column during the 10 second pyrolysis period, a portion of the column was immersed in liquid nitrogen. The heating rate of the GC oven was 4°C/min. A more detailed description of this process has been published previously by Hatcher et al. (18). Compounds were identified by comparison with published mass spectra, NBS/Wiley mass spectral library comparisons, and the mass spectra of authentic standards whenever possible.

The extracted samples were analyzed by both conventional py-GC-MS and employing the technique of simultaneous pyrolysis methylation (SPM) (19-20). The latter procedure calls for the addition of tetramethylammonium hydroxide (TMAH), at approximately 5-10 times the mass of sample to the pyrolysis boat prior to analysis (15). TMAH facilitates a methylation of acidic functional groups, which enhances chromatographic separation of pyrolyzates.

Mass percentages of samples pyrolyzed were obtained by weighing the pyrolysis boat before and after pyrolysis. The masses were obtained on a Cahn 21 Automatic Electrobalance.

## Results and Discussion

**CPMAS <sup>13</sup>C NMR.** The Goodwins, Giles Creek and Heaphy resinites were subjected to extraction to isolate an insoluble resinite polymer. This polymer represents the most highly polymerized fraction of each resinite. No doubt, the solvent used also extracts resinite polymerized to a lesser degree as well as occluded material. Both the Goodwins and Giles Creek resinites yielded very small amounts of a white powdery substance after extraction. The extracted Heaphy resinite displayed a golden yellow color. The Goodwins and Giles Creek samples yielded less than 20% of their mass as insoluble material while the Heaphy resinite was considerably less soluble, yielding 73% of its mass as insoluble polymer.

Fig. 2 contains the CPMAS <sup>13</sup>C NMR spectra of the extracted and unextracted resinite samples. Each spectrum consists of three distinct spectral regions corresponding to carboxyl carbons (190 ppm – 165 ppm), olefinic carbons (165 ppm – 100 ppm) and aliphatic carbons (100 ppm – 0 ppm). Resonances downfield of the carboxyl resonances (> 190 ppm) correspond to ketone functional groups.

**Carboxyl Carbons.** The carboxylic acid region of the spectra consists of three resonances: 186 ppm, 181 ppm, and 177 ppm. The 186 ppm resonance has been assigned to the axial carboxylic acid of diterpenoids (21) (most often non-polymeric) while the 177 ppm resonance is due to equatorial carboxylic acids, such as the C4 carboxyl group of communic acid (Fig. 1a) (10, 21-23). The 181 ppm resonance may

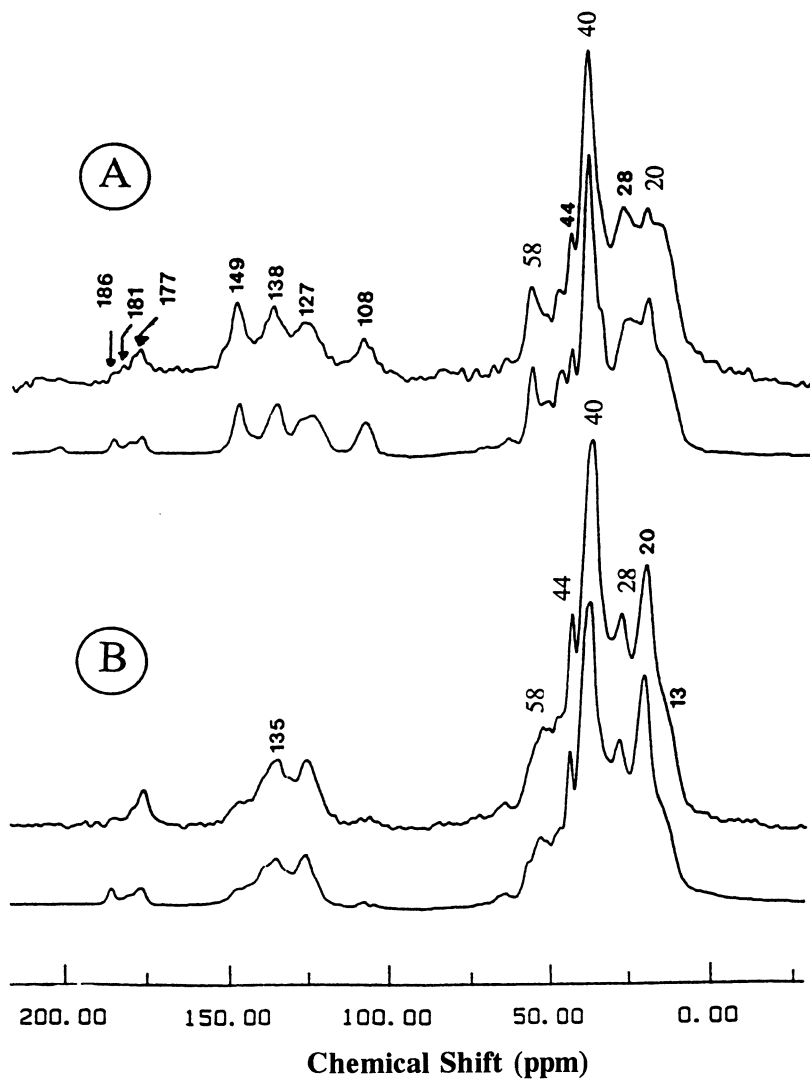


Figure 2. The CPMAS  $^{13}\text{C}$  NMR spectra for the A) extracted (top) and unextracted (bottom) Goodwins resinite and B) extracted (top) and unextracted (bottom) Heaphy resinite.

be due to configuration effects such as those observed in the spectrum of neoabietic acid (7), which contains both a 181 ppm and 186 ppm resonance. The 181 ppm resonance may also be due to carboxyl groups associated with non-polymeric components of the resinite samples.

Table II contains the integration data for the carboxyl region of the spectra. Total carboxyl content of the resinites, both as received and after Soxhlet extraction, remain relatively unchanged. In fact, the extracted resinites (spanning the rank range studied) contain an equivalent amount of carboxylic acids per diterpenoid monomer (0.5). However, carboxyl content is directly related to the original contribution of communic acid to the resinite polymer meaning that its consistency in the samples listed below may be fortuitous.

Table II.  $^{13}\text{C}$  NMR integration data for carboxyl carbons

Sample	186 <sup>a</sup> ppm	181 ppm	177 ppm	total
Goodwins	0.2 <sup>b</sup> (0.1) <sup>c</sup>	0.1 (0.1)	0.2 (0.3)	0.5 (0.5)
Giles Creek	0.1 <sup>d</sup> (ND)	0 (ND)	0.3 (ND)	0.4 (ND)
Heaphy	0.1 (0.1)	0.1 (0.1)	0.2 (0.3)	0.4 (0.5)

a = carbons per diterpenoid monomer (20 carbons); b = spectra of unextracted resinite; c = spectra of extracted resinite; d = spectra shown elsewhere (13); ND=not determined

**Olefinic Carbons.** The olefinic region contains resonances at 149 ppm, 138-135 ppm, 127 ppm and 108 ppm. The 149 ppm and 108 ppm resonances correspond to carbons 8 and 17, respectively and the 138-135 ppm and 127 ppm resonances correspond to carbons 11 and 12 of polylabdatrienes (Figure 1a-c) (1, 3, 21-22, 24-25). Table III contains the integration data for the olefinic resonances of the spectra.

Table III.  $^{13}\text{C}$  NMR integration data for olefinic carbons

Sample	149 <sup>a</sup> ppm	138-135 ppm	127 ppm	108 ppm	total
Goodwins	0.8 <sup>b</sup> (0.8) <sup>c</sup>	1.3 (0.9)	1.0 (1.1)	0.6 (0.7)	3.7 (3.5)
Giles Creek	0.5 <sup>d</sup> (ND)	0.9 (ND)	0.8 (ND)	0.4 (ND)	2.6 (ND)
Heaphy	0.4 (0.2)	1.0 (1.1)	1.1 (1.2)	0.1 (0.1)	2.6 (2.6)

a = carbons per diterpenoid monomer (20 carbons); b = spectra of unextracted resinite; c = spectra of extracted resinite; d = spectra shown elsewhere (13); ND = not determined

Total olefinic content of the extracted resinites ranges from 3.5 in the spectrum of the least mature Goodwins resinite to 2.6 in the most mature Heaphy resinite. This trend is consistent with that observed for communic acid-based resinites where total olefins per diterpenoid monomer ranged between 3.6 (unextracted) and 2.4 (extracted) for a Yallourn (lignite B) and Brunner (lignite A/subbituminous) resinite, respectively (14). Olefin reduction occurs primarily via a diminution in exocyclic methylene (C8-C17) carbons resulting in a monomer having only a single double bond. Previously reported dipolar dephasing studies have shown the remaining double bond to consist of a protonated (127 ppm) and a nonprotonated (138-135 ppm) carbon (8).

**Aliphatic Carbons.** The aliphatic region includes resonances centered around 58 ppm, 44 ppm, 40 ppm, 30 ppm, 28 ppm, 20 ppm, and 13 ppm. The 58 ppm resonance has been assigned to carbon 9 (Fig. 1) of polylabdatrienes (1).



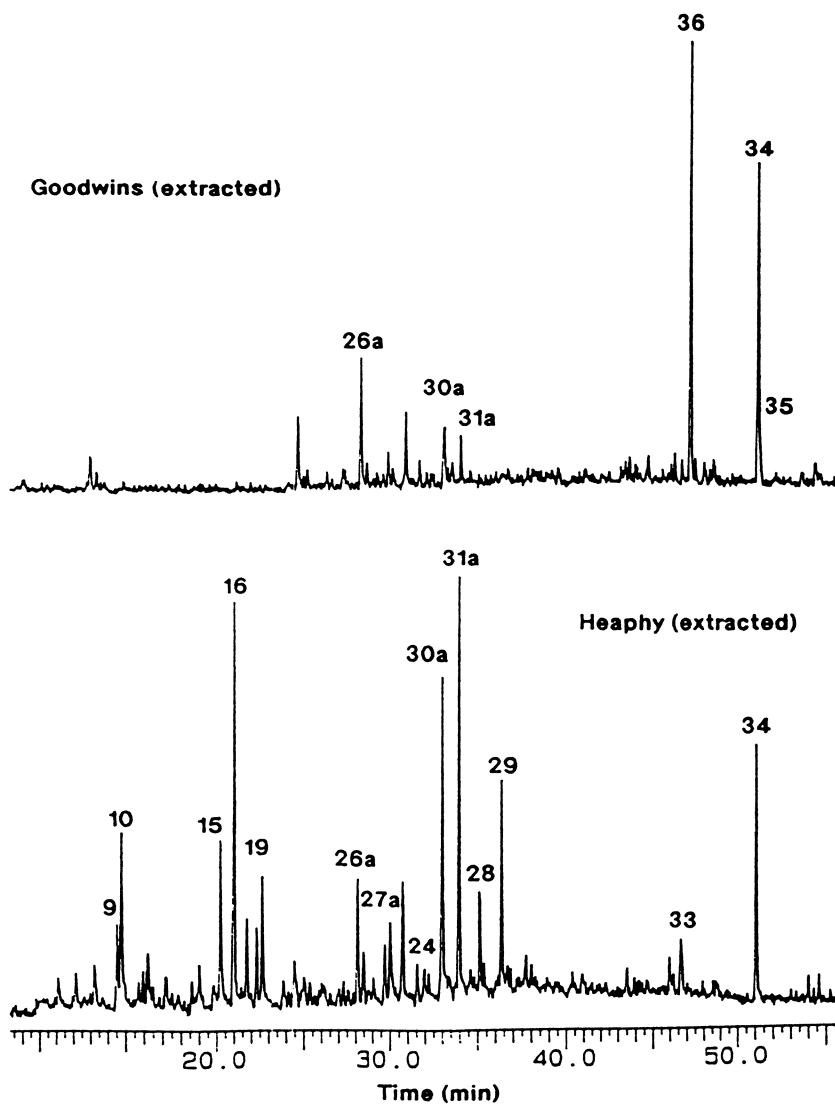


Figure 3. 540°C SPM pyrograms of the extracted Goodwins (top) and Heaphy (bottom) resinites.

The major effect of maturation evident in the spectra is the decrease in the resonance centered around 58 ppm. Decreasing abundance of the 58 ppm resonance, which has been assigned to carbon 9 of polylabdatrienes, is consistent with the depletion of exomethylene carbons with increasing rank.

**Py-GC-MS.** Polylabdanoid (Class Ib) resinites are conventionally analyzed (pyrolytically) employing the technique of *in-situ* methylation (15) with tetramethylammonium hydroxide (TMAH), a technique designed to methylate oxygenated functional groups during pyrolysis. *In-situ* derivatization (or SPM) allows for the analysis of large polar acids that are not amenable to GC as their underivatized free acids (19-20). The technique does, however, inhibit the analysis of compounds eluting in the first ten minutes of the chromatogram due to the swamping of this area by trimethylamines (from pyrolysis of TMAH). For this reason, the samples were analyzed by both conventional and SPM pyrolysis.

**SPM Pyrolysis.** The 540°C SPM pyrograms of the less mature Goodwins and more mature Heaphy resinites are shown in Fig. 3. The pyrolyzates dominating the chromatogram of the Goodwins resinite are the tricyclic acids methyl sandaracopimarate (peak 36; listed in Table IV) and methyl dehydroabietate (peak 34). The generation of primarily tricyclic acids upon pyrolysis of the extracted Goodwins resinite was unexpected. It is possible that peaks 34 and 36 are not pyrolyzates but remnants of the occluded resin acids liberated during pyrolysis. In a temperature profile of an unextracted Goodwins resinite, Anderson and Winans (15) demonstrated the emergence of hydronaphthenic acids and alcohols (representative of a commuonol/commuonic acid copolymer) at high temperature. Tricyclic acids observed in this study are, therefore, most likely the result of incomplete extraction. The pyrolyzate of the more mature Heaphy resinite, shown in Figure 3, contains a significant contribution from both alkylhydronaphthalenes as well as from naphthenic acids (26a, 27a, 30a, and 31a) and alcohols (24, 25, 28, and 29).

The effects of maturation evident in Fig. 3 include an increased abundance of alkylhydronaphthalenes (9-23) and a decrease in abundance of C3-acids (26a-27a) relative to C4-acids (30a-31a). The latter trend was first reported by Anderson et al. (3) for unextracted New Zealand resinites.

**Conventional Pyrolysis.** Results of conventional pyrolysis are shown in Fig. 4. The major pyrolyzates generated in the conventional pyrolysis of the extracted Goodwins resinite are hydronaphthenic acids (26, 27, 30, and 31) and alcohols (24, 25, 28, and 29). Both the Giles Creek and Heaphy resinites also yielded hydronaphthenic acids and alcohols along with an increased abundance of alkyl(hydro)naphthalenes (9-23).

The most notable difference between the two pyrolysis techniques (other than the lack of tricyclic acids in pyrograms of unmethylated pyrolyzates) is the significant decrease in mass of the sample being converted to pyrolyzates during SPM pyrolysis. Conventional methods yielded 71% and 69% of the Goodwins and Heaphy resinites as pyrolyzates, respectively, while SPM pyrolysis yielded only 33% and 25% of the respective samples as pyrolyzates. The lower SPM pyrolysis yields may be an indication of a decrease in pyrolysis severity as a result of TMAH/water volatilization or are possibly due to charring of the TMAH and sample resulting in underestimated values. Regardless, the pyrolysis product distributions of the two methods are comparable.

Maturation trends evident in Fig. 4 include the increasing relative abundance of the alkyl(hydro)naphthalenes with increasing maturity as well as the decreasing abundance of C3-hydronaphthenic acids (26a-27a) relative to the abundance of C4-hydronaphthenic acids (30a-31a). Also evident in this figure is the decrease in abundance of the C3-hydronaphthenic alcohols (24-25) giving rise to the C4-

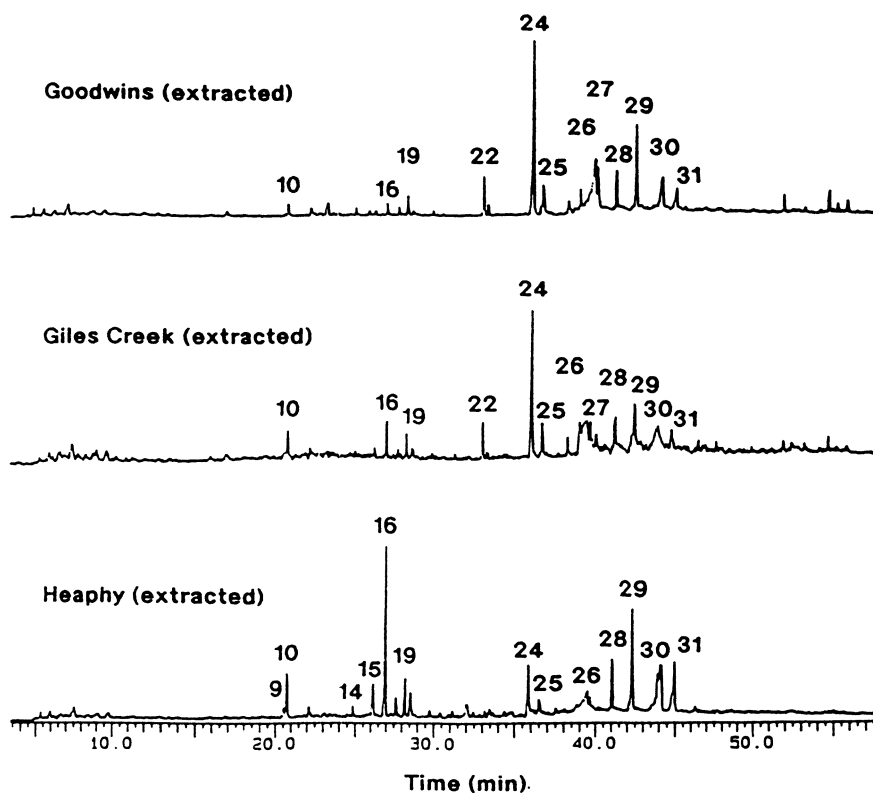


Figure 4. Conventional 540°C pyrograms of the extracted Goodwins (top), Giles Creek (middle) and Heaphy (bottom) resinites.

Table IV. Assignments for peaks labeled in Figures 3 and 4

peak	assignments	peak	assignments
1	unassigned mixture of alkenes	13	C3-hexahydronaphthalene <sup>c</sup>
2	C <sub>5</sub> H <sub>10</sub> (branched alkene) <sup>a</sup>	14	C2-tetrahydronaphthalene <sup>a</sup>
3	C <sub>6</sub> H <sub>10</sub> (branched alkene) <sup>a</sup>	15	C4-octahydronaphthalene
4	C <sub>7</sub> H <sub>12</sub> (branched alkene) <sup>a</sup>	16	C4-hexahydronaphthalene <sup>c</sup>
5	C <sub>8</sub> H <sub>14</sub> (branched alkene) <sup>a</sup>	17	C2-dihydronaphthalenes <sup>c</sup>
6	C2-benzene <sup>a</sup>	18	C4-hexahydronaphthalene <sup>c</sup>
7	terpene <sup>b</sup>	19	C4-hexahydronaphthalene <sup>c</sup>
8	C3-benzene <sup>a</sup>	20	C4-tetrahydronaphthalene <sup>c</sup>
9	C3-octahydronaphthalene <sup>c</sup>	21	C3-tetrahydronaphthalene <sup>c</sup>
10	C3-octahydronaphthalene <sup>c</sup>	22	C5-octahydronaphthalene <sup>c</sup>
11	C3-hexahydronaphthalene <sup>c</sup>	23	C3-naphthalene <sup>a</sup>
12	C3-hexahydronaphthalene <sup>c</sup>		
24	1-naphthalene-methanol-1,2,3,4,4a,7,8,8a-octahydro-1,4a,6-trimethyl [4aR-trans] <sup>d</sup>		
25	1-naphthalene-methanol-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl [4a,R-trans] <sup>d</sup>		
26	naphthalene-1(β)-carboxylic acid, 1,2,3,4,4a,7,8,8a-octahydro-1(α),4a(β),6-trimethyl <sup>d</sup>		
26a	naphthalene-1(β)-carboxylic acid, 1,2,3,4,4a,7,8,8a-octahydro-1(α),4a(β),6-trimethyl, methyl ester <sup>d,e</sup>		
27	naphthalene-1(β)-carboxylic acid, 1,2,3,4,4a,5,8,8a-octahydro-1(α),4a(β),6-trimethyl <sup>d</sup>		
27a	naphthalene-1(β)-carboxylic acid, 1,2,3,4,4a,5,8,8a-octahydro-1(α),4a(β),6-trimethyl, methyl ester <sup>d,e</sup>		
28	1-naphthalene-methanol-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl [4a,R-trans] <sup>d</sup>		
29	1-naphthalene-methanol-1,2,3,4,4a,8,8a-octahydro-1,4a,6-trimethyl-5-methylene [4a,R-trans] <sup>d</sup>		
30	naphthalene-1(β)-carboxylic acid, 1,2,3,4,4a,7,8,8a-octahydro-1(α),4a(β),5,6-tetramethyl <sup>d</sup>		
30a	naphthalene-1(β)-carboxylic acid, 1,2,3,4,4a,7,8,8a-octahydro-1(α),4a(β),5,6-tetramethyl, methyl ester <sup>d,e</sup>		
31	naphthalene-1(β)-carboxylic acid, 1,2,3,4,4a,8,8a-octahydro-1(α),4a(β)-trimethyl-5-methylene <sup>d</sup>		
31a	naphthalene-1(β)-carboxylic acid, 1,2,3,4,4a,8,8a-octahydro-1(α),4a(β)-trimethyl-5-methylene, methyl ester <sup>d,e</sup>		
32	unassigned unmethylated analog of MW=316 species <sup>c</sup>		
33	methyl-methyl podocaratrieneoate <sup>b,f</sup>		
34	methyl dehydroabietate <sup>h</sup>		
35	methylabietate <sup>b,f,g</sup>		
36	methyl sandaracopimarate <sup>b,g</sup>		

a = NBS Wiley Library; b = (7); c = tentative assignment; d = (15); e = (2); f = (6); g = (27); h = coelution with an authentic standard

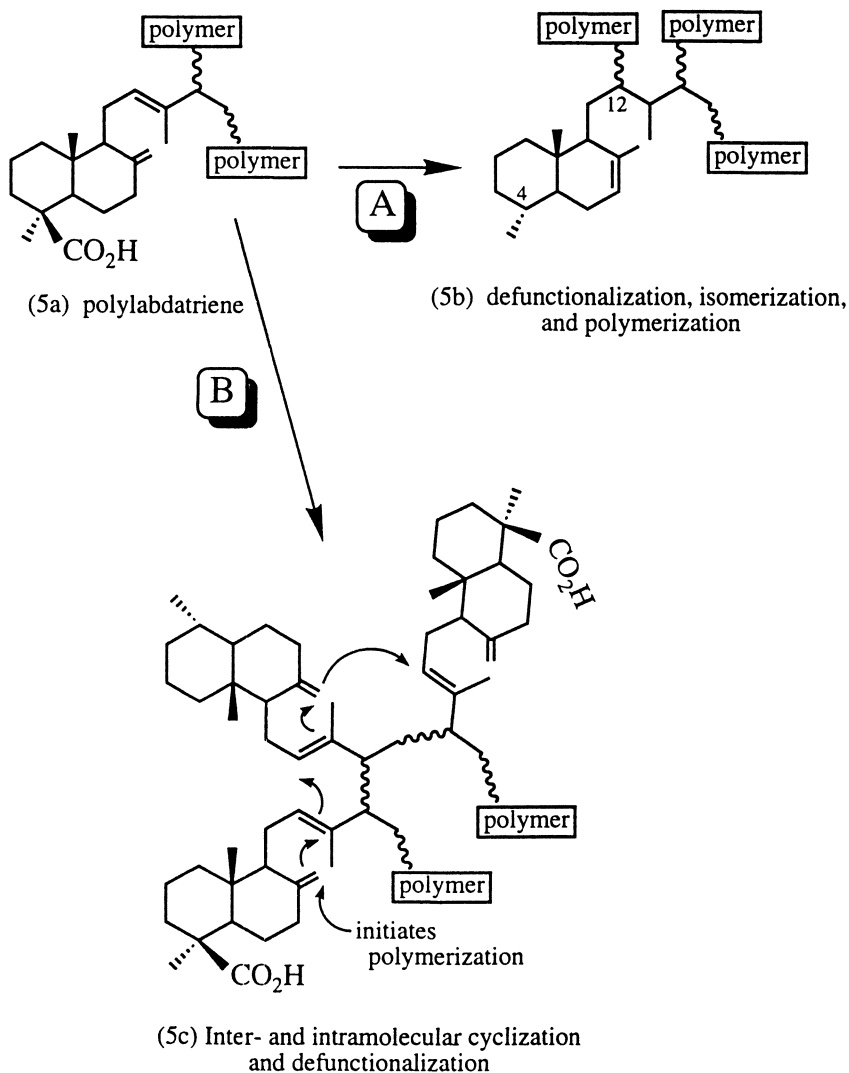


Figure 5. Maturation schemes proposed based on the results of this study.

hydronaphthenic alcohols (28-29), which is analogous to the trend observed for the acids and consistent with the results of SPM pyrolysis. Py-GC-MS results reported above are consistent with results reported previously (12) for communic acid-based resinites.

### Proposed Maturation Pathways.

Shown in Fig. 5A is a maturation scheme based on the results discussed above involving exomethylene isomerization, further polymerization of carbon 12, and defunctionalization of carbon 4. Depletion of exomethylene functionality is facilitated by the isomerization reactions while "loss" of olefinic carbons can be accounted for by the polymerization of carbon 12. The presence of alkyl(hydro)naphthalenes in the pyrolyzate of mature resinites can be attributed to the defunctionalization of carbon 4. Support for defunctionalization reactions has recently been presented by Anderson (26) based on decarboxylation of pyrolyzates of a Yallourn resinite

For the scheme shown as Figure 5A to be consistent with all available data, the isomerized exomethylene (C8-C17) must occupy the position between carbons 8 and 9. This criterion is based on the remaining olefin having a protonated and a nonprotonated carbon. With the only double bond allowed (by NMR) already placed in the monomer, it is necessary for defunctionalization to occur reductively so as not to add additional olefinic carbons to the matrix.

The major inconsistency of this scheme with the observed trends is its inability to account for the presence of tetra and dihydroalkylnaphthalenes as well as fully unsaturated alkylnaphthalenes in the pyrolyzate of mature resinites. These compounds can be better accounted for by the maturation scheme shown in Figure 5B. Proposed in this scheme (5B) are cyclization reactions occurring in the side chains as well as possible intramolecular cyclizations. The arrows shown in the figure indicate the general idea – they are not suggesting an actual reaction mechanism but show the possibility of condensation due to the proximity of olefinic carbons. Defunctionalization reactions have also been included in this scheme.

The resulting polymer, following this type of pathway, would be a multi-ringed system that could generate hydronaphthenic acids (26a, 27a, 30a, and 31a) as well as tricyclic acids (33-35) and alkyl(hydro)naphthalenes (9-23) upon pyrolysis. Cyclization reactions could also account for tetra- and dihydronaphthalenes as well as fully unsaturated alkylnaphthalenes in the pyrolyzates of the most mature resinites, which cannot be accounted for by defunctionalization of polylabdatriene monomers alone.

Condensation of polylabdanoid resinites can also account for the decreased solubility of the most mature resinites. With age and subsequent maturity, resinites are known to become increasing less soluble, as demonstrated in the results of this study. The Goodwins resinite, the least mature resinite analyzed in this study, was more than 90% soluble. The most mature sample, viz. the Heaphy resinite, yielded only 27% of its mass as low molecular weight soluble materials.

### Conclusions

- 1) CPMAS  $^{13}\text{C}$  NMR and py-GC-MS maturation trends observed are consistent with those observed for communic acid-based resinites (12).
- 2) Maturation results in the depletion of exomethylene (C8-C17) carbons. Total olefins per diterpenoid monomer approaches 2 (i.e. one double bond per 20 carbons).
- 3) Pyrolysis of a mature (subbituminous) resinite yields alkyl(hydro)naphthalenes along with pyrolyzates indicative of labdatriene precursors.

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## Chapter 6

# New Evidence Concerning the Structure, Composition, and Maturation of Class I (Polylabdanoid) Resinites

Ken B. Anderson<sup>1</sup>

Amoco Oil Company, P.O. Box 3011, Naperville, IL 60566-7011

Pyrolysis-Gas Chromatographic-Mass Spectrometric (Py-GC-MS) analysis of a range of Class I resinites indicates that the macromolecular structures of these resinites are typically derived from copolymers of labdanoid carboxylic acids, alcohols and hydrocarbons. Class Ia and Ib resinites are derived from copolymers of *regular* [1S, 4aR, 5S, 8aR] labdanoid diterpenes, especially communic acid, communol and/or biformenes. Class Ic resinites are based on structures derived from *enantio* [1S, 4aS, 5R, 8aS] labdanoids, especially ozic acid, ozol and/or *enantio* biformenes. The monomeric components of Class I resinites are readily determined in Py-GC-MS analyses by recognition of characteristic bicyclic products derived from the A/B ring system of the original labdanoid monomers.

In addition to bicyclic products derived directly from the labdanoid A/B ring system, products derived by modification of this ring system are also observed in Py-GC-MS analyses of some Class I resinites. These include: bicyclic methyl ethers produced by pyrolytic hydrolysis/O-methylation of succinylated (esterified) communol monomers within the macromolecular structure of Class Ia resinites; and, in samples of moderate maturity, C<sub>13</sub> and C<sub>14</sub> bicyclic products derived from A-ring defunctionalized labdanoids. The existence of these A-ring defunctionalized products indicates the existence of a heretofore unrecognized maturation pathway in polylabdanoid resinites.

NOTE: This chapter is Part V of a series entitled "The Nature and Fate of Natural Resins in the Geosphere." Part IV of this series is by Anderson, K. B. *Org. Geochem.* **1994**, *21*(2), 209-212.

<sup>1</sup>Current address: Chemistry Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439

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## Introduction and Background.

Amber has long been prized by entomologists and paleontologists for the extraordinary degree of preservation of fossil materials entrapped within it, especially insects and plant matter. Recent studies have shown that this high degree of preservation includes preservation of individual soft tissues and organs (1) and even preservation of molecular characteristics and DNA (2-4). Less well recognized however, is the fact that fossil resins preserve details of their own original molecular structure to a greater degree than perhaps any other form of sedimentary organic matter. The preservation of molecular structure in these materials, and the changes to the original structures which do occur in response to (primarily) thermal stress, have the potential to be of significant value in organic geochemical studies. For this reason, the structural characteristics of resinates are currently receiving considerable attention (5-14).

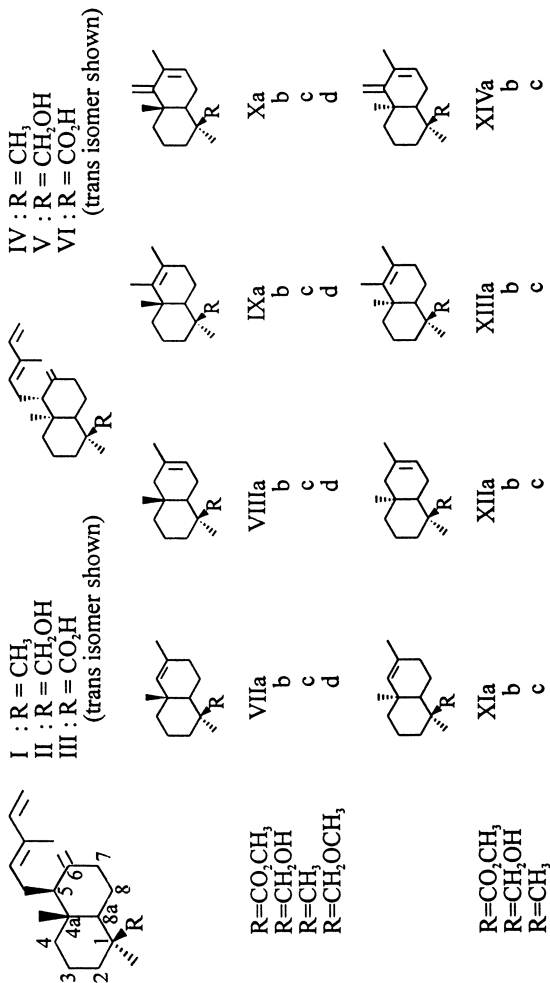
In earlier reports in this series it has been shown that most resinates can be classified into one of five classes based on the nature of their (macro)molecular structure (6-8). It has also been demonstrated that resinates derived from resins based on polymers of labdanoid diterpenes (Class I resinates), which are by far the most common and abundant class of resinite, can be further divided into three subclasses (6,8).

The macromolecular structures of all Class I resinates are based on polymers of labdanoid diterpenes. In many cases they also contain occluded mono-, sesqui-, di-, and/or triterpenoids and in some samples, especially immature samples, these may be more abundant than the polymeric component of the resinite. The composition of these occluded materials varies widely in both recent and fossil resins. The nature of the polylabdanoid macromolecular structure however, is highly conservative across a wide range of modern and especially fossil resins. Hence although low molecular weight non polymeric materials may be highly useful for provenance and paleobotanical studies, their usefulness for the purposes of classification and geochemical studies is limited.

Sub-classification of Class I resinates is based on details of the structural characteristics of the labdanoid diterpenes from which the polymeric component of the resinite is derived. The macromolecular component of Class Ia and Ib resinates is based on polymers of labdanoid diterpenes having a *regular* configuration (ie, [1S, 4aR, 5S, 8aR]) especially commun acid (III) and communol (II). These two subclasses are then further differentiated by the presence of succinic acid, which is incorporated in significant amounts into the macromolecular structure of Class Ia resinates (presumably as a cross-linking agent between labdanoid alcohols, especially communol). Conversely, Class Ic resinates are based on polymers of labdanoid diterpenes with the *enantio* [1S, 4aS, 5R, 8aS] configuration such as ozic acid (VI) (6,8).

In Py-GC-MS analyses, differentiation of sub-classes of Class I resinates is achieved by recognition and identification of characteristic bicyclic products derived from the A/B ring system of the original labdanoid monomers. A large percentage of Class I resinates contain significant amounts of polymerized commun acid (III), and in some cases, the macromolecular component of the resinates is derived virtually entirely from this monomer. Pyrolysis of such materials results in generation of four characteristic bicyclic acids (in addition to release of occluded material) which are

Structures Cited in Text



Victorian Brown Coal  
(Yallourn seam) Resinite

Unextracted

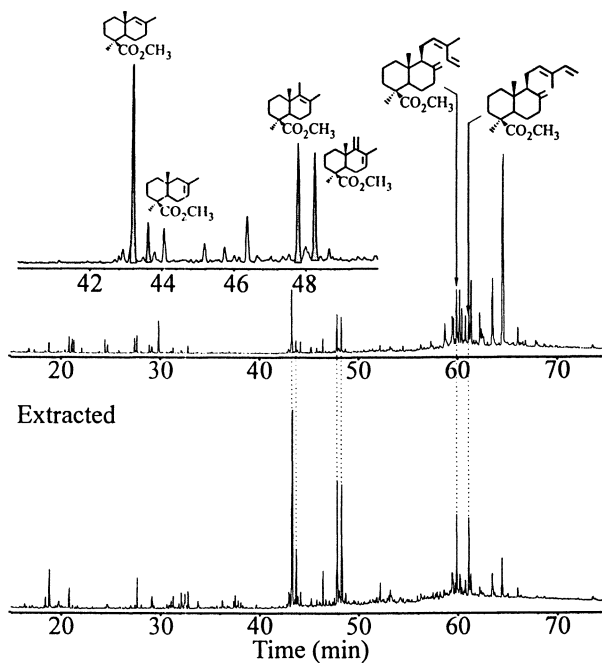


Figure 1. Total ion chromatograms obtained by Py-GC-MS analysis of whole (unextracted) and extracted Victorian Brown Coal resinite (Yallourn seam). Expansion of chromatogram showing elution of characteristic bicyclic acids is inset. Products observed in the chromatogram of the extracted sample are derived from the macromolecular component of the resinite.

readily identified from the mass spectral characteristics and chromatographic behavior of their corresponding methyl esters VIIa - Xa (6). An example is illustrated in Figure 1. That these characteristic bicyclic products are derived from the macromolecular component of the resinites is readily established by pyrolysis of thoroughly extracted samples, as also illustrated in Figure 1. Pyrolysis of ambers derived from resins based on ozic acid (VI) results in generation of four analogous epimeric bicyclic acids, which are readily differentiated from those derived from communic acid on the basis of the chromatographic behavior of their corresponding methyl esters XIa - XIVa (6).

Until recently, the presence of these characteristic bicyclic acids (methyl esters) in the pyrolysate of a resinite was a primary basis for classification of Class I resinites (6). However, in a recent report in this series (8), Py-GC-MS data for an Upper Cretaceous resinite from the Taimyr Peninsula (Siberia) were presented which indicate the presence of series of bicyclic hydrocarbons (VIIc - Xc) and alcohols (VIIb - Xb) in the pyrolysate of this sample. These data, and data from two additional related Middle and Upper Cretaceous samples from the same region are illustrated in Figure 2. The pyrolysates of all of these samples are dominated by bicyclic hydrocarbons (VIIc - Xc) and alcohols (VIIb - Xb) which are clearly analogous to the characteristic bicyclic acids observed in the pyrolysates of samples such as that illustrated in Figure 1. The presence of these characteristic bicyclic alcohols and hydrocarbons are interpreted as indicating that the macromolecular structures of these ambers are derived from a copolymeric structure in which the primary monomers are biformene (I) and communol (II). The absence of compounds VIIa - Xa indicates that communic acids are absent. These observations suggest that these samples represent a previously unrecognized form of Class Ib resinite. (A third series of compounds, viz, A-H, will be discussed later in this text).

The observation and assignment of compounds VIIb - Xb and VIIc - Xc immediately raises the question of the significance of these products in the pyrolysates of other Class I resinites, and hence the significance of labdanoids other than communic and ozic acids in the macromolecular structures of other Class I resinites. A review of unpublished Py-GC-MS data generated by the author over the past several years suggested that these characteristic alcohols and hydrocarbons were present in a significant number of Class I resinites. Therefore, in order to determine the significance of diterpenoids other than communic and ozic acids in the macromolecular structures of other Class I resinites, Py-GC-MS analyses of a diverse series of samples have been undertaken. All samples were reanalyzed under identical conditions, and as much as possible, series of samples were analyzed "back-to-back" to ensure that both chromatographic and mass spectral data were directly comparable.

The primary purpose of this report is to discuss the observation of characteristic products derived from communol and biformene in a wide range of Class Ia and Ib resinites, and to report identification of analogous *enantio* bicyclic alcohols and hydrocarbons in the pyrolysates of Class Ic resinites. Furthermore, insights gained during identification of these characteristic alcohols and hydrocarbons have subsequently enabled identification of a number of other significant series of compounds. The identification of these compounds, which are described in detail below, has significant implications for the structure, maturation, and analysis of Class I resinites.

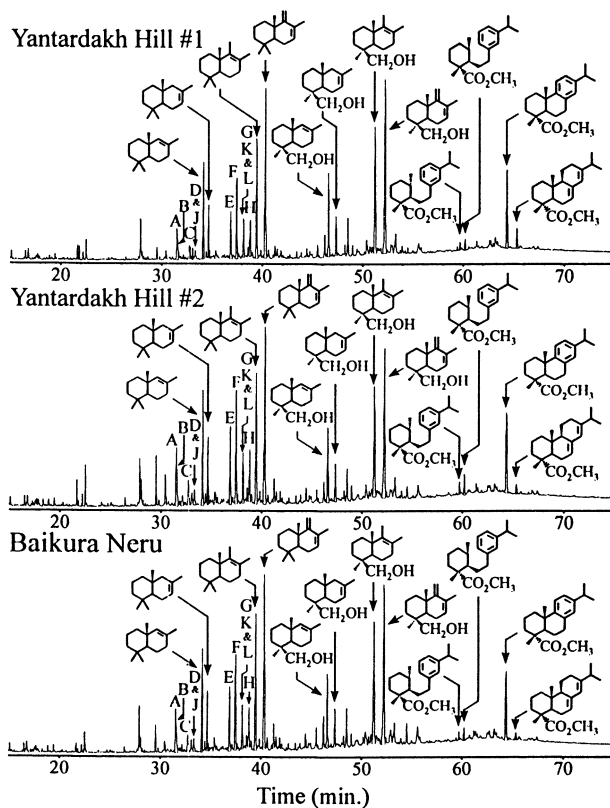


Figure 2. Total ion chromatograms obtained by Py-GC-MS of commuonl/biformene copolymeric Class Ib resinites obtained from Middle and Upper Cretaceous sediments in the Taimyr Peninsula, Siberia. See also reference 8.

### Experimental.

Py-GC-MS analyses were conducted using an HP 5890(II) GC directly coupled to an HP 5970 or 5971 mass selective detector. Pyrolyses were carried out using a CDS "Pyroprobe" pyrolysis system.  $T_{\text{Py}} = 480^{\circ}\text{C}$  in all cases reported herein. Methylation of acidic components of the pyrolysates was achieved in-situ by co-pyrolysis with tetramethyl ammonium hydroxide (TMAH) as described elsewhere (5).

Chromatographic conditions used to obtain the data reported herein are as follows: Column = 60m DB-1701, Temperature program:  $T_{\text{(initial)}} = 40^{\circ}\text{C}$  (1.5 min), Ramp rate =  $4^{\circ}\text{C}/\text{min}$ ,  $T_{\text{(final)}} = 280^{\circ}\text{C}$ . Additional data, (not illustrated) have also been generated using 60m DB-5 and RTx-200 capillary GC-columns.

Off-line pyrolysis was carried out as follows. Pre-extracted Victorian Brown Coal resinite (0.75g) was loaded into an  $\text{N}_2$  purged pyrex pyrolysis tube. After allowing sufficient time for all air to be purged, the pyrolysis tube was then placed into a furnace preset at  $450^{\circ}\text{C}$  for 2 minutes. (Note: pyrolysis of this sample was found to be significantly exothermic. Direct measurement of the temperature at the sample indicated that after this period of time an actual  $T_{\text{max}}$  of  $600^{\circ}\text{C}$  had been reached). The tube was removed and allowed to cool to ambient temperature. Volatile products evolved during pyrolysis were trapped using a coil type water cooled glass condenser, loosely packed with glass wool. (Note: trapping of volatile products was imperfect, therefore, no attempt was made to calculate pyrolysis yields.) The pyrolysate was then collected in THF (20mL) and reacted with 1.0M  $\text{LiAlH}_4$  (10 mL) for 2.5 hours. The product of this reaction was then filtered and concentrated by rotary evaporation. GC-MS analysis confirmed that the desired alcohol products had been generated as the primary products of this reaction. A small aliquot of this product (re-dissolved in  $\text{CH}_2\text{Cl}_2$ ) was then methylated using  $(\text{CH}_3)_3\text{SiCHN}_2/\text{HBF}_4$  (15). GC-MS analysis confirmed that the desired methyl ether products had been prepared. The identity of these products with those observed in the pyrolysates of Class Ia (and Ib) resinites was confirmed by co-pyrolysis.

Succinylation of Baikura-Neru Resinite: Resinite (132.7 mg) was placed in a small flask together with  $\text{CH}_2\text{Cl}_2$  (10 mL). After stirring for 30 min to allow time for the resinite to swell, succinic anhydride (126 mg) was added and the reaction was then stirred overnight at room temperature.  $\text{CH}_3\text{OH}$  (10 mL) was then added to the reaction. This resulted in complete dissolution of the product. Therefore, the product was diluted with  $\text{CH}_3\text{OH}$  (70 mL) and  $\text{H}_2\text{O}$  (15mL) to precipitate the product and concentrated by rotary evaporation. The precipitate was then filtered through a  $0.45\ \mu\text{m}$  filter, washed with a few mL of  $\text{H}_2\text{O}$ , and dried under vacuum at room temperature. Yield = 119 mg.

Reductive decarboxylation products were prepared from the off-line pyrolysate of extracted Victorian Brown Coal resinite using the method initially described by Barton and co-workers (16). Briefly, the off line pyrolysate of extracted VBC resinite ( $\sim 0.5\text{mmol}$ ) was reacted with (1) oxalyl chloride (2M in Benzene) and DMF (trace) to produce the corresponding acid chlorides. The product of this reaction was then allowed to react with a dry suspension of 2-mercaptopyridine N-oxide sodium salt (250mg) and DMAP (26mg) in benzene (60mL) ( $\text{N}_2$ ). After stirring for 30 minutes, freshly distilled t-butyl mercaptan (500  $\mu\text{L}$ ) was added and the reaction was then refluxed for 2.5 hours. The product was then extracted with  $\text{H}_2\text{O}$  (2X40mL), dried over  $\text{MgSO}_4$  and chromatographed over silica gel with n-pentane in the usual way.

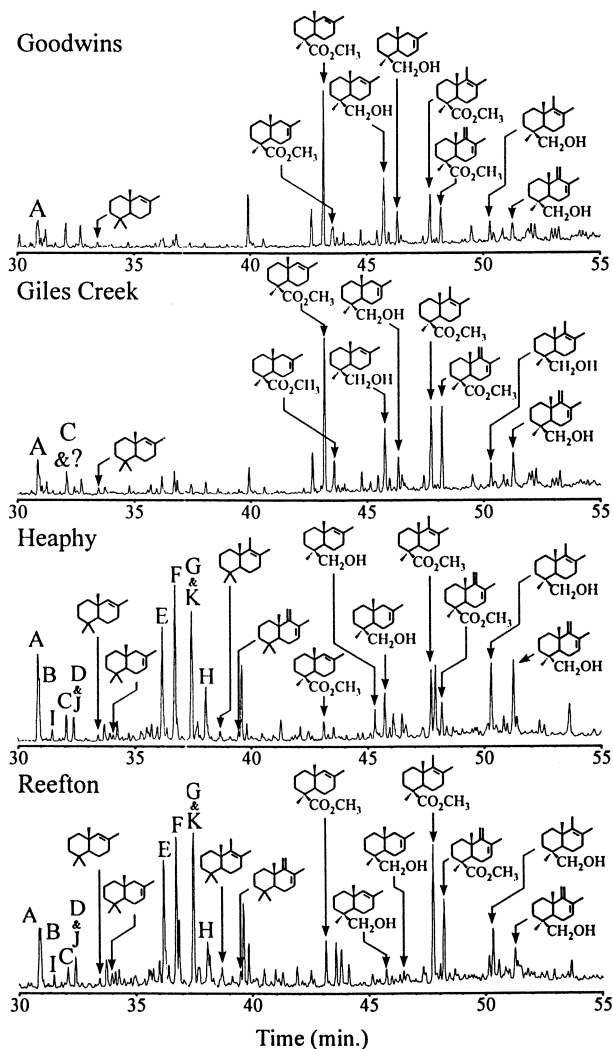


Figure 3. Partial chromatograms obtained by Py-GC-MS analysis of Class Ib resinites of increasing rank collected from a series of New Zealand coal fields. Characteristic bicyclic acids, alcohols, and hydrocarbons reflecting the distribution of labdanoid monomers in the original resin are indicated.

### Results and Discussion.

Partial Py-GC-MS total ion chromatograms for four Class Ib resinites collected from coal fields in New Zealand are illustrated in Figure 3. These data illustrate the distribution of polymer-derived characteristic bicyclic products in the pyrolysates of these samples. (Some data generated from earlier analyses of these samples has been reported previously (5,6)). These samples are all likely to be derived from a common botanical source (*Agathis*) and hence give an indication of the variability of the composition of related resinites. As illustrated in this Figure, in addition to characteristic bicyclic acids derived from structures related to communic acid, varying amounts of the bicyclic alcohols and hydrocarbons observed in the pyrolysates of the Siberian resinites (see Figure 2) are also observed in the pyrolysates of all of these samples, indicating that the polymeric components of these resinites incorporate communol and biformene in addition to communic acid. (The effects of increasing maturity on the distribution of products observed in the pyrolysates of these four resinites will be discussed latter in this text. (See also reference 6).

Communol and biformene derived characteristic bicyclic products are also observed in the pyrolysates of Class Ia resinites, as illustrated in Figure 4. A number of previous workers have suggested that the macromolecular structure of succinite probably incorporates communol units to allow for cross linking of the structure by succinic acid (17,18). The observation of compounds VIIb-Xb in the data illustrated in Figure 4 provides direct evidence confirming that this monomer is abundant in the macromolecular structure of Class Ia resinites. The presence of abundant biformene monomers, (indicated by the observation of compounds VIIc -Xc) in the structures of these resinites has not been previously reported.

In addition to compounds VII(a-c) - X(a-c), a fourth series of analogous compounds was also observed in the pyrolysates of these samples. Based on interpretation their mass spectra, these compounds were tentatively assigned as methyl ethers analogous to the bicyclic alcohols derived from communol. (See structures VIId - Xd.) Mass spectra for these compounds are given in Table I below.

In order to confirm the identification of compounds VIId - Xd and to substantiate the assignment and stereochemistry of compounds VIIb-Xb, mixtures of these compounds were prepared synthetically by sequential reduction ( $\text{LiAlH}_4$ ) and methylation ( $(\text{CH}_3)_3\text{SiCHN}_2/\text{HBF}_4$ ) of the off-line pyrolysate of an extracted Victorian Brown Coal resinite derived virtually entirely from communic acid. (See Figure 1) The chromatographic behavior and mass spectral characteristics of the products from these reactions were identical to those of the products generated *in-situ* by Py-GC-MS analysis. (Note: Analogous epimeric bicyclic methyl ether products have now also been observed in the pyrolysates of fossil resins derived from *Pseudolarix*. (See Part VI of this series, Anderson and Lepage, in this volume.)

A search of the literature indicates that although a small number of labdanoid methyl ethers are known (19-21) these compounds are rare and generally occur in low abundance. Furthermore, those which have been characterized lack the side-chain characteristics necessary for polymerization into polylabdanoid structures. This suggested that compounds VIId - Xd might be secondary products resulting from the *in-situ* methylation procedure used for these analyses. To verify this premise, Py-GC-MS analyses were carried out using  $\text{D}_{13}$ TMAH. The results of these experiments



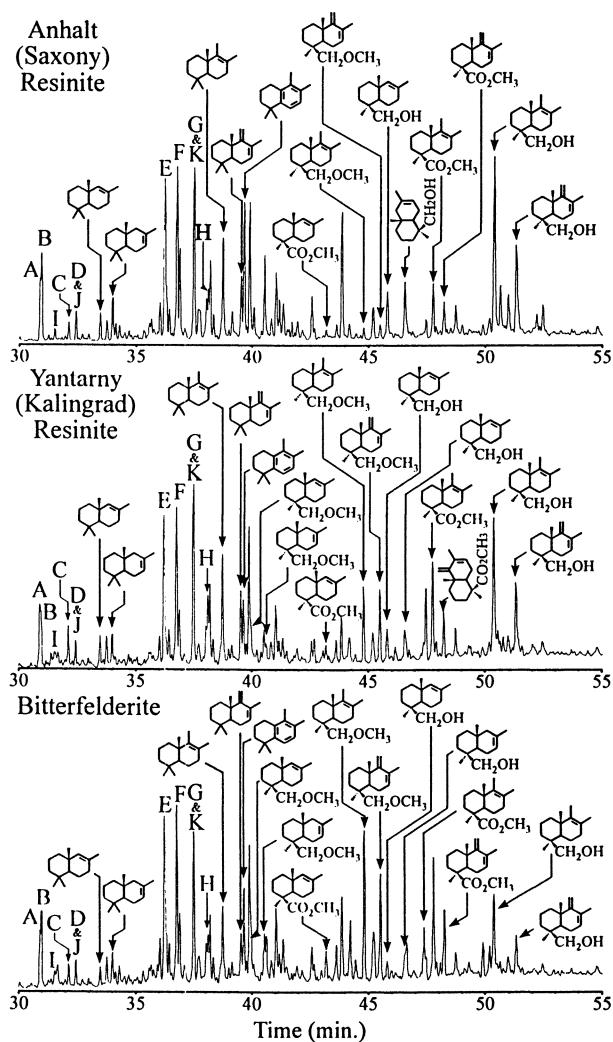


Figure 4. Partial chromatograms obtained by Py-GC-MS analysis of Class Ia resinites, illustrating the distribution of bicyclic products derived from communic acid, communol, and biformene. Succinite = USNM 353431. See text for discussion of the origin of bicyclic methyl ethers.

unequivocally established that the O-methyl group in these compounds derived from the methylation procedure. However, the low to very low abundance of these products in the pyrolysates of other Class Ib resinites, including those illustrated in Figures 2 and 3, indicates that direct methylation of communol or products derived from communol (eg, compounds VIIb-Xb) by TMAH is not significant under the conditions used for these analyses.

**Table I. MS Data for Characteristic *Regular* Bicyclic Methyl Ethers**

Compound	MS Data and Assignment
<b>VIIId</b>	222(8), 207(4), 190(12), 77(93), 175(27), 161(10), 147(15), 135(15), 133(20), 121(35), 119(38), 107(70), 105(65), 98(64), 95(100), 93(59), 91(65), 81(67), 79(52), 77(43), 69(16), 67(36), 65(19), 55(52), 53(29), 45(85), 41(73), 39(33), 29(37), 15(9). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1-methoxymethyl-1,4a,6-trimethyl [1S, 4aR, 8aR]
<b>VIIIId</b>	222(7), 190(30), 177(22), 175(17), 161(13), 147(7), 133(26), 121(23), 120(43), 119(68), 109(38), 107(100), 105(81), 97(26), 95(46), 93(38), 91(55), 81(38), 79(43), 77(33), 69(14), 67(37), 65(15), 55(63), 53(27), 45(72), 41(65), 39(29), 29(32), 15(7). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-1-methoxymethyl-1,4a,6-trimethyl [1S, 4aR, 8aR]
<b>IXd</b>	236(11), 221(10), 204(6), 191(36), 189(30), 161(18), 149(21), 147(17), 135(47), 133(32), 121(62), 119(62), 109(64), 107(53), 105(40), 98(34), 95(84), 93(46), 91(58), 83(22), 81(28), 79(42), 77(36), 69(19), 67(42), 65(16), 59(25), 55(61), 53(29), 45(100), 41(83), 29(36), 15(8). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1-methoxymethyl-1,4a,5,6-tetramethyl [1S, 4aR, 8aR]
<b>Xd</b>	234(16), 202(6), 189(12), 187(37), 173(17), 159(35), 146(26), 145(43), 133(68), 132(100), 131(33), 120(28), 119(92), 117(29), 115(19), 107(36), 105(53), 95(19), 93(23), 91(76), 81(20), 79(39), 77(46), 67(22), 65(20), 55(47), 53(28), 45(87), 41(65), 39(31), 29(35), 27(16), 15(10). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-1-methoxymethyl-1,4a,6-trimethyl-5-methylene [1S, 4aR, 8aR]

There is strong evidence from infrared analyses to suggest that succinic acid is present in Class Ia resinites at least partially in its esterified form (22), and as noted above, it has been suggested that communol units within the structures of Class Ia resinites are likely to be at least partially esterified with succinic acid. This suggested that the abundance of compounds VIIId - Xd in the pyrolysates of the Class Ia resinites illustrated in Figure 4, may reflect partial esterification of communol units with succinic acid.

To test this hypothesis, succinyl-Baikura Neru resinite was prepared by reaction

with succinic anhydride. A partial total ion chromatogram illustrating the distribution of characteristic bicyclic methyl ethers and acids observed in the pyrolysate of this product is illustrated in Figure 5. This analysis demonstrates that pyrolysis of this succinylated product in the presence of TMAH results in formation of abundant methyl ether products which are not observed in the pyrolysate of the underivatized resinite (cf. Figure 2). It has not yet been determined if pyrolysis with *in-situ* methylation of esters other than succinyl esters gives the same distribution of products as is observed in these analyses. However, the presence of low levels of compounds VII d -X d in resinites which do not contain detectable levels of succinic acid suggests that this may be the case. It is also apparent from the data illustrated in Figure 5 that methyl esters (ie compounds VII a -X a) are also abundant in the pyrolysate of this product. This suggests that the abundance of compounds VII a -X a in the pyrolysates of Class Ia resinites may not necessarily directly reflect the abundance of communic acid moieties in the macromolecular structures.

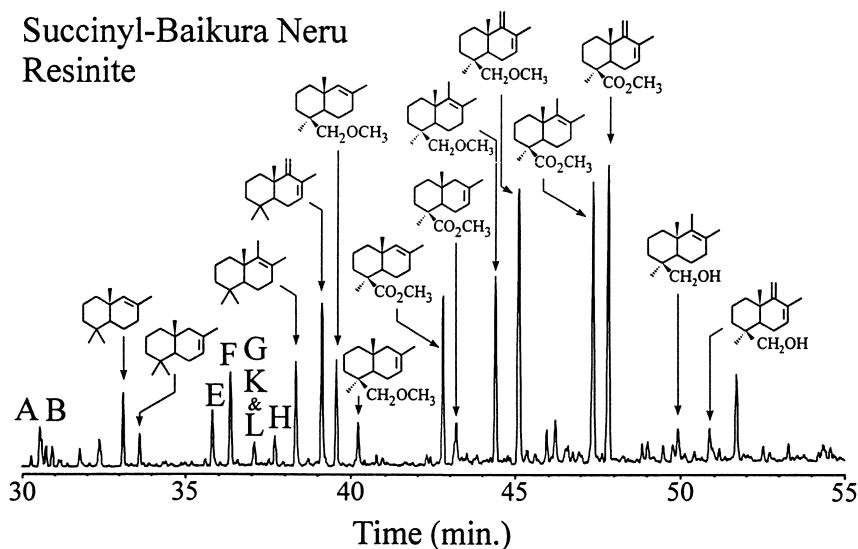


Figure 5. Partial Py-GC-MS chromatogram illustrating the distribution of bicyclic products observed in the pyrolysate of succinylated Baikura Neru (Cretaceous, Taimyr Peninsula, Siberia) resinite. Characteristic bicyclic methyl ethers and acids are not observed in the pyrolysate of the original resin (see Figure 1).

Additional analysis of this reaction is currently in progress, but it is clear from these observations that the characteristic bicyclic methyl ethers observed in the pyrolysates of Class Ia resinites reflect (at least in part) succinylation of communal units within the macromolecular structure.

In order to determine if *enantio* bicyclic products analogous to compounds VII(b-d)-X(b-d) could be observed in the pyrolysates of Class Ic resinites, an investigation of a series of Class Ic resinites was also undertaken. (Characteristic *enantio* bicyclic acids derived from polymerized ozic acid (XIa-XIVa) have previously been identified (6)). Py-GC-MS data from two well known Class Ic resinites are illustrated in Figure 6. As illustrated in this figure, *enantio* bicyclic alcohols (XIb - XIVb) and hydrocarbons (XIc - XIVc) are very abundant in the pyrolysates of these resinites and, in these samples, are significantly more abundant than their analogous bicyclic acids. (As discussed below, it is not possible to differentiate compounds VIc - Xc and compounds XIc - XIVc on the basis of chromatographic behavior. In this case, the stereochemistry of compounds XIc - XIVc has been assigned by analogy with the corresponding bicyclic alcohols and acids. This assumes that the stereochemistry of all of the original labdanoid precursors incorporated into the macromolecular structures of the resins from which these ambers are derived was equivalent. Additional analyses to confirm this assumption and hence, the stereochemistry of these characteristic bicyclic hydrocarbons is currently in progress. See also additional discussion below.) These products are obviously analogous to those derived from communal and biformene in the pyrolysates of Class Ia and Ib resinites and hence reflect the presence of ozol (V) and *enantio* biformenes (IV) in the polymeric structure of these resinites. Mass spectral data for compounds XI(a-c) - XIV(a-c) are given in Table II.

Comparison of the data given in Table II with that reported in earlier publications in this series (6-8) demonstrates that in most cases, mass spectral data alone are often insufficient to distinguish between enantiomeric products observed in the pyrolysates of Class I resinites. Therefore, in order to differentiate differing forms of polylabdanoid resinite, careful comparison and analysis of chromatographic data is also essential.

Chromatographic differentiation between the bicyclic acids (methyl esters) derived from communic acid (XV-XVIII) and those derived from ozic acid (XIII-XXVI) has been discussed previously (6). *Regular* and *enantio* bicyclic alcohols can also be distinguished on the basis of their chromatographic behavior as demonstrated by data illustrated in Figure 7. However, the characteristic *regular* and *enantio* series

**Table II. MS Data for Characteristic *Enantio* Bicyclic Methyl Esters, Alcohols and Hydrocarbons**

Compound	MS Data and Assignment
<b>XIa</b>	236(8), 221(3), 204(2), 177(42), 176(26), 161(100), 147(6), 133(9), 121(20), 119(12), 107(38), 105(30), 95(24), 93(20), 91(31), 81(22), 79(22), 77(20), 69(8), 67(13), 65(8), 59(11), 55(15), 53(9), 41(23), 39(10), 29(7). Naphthalene-1-carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,6-trimethyl methyl ester [1S, 4aS, 8aS]
<b>XIIa</b>	236(8), 221(1), 204(6), 177(100), 161(61), 145(6), 133(15), 121(74), 120(32), 119(28(109(68)), 107(49), 105(58), 95(36), 93(35), 91(51), 81(30), 79(35), 77(35), 69(16), 67(24), 65(14), 59(15), 55(33), 53(21), 41(40), 39(21), 29(17), 27(13). Naphthalene-1-carboxylic acid-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl methyl ester [1S, 4aS, 8aS]
<b>XIIIa</b>	250(11), 235(15), 207(4), 203(8), 191(25), 190(15), 175(100), 161(4), 149(5), 147(11), 135(22), 133(15), 121(30), 119(54), 109(24), 107(31), 105(21), 95(18), 93(18), 91(31), 83(14), 81(15), 79(22), 77(20), 69(11), 67(17), 65(8), 59(15), 55(22), 53(11), 41(32), 39(10), 29(8), 15(6). Naphthalene-1-carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl methyl ester [1S, 4aS, 8aS]
<b>XIVa</b>	248(18), 233(2), 216(3), 189(19), 188(41), 174(15), 173(100), 161(11), 159(17), 148(15), 147(14), 146(15), 145(28), 134(16), 133(83), 132(51), 121(17), 120(22), 119(58), 117(20), 115(20), 109(17), 107(29), 105(40), 95(15), 93(22), 91(58), 81(15), 79(31), 77(36), 69(13), 67(13), 65(15), 59(19), 55(24), 53(15), 41(35), 39(17), 29(10), 15(9). Naphthalene-1-carboxylic acid -1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl-5-methylene methyl ester [1S, 4aS, 8aS]
<b>XIb</b>	208(6), 193(12), 177(100), 175(35), 161(7), 147(9), 135(10), 133(11), 121(23), 119(19), 109(16), 107(60), 105(40), 95(81), 93(44), 91(52), 81(66), 79(39), 77(30), 69(17), 67(32), 65(15), 55(46), 53(24), 43(33), 41(65), 39(30), 31(57), 29(27), 27(15). 1-Naphthalenemethanol-1,2,3,4,4a,7,8,8a-octahydro-1,4a,6-trimethyl [1S, 4aS, 8aS]
<b>XIIb</b>	208(17), 190(26), 177(41), 175(25), 161(33), 147(11), 135(16), 133(29), 121(30), 120(43), 119(65), 109(98), 107(59), 106(39), 105(90), 97(69), 95(98), 93(52), 91(72), 81(70), 79(50), 77(46), 69(20), 67(51), 65(21), 55(100), 53(35), 43(40), 41(81), 39(41), 31(75), 29(40), 27(24). 1-Naphthalenemethanol-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl [1S, 4aS, 8aS]

Table II (continued).

Compound	MS Data and Assignment
<b>XIIIb</b>	222(15), 207(28), 191(42), 189(18), 179(6), 161(11), 149(14), 147(13), 135(28), 133(17), 121(44), 119(29), 109(55), 107(36), 105(31), 95(100), 93(33), 91(45), 83(24), 81(30), 79(32), 77(26), 69(15), 67(36), 65(15), 57(18), 55(48), 53(24), 43(30), 41(67), 39(24), 31(53), 29(28), 27(13). 1-Naphthalenemethanol-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl [1S, 4aS, 8aS]
<b>XIVb</b>	220(32), 189(16), 187(33), 173(23), 161(23), 159(28), 147(19), 146(16), 145(42), 135(22), 133(66), 132(82), 131(31), 121(39), 120(27), 119(82), 117(31), 115(24), 109(23), 108(36), 107(74), 105(73), 95(43), 93(48), 91(100), 81(30), 79(54), 77(63), 69(14), 67(28), 65(27), 55(68), 53(38), 43(33), 41(89), 39(46), 31(87), 29(39), 27(26). 1-Naphthalenemethanol-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl-5-methylene [1S, 4aS, 8aS]
<b>XIc</b>	192(26), 177(85149(17), 136(12), 135(11), 123(37), 122(33), 121(35), 109(25), 107(82), 105(22), 95(72), 93(51), 91(54), 81(86), 79(42), 77(40), 69(62), 67(35), 65(19), 55(49), 53(33), 43, 19), 41(100), 39(41), 29(37), 27(23). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1,1,4a,6-tetramethyl [4aS, 8aS]
<b>XIIc</b>	192(22), 177(18), 149(4), 137(8), 136(8), 124(30), 123(12), 121(11), 109(100), 107(34), 95(24), 93(24), 91(30), 81(36), 79(24), 77(19), 69(24), 67(23), 65(12), 55(37), 53(19), 43(13), 41(59), 39(25), 29(18), 27(15). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-1,1,4a,6-tetramethyl [4aS, 8aS]
<b>XIIIc</b>	206(23), 191(59), 163(9), 150(13), 149(9), 135(28), 121(75), 109(46), 107(43), 105(22), 95(100), 93(25), 91(42), 83(28), 81(27), 79(29), 77(27), 69(35), 67(29), 65(13), 56(19), 55(49), 53(26), 43(22), 41(86), 39(28), 29(32), 27(18). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1,1,4a,5,6-pentamethyl [4aS, 8aS]
<b>XIVc</b>	204(47), 189(21), 161(37), 148(16), 147(17), 135(31), 133(48), 122(24), 121(38), 120(28), 119(72), 109(24), 108(48), 107(51), 105(85), 95(27), 93(48), 91(82), 79(43), 77(52), 69(20), 67(21), 65(27), 55(56), 53(34), 43(19), 41(100), 39(44), 29(35), 27(28). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-1,1,4a,6-tetramethyl-5-methylene [4aS, 8aS]

bicyclic hydrocarbons (VIc - Xc and XIc - XIVc) are true enantiomers and hence cannot be resolved using normal chromatographic phases. (Corresponding *regular* and *enantio* series characteristic bicyclic acid and alcohols are epimers rather than true enantiomers, and hence are separable under normal chromatographic conditions.) Therefore, differentiation of these compounds requires specialized chromatographic media.

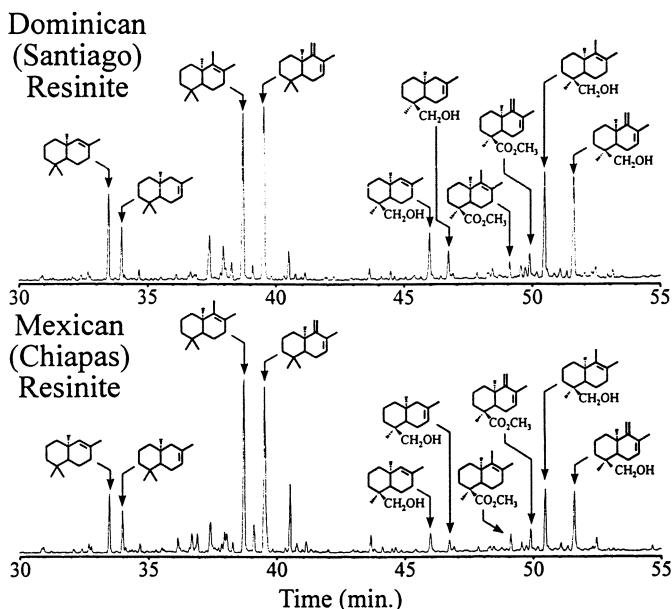


Figure 6. Partial chromatograms obtained by Py-GC-MS analysis of Class Ic resinites illustrating that the macromolecular structures of these products are derived from copolymeric structures based primarily on ozol and *entio* biformenes.

It is apparent from the data reported herein, and from published and unpublished analyses of a significant number of other resinites (5-8, 12-14, Anderson, unpublished results), that Class I resinites are normally derived from polylabdanoid structures based on either *regular* [1S, 4aR, 5S, 8aR] or *entio* [1S, 4aS, 5R, 8aS] labdanoids. It is noteworthy, however, that in one resinite, collected from undated<sup>1</sup>

#### I. Note Added in Proof.

After preparation and review of this manuscript, radiocarbon dating data for these resinites became available. The results of these analyses indicate the following ages for the samples tested by the author:

Kenyan Resinite = 40-50 ybp

Columbian Resinite = 180 ± 50 ybp

These results indicate that these resins are recent rather than true fossil materials. While these results do not alter any of the conclusions reported herein, it is clearly important to be aware of these data when attempting to correlate the data discussed in this report with analyses of other samples.

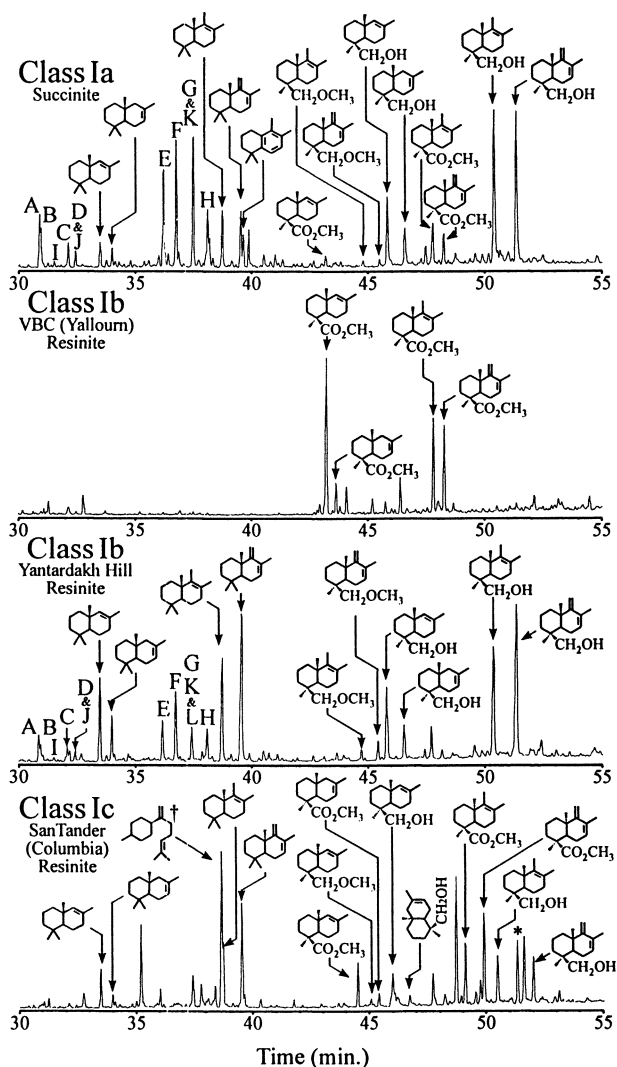


Figure 7. Partial Py-GC-MS chromatograms of Class Ia, Ib and Ic<sup>1</sup> resinites illustrating differences in the chromatographic behavior of enantiomeric characteristic bicyclic products. †  $\beta$ -Bisabolene is a common sesquiterpenoid component of many resins and is not part of, nor derived from, the macromolecular structure of this resinite. \* =  $nC_{15}CO_2CH_3$ .



sediments near Mombasa, Kenya, Py-GC-MS analysis indicates the presence of *both regular* and *enantio* labdanoid structures. Data from this resinite are illustrated in Figures 8 and 9. The identification of compounds VIIc - Xc and XIc - XIVc in the data illustrated in Figure 8 was confirmed by both comparison of mass spectral data and coprolysis with known Class Ib and Class Ic samples (Figure 9). The presence of both *regular* and *enantio* labdanoids in the macromolecular structure of this resinite is at least highly unusual (unique based on currently available data), and at present places this sample outside the classification system for resinites described in earlier reports in this series (6-8). If additional resinites incorporating both *regular* and *enantio* labdanoids are identified in the future, it will become necessary to modify the present form of the classification system for class I resinites to incorporate these materials.

The relative abundances of the C<sub>14</sub> and C<sub>15</sub> *regular* and *enantio* characteristic bicyclic alcohols (VIIb-Xb, and XIb-XIVb), especially the relative abundances of the *regular* and *enantio* C<sub>15</sub> bicyclic alcohols, in the pyrolysate of this Kenyan resinite merits some discussion. If one assumes that the *regular* and *enantio* bicyclic alcohols (VIIb-Xb, and XIb-XIVb) observed in the pyrolysate of this resinite are derived from communol (II) and ozol (V) respectively, and given that both have experienced the same burial history, then it could reasonably be anticipated that the distributions of *regular* and *enantio* C<sub>14</sub> and C<sub>15</sub> characteristic bicyclic alcohols should be equal. In this case, however, the observed distributions are unequal, (see especially Figure 9) and are inconsistent with the results previously reported for other immature resinites (6).

In a previous publication in this series (6) it has been reported that the distribution of the characteristic bicyclic acids observed in the pyrolysates of Class Ib resinites varies consistently as a function of increasing maturity (6). In that report it was suggested that the abundances of the various C<sub>14</sub> and C<sub>15</sub> characteristic bicyclic acids (VIIa-Xa) were controlled by the relative abundances of several proposed isomeric monomer structures differing in the positions of the two C=C double bonds initially present in each polymerized labdanoid monomer. The ratio of the two C<sub>15</sub> characteristic bicyclic acids, ie, IXa:Xa, was not discussed in that report, however, it might be assumed that this ratio should also be controlled by the relative abundances of these isomeric monomers. In fact, from the data illustrated in Figure 3, this ratio does systematically increase from ~1.2 (Giles Creek) to ~2.1 (Heaphy) in the pyrolysates of the New Zealand resinites from which those original conclusions were drawn.

The data for the Kenyan resinite illustrated in Figures 8 and 9, especially the ratios of the *regular* and *enantio* C<sub>15</sub> bicyclic alcohols, might initially be interpreted as suggesting that the series of olefin isomerizations proposed in our earlier report may be

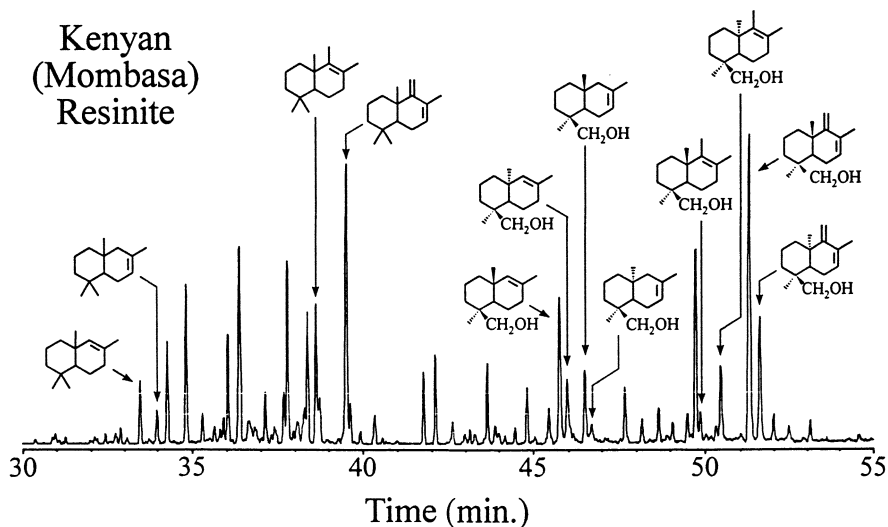


Figure 8. Partial Py-GC-MS chromatogram of Kenyan resinite<sup>1</sup> (Mombasa) illustrating the presence of *regular* and *enantio* bicyclic products in the pyrolysate of this resinite. Bicyclic hydrocarbons are illustrated in the non-stereospecific form since both *enantio* and *regular* may be present under these peaks.

in error. Firstly; although both the *regular* and *enantio*  $C_{15}:C_{14}$  ratios, as determined from the distributions of the characteristic bicyclic alcohols VIIb-Xb and XIb-XIVb (ie, [(XIIIb+XIVb):(XIb+XIIb)] and [(IXb+Xb):(VIIb+VIIIb)]), are equal to within experimental error, the value of this ratio ( $C_{15}:C_{14} > 1$ ) is inconsistent with the values observed in other immature resinites. And secondly; the distributions of *regular* and *enantio*  $C_{15}$  bicyclic alcohols are dramatically unequal (IXb:Xb  $\sim$  29; XIIIb:XIVb  $\sim$  1.7).

The fact that in this sample, the *regular* and *enantio*  $C_{15}:C_{14}$  ratios in this sample are equal, and that the ratios of the *regular* and *enantio*  $C_{15}$  bicyclic alcohols are unequal, indicates that the ratio of the two characteristic  $C_{15}$  bicyclic products is apparently independent of the  $C_{15}:C_{14}$  ratio. Never-the-less, the ratio of the  $C_{15}$  products IXb:Xb and XIIIb:XIVb is also likely to be controlled by the position of the olefinic structures in the B ring and side chain, although the relative importance of the various possible positional isomers in determining the observed distribution of  $C_{15}$  products is unclear from the presently available data. In this case, the differences in the ratios of the  $C_{15}$  alcohols IXb:Xb and XIIIb:XIVb strongly suggests that the original

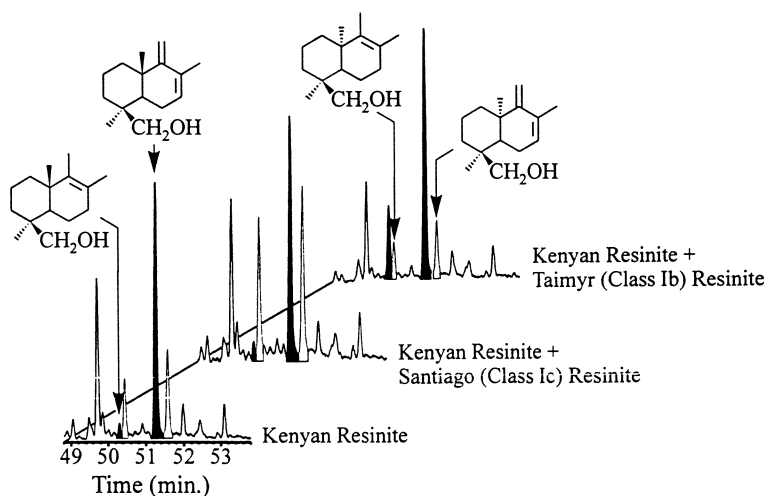


Figure 9. Partial Py-GC-MS chromatograms illustrating the distribution of  $C_{15}$  bicyclic alcohols in the pyrolysates of (A) Kenyan resinite, (B) co-pyrolysis of Kenyan resinite and Dominican Republic (Santiago) resinite (See Figure 4) and (C) co-pyrolysis of Kenyan resinite and Yantardakh Hill (Taimyr Peninsula, Siberia) resinite (See Figures 1 and 5). These data demonstrate co-elution of known *regular* and *enantio* bicyclic alcohols with those observed in the pyrolysate of the Kenyan resinite.

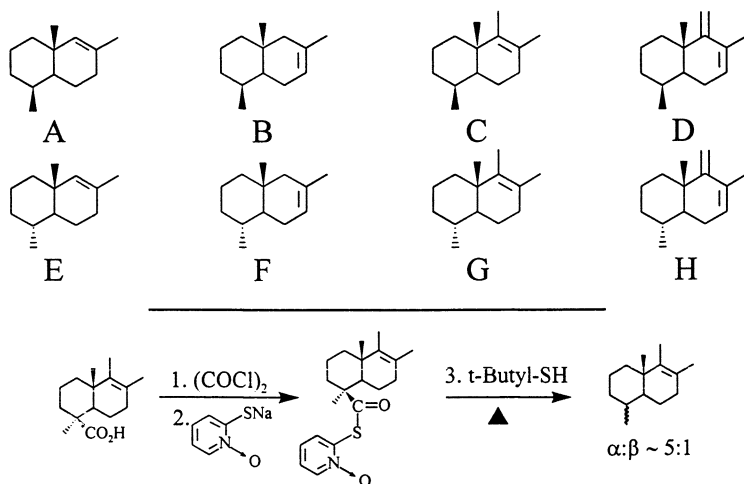


Figure 10. Structures of A-ring defunctionalized bicyclic products observed in the pyrolysates of Class Ia and Ib ambers. Reaction scheme used for confirmation of these structures is also illustrated.

*regular* and *enantio* labdanoid alcohols incorporated into the macromolecular structure of this resinite differ in their double bond configuration as well as their stereochemistry. That is, in this case the assumption that the labdanoid alcohols incorporated into the macromolecular structure of this resinite are derived from communol (II) and ozol (V) does not appear to be valid.

This may also account for the unusual  $C_{15}:C_{14}$  ratio observed in the pyrolysate of this sample. As noted above, the  $C_{15}:C_{14}$  ratio is likely to be controlled by the positions of the olefinic structures in either or both the B ring and the side chain (6). In resinites derived from labdanoids analogous with communic or ozic acids, the distribution of double bond isomers amongst monomers incorporated into the macromolecular structure will be determined by the age and thermal history of the sample. This distribution may be skewed, however, by incorporation of isomeric labdanoids in which the double bond configuration differs from that of communic or ozic acids. This indicates that although these ratios may be useful for comparing the maturity of related resinites, care must be exercised when attempting to use these ratios to gauge the relative maturity of unrelated samples.

Up to this point, this report has focused primarily on discussion of the occurrence and significance of bicyclic products which can be directly related to the A/B ring system of labdanoid diterpenes. However, in addition to these compounds a series of eight  $C_{13}$  and  $C_{14}$  products (labeled as A-H in Figures 1-6) are also observed in the pyrolysates of several Class Ia and Ib resinites, especially in samples of moderate thermal maturity. (In several samples these compounds co-elute with other unidentified minor components (compounds I-L) These however can be easily resolved by analysis using alternative chromatographic phases). The regular distribution and occurrence of compounds A-H in resinites of diverse origins suggests that they are likely to be derived from the macromolecular structure of the resinite. These compounds are especially abundant in the pyrolysates of Class Ia and Ib resinites which are relatively rich in communic acids (see Figures 2 and 3), but identical products are also observed, albeit at relatively lower abundance, in the pyrolysates of the Siberian Class Ib resinites, illustrated in Figure 2, in which communic acids are absent. Based on these observations and on interpretation of mass spectral and chromatographic data, these products, were tentatively assigned as A-ring (reductively) defunctionalized analogues of the typical bicyclic products derived from polylabdanoid structures (see Figure 10). Mass spectral data for these compounds are given in Table III. In order to confirm the structures of these products, the off line pyrolysate of Victorian brown coal resinite was subjected to reductive decarboxylation (16). The reaction sequence employed is also illustrated in Figure 10. The products obtained from this reaction were found to be identical (mass spectra and chromatographic behavior) to compounds A-H.

Table III.

## MS Data for Characteristic A-Ring Defunctionalized Bicyclic Hydrocarbons

Compound	MS Data and Assignment
<b>A</b>	178(16), 163(49), 149(11), 135(11), 121(18), 108(17), 107(51), 93(26), 91(24), 81(100), 79(77), 69(6), 67(13), 65(7), 55(15), 53(10), 41(32), 39(15). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1,4a,6-trimethyl [1S, 4aS]
<b>B</b>	178(26), 163(29), 149(4), 135(8), 123(21), 122(13), 110(34), 109(40), 107(15), 105(15), 95(100), 93(22), 91(23), 81(40), 79(24), 77(19), 67(23), 65(11), 55(20), 53(17), 41(36), 39(19). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1,4a,6-trimethyl [1R, 4aS]
<b>C</b>	178(25), 163(52), 149(31), 135(19), 122(23), 121(36), 109(23), 108(31), 107(79), 105(16), 95(35), 93(51), 91(38), 81(100), 79(45), 77(36), 69(13), 67(34), 65(14), 55(31), 53(19), 41(52), 39(34). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl [1S, 4aS]
<b>D</b>	178(18), 163(41), 149(7), 135(10), 123(24), 122(19), 110(41), 109(27), 107(26), 95(100), 93(28), 91(30), 81(55), 79(25), 77(26), 67(34), 55(35), 41(46), 39(25). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl [1R, 4aS]
<b>E</b>	192(14), 177(40), 161(6), 149(8), 143(5), 136(12), 121(41), 109(8), 107(14), 105(9), 95(100), 93(11), 91(14), 83(7), 81(8), 79(11), 77(9), 69(6), 67(10), 55(13), 41(16). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl [1S, 4aS]
<b>F</b>	190(62), 175(67), 161(19), 147(34), 133(35), 121(41), 119(74), 108(100), 105(63), 93(82), 91(58), 81(26), 79(34), 77(35), 67(15), 65(14), 55(27), 41(38). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-5-methylene-1,4a,6-trimethyl [1R, 4aS]
<b>G</b>	192(20), 177(37), 149(22), 136(23), 121(50), 109(9), 107(18), 105(14), 95(100), 93(16), 91(21), 83(22), 81(12), 79(14), 67(16), 55(26), 41(42). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl [1S, 4aS]
<b>H</b>	190(67), 175(70), 161(22), 147(48), 133(35), 121(61), 119(66), 108(95), 105(74), 93(100), 81(38), 79(48), 77(43), 69(14), 67(24), 65(18), 55(38), 53(21), 41(50). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-5-methylene-1,4a,6-trimethyl [1R, 4aS]
<b>I</b>	176(17), 161(21), 133(19), 120(23), 119(39), 105(100), 91(28), 79(13), 77(21), 67(10), 65(12), 55(14), 41(18), 39(14). Unassigned.
<b>J</b>	176(39), 161(45), 148(12), 133(29), 119(56), 105(100), 93(15), 91(39), 79(11), 77(22), 65(11), 53(12), 41(10). Unassigned.
<b>K</b>	190(47), 175(34), 161(24), 148(60), 147(38), 133(76), 121(21), 119(100), 107(19), 105(60), 95(26), 93(54), 91(75), 79(62), 77(48), 67(22), 65(20), 55(26), 53(21), 41(58), 29(16), 27(18). Unassigned.

The existence and widespread occurrence of compounds A-H in geographically and chronologically diverse samples indicates that a here-to-fore unrecognized pathway involving progressive loss of A ring functionality (especially carboxyl functionality) is active in the maturation of Class I resinites. The quantitative significance and structural consequences of this maturation pathway can not yet be clearly established. However, it is possible that in addition to formation of A-nor labdanoid monomers, A-ring defunctionalization may also lead to condensation and cross-linking of the macromolecular structure. This may account for the decreasing pyrolysis yields obtained from Class I resinites with increasing rank which has been reported by Clifford and Hatcher (9) and which has also been observed in studies carried out by the author. Additional studies are required to evaluate the significance of this reaction pathway in the maturation of Class I resinites.

To date, analogous defunctionalized products have not been observed in the pyrolysates of Class Ic resinites. However, there is no apparent reason why these compounds should not be able to be generated in this form of Class I resinite. Hence, in the opinion of the author, the absence of this type of product in the pyrolysates of Class Ic resinites probably reflects the inadequate data-base available due to the limited number of samples so far analyzed. It is likely that as additional data become available, especially data for more mature Class Ic resinites, analogous products will be identified in Class Ic resinites.

### Conclusions.

The macromolecular components of the majority of Class I resinites are based on copolymeric structures derived from mixtures of labdanoid diterpenes. Class Ia and Ib resinites are typically based on copolymers derived primarily from *regular* [1S, 4aR, 5S, 8aR] labdanoid diterpenes; including especially, communic acid, communol and/or biformene. Conversely, Class Ic resinites are based on copolymers of *enantio* [1S, 4aS, 5R, 8aS] labdanoids including ozic acid, ozol, and *enantio* biformenes. It is also possible that as additional data become available, additional monomeric structures will be identified. However, currently available data suggest that these compounds are the predominant monomers from which the macromolecular structures of Class I resinites are derived. The classification system for Class I resinites which has been previously described (8) is sufficiently general that none of these observations necessitates further revisions. In one case, data have been generated which indicate incorporation of both *regular* and *enantio* labdanoids into the macromolecular structure, but to date this has only been observed in a single sample.

Class I resinites are conveniently analyzed by Py-GC-MS and the labdanoid components of the macromolecular structure identified by recognition of characteristic bicyclic products derived directly from the A/B ring system of the original labdanoid monomers. Characteristic *regular* and *enantio* bicyclic acids, alcohols and hydrocarbons have been identified in the pyrolysates of a diverse suite of Class I resinites and it is clear from these data that in addition to communol and ozonides, labdanoid alcohols (such as communol and ozonol) and hydrocarbons (such as *regular* and *enantio* biformentenes) are widespread and typical components of Class I resinites. In many cases, these materials are more significant in the macromolecular structure than labdanoid carboxylic acids. In most instances, mass spectral data alone are inadequate for confident differentiation of *regular* and *enantio* bicyclic products. However, these products can normally be distinguished on the basis of chromatographic behavior, in most cases in a single analysis.

In addition to primary bicyclic products derived directly from the labdanoid A/B ring system, products derived by modification of this ring system are also observed in Py-GC-MS analyses of some Class I resinites. Bicyclic methyl ether analogues of communol derived bicyclic alcohols are observed in the pyrolysates of Class Ia resinites. These are produced by pyrolytic hydrolysis/O-methylation of succinylated (esterified) communol monomers within the macromolecular structure of these resinites by TMAH. A-ring defunctionalized C<sub>13</sub> and C<sub>14</sub> bicyclic products are also observed in the pyrolysates of Class I resinites of sufficient maturity. The existence of these products indicates the existence of a heretofore unrecognized maturation pathway in polylabdanoid resinites.

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## Chapter 7

# Gedanite and Gedano-Succinite

Edith C. Stout<sup>1</sup>, Curt W. Beck<sup>1</sup>, and Barbara Kosmowska-Ceranowicz<sup>2</sup>

<sup>1</sup>Amber Research Laboratory, Department of Chemistry, Vassar College,  
Poughkeepsie, NY 12601

<sup>2</sup>Polish Academy of Science, Museum of the Earth,  
PL-00 488 Warsaw, Poland

Helm described two fossil resins occurring with succinite: Gedanite, which he thought to have a botanical source other than that of succinite, and "friable amber", which he held to be a diagenetic variant of succinite. Savkevich considered both resins to be diagenetic variant of succinite and named the "friable amber" gedano-succinite. This reports the study by FTIR and GC-MS of the nine specimens labeled 'gedanite' in mineralogical collections of Europe and the U.S.A. Seventy identified components, including 18 diterpene resin acid derivatives, are essentially the same as have been reported in the soluble fraction of succinite. Gedanite and gedano-succinite can be distinguished by their succinic acid content and by their IR spectra. Most of the presumed gedanites in the collection are, in fact, gedano-succinite; one is ordinary succinite. The close similarity of the components distribution of all three resins suggests that all share a common botanical source.

The properties, chemical composition, and botanical sources of the fossil resins gedanite=brittle amber (German: das Spröde) and gedano-succinite=friable amber (German: mürber Bernstein), and particularly their relationship, if any, to succinite=Baltic amber have been studied for more than a century. We here report on their chemical composition as established by gas chromatography-mass spectrometry and their identification by infrared spectroscopy.

The first appearance in the literature of these varieties of 'friable amber' from the Eastern Baltic is in 1877 in a paper by Helm that deals principally with the chemical and physical properties of succinite (*1*). At the end of the paper, he describes a resin occurring "here and there along the Baltic coast" and called "*mürber Bernstein*" (friable amber) by the local amber workers. He writes that it is "barely distinguishable" from succinite in its appearance and in its density, but that it "is less

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hard, splinters easily on breaking or cutting, and is therefore less suited for working".

The pieces in his possession were of "a more or less light amber color". Helm goes on to report that friable amber becomes cloudy on heating and that at 140°C it begins to suffer decomposition with gas evolution, then melts and emits gases that have the same odor as those produced by succinite, except that they are not acrid, indicating the absence of succinic acid. Helm obtained no succinic acid from the resin by pyrolysis. The distillate was an oil similar in appearance and odor to oil of amber; the residue, however, was dark yellow and transparent, not dark brown and opaque as it is in the case of succinite. At room temperature, alcoholic potassium hydroxide (of unspecified concentration) dissolved 30 % of the friable amber. In four of these extracts, Helm found 0, 0, 0.3, and 0.4 % succinic acid, respectively. Hot diethyl ether dissolved 53 % in one trial, but only 39.3 % in another. Hot ethanol dissolved 24.4 %; from the residue, diethyl ether dissolved a further 22.3 %. The fraction soluble only in ether melted at 162 - 170°C without decomposition; the ethanol-soluble fraction at 100 - 105°C; the residue insoluble in both alcohol and ether melted "at a high temperature" with decomposition. In turpentine, friable amber was more soluble than succinite. It contained 0.06 % ash. Helm concluded that friable amber is intermediate between succinite and copal in hardness, melting point, and solubility, that the ether-soluble fraction has a higher melting point than that of succinite, and that its succinic acid content is lower than that of succinite. He rejected the notion that it may be an incompletely fossilized succinite, since it occurs along with succinite in deposits of the same geological age, and suggested that it has different botanical origin than succinite.

In the following year, Helm briefly refers to "a mineral closely related to succinite, which I have called gedanite", but only gives its sulfur content as 0.22 to 0.28 % (2). In a companion paper (3), he assigns the name gedanite (from Gedanum, the Latin name of the city of Gdansk = Danzig) to the resin which he identifies as the material that the local "amber workers call *mürber Bernstein* (friable amber) or *unreifer Bernstein* (unripe amber)". The latter term introduces an unfortunate confusion with a quite different fossil resin properly called unripe amber that was reported by Spirgatis (4,5) from the Baltic Sea near Brüsterort (54.58 N 20.00 E), now Taran Mys or Mayak lighthouse on the northwestern corner of the Sambian peninsula and equated with the resin occurring in lignite near Bernburg (51.49 N 11.44 E) in the then Duchy of Anhalt in Saxony that had been named krantzite by Bergemann (6). The confusion has persisted in the literature: in his comprehensive compilation of coals and organic minerals, Zincken (7) lists a 'gedanite' that is clearly Spirgatis' Brüsterort resin. Proper unripe amber is soft and elastic, but hardens on exposure to air. It merits further study, but it has nothing to do with gedanite or friable amber and it is not the subject of this report.

Helm (3) describes his newly named gedanite by repeating some of the data in his earlier paper (1) and revising others: he now gives the solubility of gedanite in hot diethyl ether as 40.52 %, in hot ethanol as 18 - 25 %, with the residue yielding another 20 - 24 % to ether, and adds the solubility in chloroform as 34 %. He makes one important correction: pyrolysis of a large sample (20 g) of gedanite yielded *no succinic acid*. He surmises that his earlier samples (in which he had found small amounts of this acid in two out of four trials) must have been contaminated with succinite. That statement is important in that it admits that gedanite and succinite are not always easily distinguishable by appearance alone.

But in 1896 Helm revokes his identification of gedanite with friable amber (8). He retains gedanite as a distinct mineral species but now considers friable amber to be a mere variety of succinite because it, unlike gedanite, does contain small amounts of succinic acid. He now refers to the friable amber explicitly as "friable succinite" which he distinguishes from "real succinite" which he also calls "hard succinite". He is extremely careful in his pronouncements about the botanical origin of the two

resins. For gedanite, he suggests a different botanical source from succinite because of the differences in its physical and chemical properties, most notably the absence of succinic acid, but allows that "no plant remains have been found [in gedanite] that allow the inference of a distinct source tree". For the "friable succinite", he "cannot decide" whether its botanical source is the same as that of "real succinite". He warns that "the two [resins], gedanite and friable succinite, are very similar [in appearance] and difficult to distinguish from one another", and that "mineralogists and collectors often mistake one for the other". The properties given by Helm (8) for gedanite, friable amber, and succinite, are summarized in Table I, to which we have added the elemental composition of gedano-succinite as determined in the Museum of the Earth for a specimen (Inventory Number 2222) identified as such by Savkevich. Helm does not seem to have been aware of the chemical studies by Aweng (9,10) who had concluded that gedanite and succinite have "very probably the same composition and the same [botanical] origin". That conclusion was also reached later by Tschirch (11), but the identity of his gedanite samples is questionable, since he reports that it contains 2 % succinic acid.

In the same year Klebs contradicts Helm on two points (12). First, while conceding that gedanite is practically devoid of succinic acid, he reports an analysis of a very large sample (200 g) which yielded 0.0015 % of that acid. Secondly, he gives the melting of gedanite point as 348°C and adds that, in a series of tests, he never found it to be below 300°C. He questions the homogeneity of his samples and states that he considers gedanite to be "a group of fossil resins" rather than a single resin. This heterogeneity appears to be responsible for the wide range of melting points reported by other workers: as high as 356.1°C (13) and as low as 296°C (14).

Helm's careful distinction between gedanite and friable amber is often lost in the later literature. Dahms uses the two terms as synonyms of each other as well as of unripe amber (15). Schmid does distinguish between them and uses *spröder Bernstein* (brittle amber) as a synonym of gedanite (16). In recent publications, friable amber is generally ignored and only gedanite = brittle amber is listed, without new data but with surmises of its botanical origin. Andrée states that gedanite has been assigned to *Pinites stroboïdes*, i.e. an extinct form of *Pinus strobus*, the Eastern white pine now limited to the Eastern part of Canada and of the United States, but cites no source for this assertion (17). Paclt (18) gives the botanical source of both gedanite and succinite as *Pinus succinifera* (18), a collective species defined by Conwentz as the source tree of succinite (19), but called into question on chemical grounds (20,21). His thorough histological examination of the wood and bark associated with fossil resins led Schubert to the tentative conclusion that gedanite arises from trees of the genus *Pinus* that are close to the genus *Picea* but that are distinct from the trees that produced succinite (22). Hey (23) does not rank gedanite as a species, but as a variety of succinite which he still traces to Conwentz' *Pinus succinifera* (23). Lastly, Savkevich has interpreted the differences between gedanite, friable amber, and succinite as diagenetic, rather than botanical (24,25). Based on his studies of the fossilization process, he concludes that exudations from a single botanical source may be converted into different fossil resins depending on whether they are deposited in an anaerobic and alkaline environment (e.g. a swamp) or whether they are exposed to atmospheric oxygen. Because, in his view, friable amber is diagenetically intermediate between gedanite and succinite, he gave it the new name gedano-succinite and postulated that this resin was subject to aerobic conditions longer than gedanite but not as long as succinite. It must be pointed out that Savkevich's hypothesis is not supported by the elemental analyses in Table I, which show succinite to have the lowest oxygen content of 7.33 %, while gedanite is higher with 10.47 %, and gedano-succinite highest with 16.60 %.

**Table 1. Properties of Gedanite, Gedano-Succinite, and Succinite**

Property	Gedanite	Friable Amber or GedanoSuccinite	Succinite
Hardness	1.5 - 2.0	1.5 - 2.0	2.0 - 2.5
Color	light yellow to golden yellow; rarely darker	light yellow, reddish yellow; rarely dark yellow	wide range of colors from light yellow to red-brown
Clarity	transparent; rarely semi-transparent	transparent to semi-transparent; rarely opaque	all degrees of transparency and opacity
Fluorescence	no	no	yes
Static Electricity	yes	yes	yes
Density	1.058 - 1.068	1.060 - 1.066	1.050 - 1.096
Melting Point	260 - 270	280 - 287	287 - 300
Succinic Acid Solubility	softens and swells at 140 - 180	1.13 and 1.70 %	3 - 8 %
Ethanol	42 %	30 %	20 - 25 %
Diethyl ether	63 %	53 %	18 - 23 %
Chloroform	45 %	33 %	20.6 %
Benzene	42 %	38 %	9.8 %
Carbon disulfide	58 %	39 %	24 %
Turpentine	> 58 % (swells)	45 %	25 %
Linseed oil	100 %	38 %	18 %
Elemental analysis			
Carbon	78.63 %	73.88 %	81.01 %
Hydrogen	10.48 %	9.22 %	11.41 %
Oxygen	10.47 %	16.60 %	7.33 %
Sulfur	0.42 %	0.3 %	0.25 %

### Materials used in this study

In the course of collecting fossil resins for a data base of infrared spectra, we have found eight specimens labeled 'gedanite' in the major mineralogical collections of the United States and Europe. Except for five specimens in the Museum of Earth, including Savkevich's holotype Inventory No. 2222, we did not find a single specimen labeled 'friable amber' or 'gedano-succinite'.

There are three 'gedanite' samples in the Department of Mineralogy of the French National Museum of Natural History (Musée National d'Histoire Naturelle), Paris. All of the specimens are recorded as having come from the extensive, formerly East Prussian amber deposit at Palmnicken (54.52 N 19.57 E), now Yantarnyy, Russia, from the Russian word yantar = amber (United States Board on Geographic Names, various dates). Paris Inventory No. 100.1365 identifies several large, spherical pieces of almost uniformly dull yellow color with very little streaking. The resin is tough rather than friable and its solubility in diethyl ether is 14 %. It melted with decomposition at 380°C. Paris Inventory No. 100.1366 consists of several large spherical pieces with deep cracks. The resin is transparent and yellow-orange in color. It is tough, breaks conchoidally, and melts with decomposition over a wide range from 320 - 370°C. Its solubility in diethyl ether is 17 %. Paris Inventory No. 100.1367 is very different in appearance from the two specimens above. The fist-sized piece is yellow and opaque, resembling the succinite variety called "kumst" in the amber trade. New fractures are almost white and the material is exceedingly tough. Its solubility in diethyl ether is 16 %. It melts with decomposition at 380°C.

Two samples are from the Roebing Collection in the Department of Mineralogy of the Smithsonian Institution, Washington, D.C. Inventory No. R[oebling] 7293 is labeled as having been found at Sassau (54.57N 20.06E) which is also on the Samland peninsula. The sample is uniformly dull yellow in color and very tough. It melts to a dark red liquid at 300°C and 49 % of it is soluble in diethyl ether. Inventory No. R[oebling] 7296 is reported to come from the vicinity of Gdansk (Danzig; 54.22 N 18.41 E.) in Poland. The color ranges from yellow-orange to reddish-brown. The sample is friable, decomposed beginning at 300°C, and had a solubility in diethyl ether of 31 %.

One 'gedanite' was furnished by the Department of Mineralogy of the American Museum of Natural History, New York, N.Y. The Inventory No. is 17339. The label gives no geographic origin. The sample is red-brown in color, with yellow streaks, but new fractures are almost colorless or very pale yellow. It is tougher than R 7296 above, begins to decompose at 290°C, and has a solubility in diethyl ether of 17 %.

The sample from the British Museum of Natural History, South Kensington, London, Inventory No. 53944 was purchased by the museum from the mineral dealer A. Krantz, Bonn, Germany, and is reported to have come from Sassau on the Samland peninsula. It is of uniformly dull yellow color, very difficult to break, melts with decomposition below 380°C, and has a solubility in diethyl ether of 27 %.

The sample identified in Tables II as 'Vassar' is part of a teaching collection of amber in the Department of Geology at Vassar College. It was assembled and sold in kit form as a teaching aid some fifty years ago by a Professor Drenckhahn of Fehmarn, Germany (54.28 N 11.08 E), an island in the Western Baltic Sea. Drenckhahn does not give a geographic origin for this 'gedanite', which is transparent and yellow to light orange in color. The sample is very tough, decomposes below 380°C, and has a solubility of 14 % in diethyl ether.

The ninth specimen of gedanite is from the collection of the Museum of the Earth (Inventory No. 20710) and comes from the Sambian peninsula. It is very pale yellow, transparent, brittle, and has a solubility in diethyl ether of 38 % and starts to decompose at 300°C.

### Experimental Techniques: GC-MS

Each sample was exhaustively extracted with diethyl ether, the extract methylated with diazomethane, and the resulting sample injected into a Hewlett-Packard Model 5995 gas chromatograph - mass spectrometer. The injection port was held at 250°C, the mass analyzer at 180°C, and the source temperature at 172°C. Fragmentation was by electron-impact at 70eV. The optimum column temperature program was found to be an initial temperature of 40°C, held constant for 4 minutes and then ramped at the rate of 8°C/minute to 250°C. The capillary column (15 m) had a stationary phase of RSL-150 poly(dimethylsiloxane). The carrier gas was helium at 10 psi. Because quantification from the total ion chromatograms of a GC-MS system is far from accurate, all samples were also run on a Hewlett-Packard Model 5880 gas chromatograph equipped with a flame ionization detector, using an identical capillary column and the same temperature program. Identification of structures from the mass spectra was made by using the instrument library, standard collections of mass spectra (26,27), specialized data collections (28,29), and, most usefully, the ground-breaking GC-MS analysis of succinite by Mills *et al.* (21).

### Results and Discussion

The results of the GC-MS analyses are summarized in Table II. A total of 70 components have been identified; their structures are shown in the Appendix. The table lists the component number, the retention time, the structural identification, the percentage of the component, if any, in each of the nine 'gedanite' samples, and, in the last column, whether a component was (+) or was not (-) found in succinite by Mills *et al.* (21). Of the 70 components, we have been able to assign chemical structures to 62. The other eight yield mass spectra that are clearly identical to spectra published by Mills *et al.* (21), but which neither the British authors nor we could identify. They are nevertheless significant in that they add to the evidence of the chemical and hence botanical similarity of the three resins.

The data permit two major conclusions: First, few of the specimens that are labeled 'gedanite' in the mineralogical collections are, in fact, gedanite. Of the nine samples, Paris Inv. No. 1367 is actually succinite. This is shown by the large amount (3 %) of succinic acid in the form of its dimethyl ester (Compound No. 4) which is further enhanced by the presence of the monoterpenol esters methyl fenchyl succinate (Compound 32), methyl bornyl succinate (Compound 35), fenchyl bornyl succinate (Compound 62), and dibornyl succinate (Compound 66), and methyl dehydroabietyl succinate (Compound 70). All these esters, plus difenchyl succinate, were found by Mills *et al.* (21) in succinite. Paris Inv. No. 1367 is the *only* specimen in the present study to contain all of these compounds. Their aggregate amount is 5.36 %, corresponding to 3.44 % of free succinic acid in this sample, or well within the range known to be characteristic of Baltic succinite. Of the remaining eight 'gedanites', only three are true gedanite, namely Smithsonian Inv. Nos. R 7293 and R 7296 and, of course, the authentic sample from the Museum of the Earth, Inv. No. 20710. They contain little or no succinic acid and none of the monoterpenol esters of this acid. The remaining five 'gedanites', i.e. the other two from the Paris collection and the specimens in the American Museum of Natural History, the British Museum, and the Vassar College collection, are neither gedanite nor succinite but are friable amber or, in Savkevich's nomenclature (24,25), gedano-succinite. They contain small amounts of succinic acid (<0.07 % to 0.63 %) as well as small amounts of the fenchyl and bornyl half esters (0.05 % to 1.49 %).

The second conclusion goes to the botanical origin of both gedanite and gedano-succinite. As Table II shows, the succinic acid esters just discussed represent the *only* significant compositional difference between all the nine samples studied. What is

Table II. Constituents of Gedanite, Gedano-Succinite, and Succinite

No.	CONSTITUENT	R <sub>i</sub> (min)	100.1365	100.1366	100.1367	R7293	R7296	17339	53944	Vassar	20710	Mills
1	1,4-Cineole	5.1			trace							-
2	m- and p-Cymene	5.3		trace	0.16			trace	trace	trace	trace	+
3	Cineole = 1,8-Cineole	5.4			0.39	trace		0.07	0.14	0.14	0.16	+
4	Dimethyl succinate	5.4	0.14	<0.07	3.00	<0.20	<0.05	0.63	0.15	0.15	0.15	+
5	o-Cymene	5.6		trace			trace	0.36	0.09	0.22	0.03	+
6	trans-Decahydronaphthalene	6.0				0.20		trace				-
7	Dimethyl methylsuccinate	6.4				trace		trace		0.03		-
8	Fenchone	6.6		0.07	0.27	0.28	0.05	0.24	0.29	0.03		+
9	Methyl benzoate	6.7		trace		trace		trace	0.13	trace	trace	-
10	cis-Decahydronaphthalene	6.9				0.20		0.06				-
11	Fenchyl alcohol	7.3		0.35	0.61	0.57	0.11	0.54	1.44	0.32	0.16	+
12	Camphor	7.7	0.04	0.53	1.15	1.56	0.21	1.07	1.62	0.20	0.05	+
13	Isoborneol	8.2		0.05	0.08		0.09	0.07	0.06	0.05	0.03	+
14	Borneol	8.4	0.09	1.01	1.30	0.95		1.33	3.00	0.76	0.32	+
15	Mills scan #248	9.7		trace						0.08		+
16	Bornyl formate	9.7		0.05	0.08			0.12	0.22	0.13		+
17	4-(1-Methylethyl)-benzaldehyde	9.8			0.06							-
18	Bornyl acetate	10.8		0.01	0.14			0.14	0.11	trace		+
19	Methyl cinnamate	10.9							0.39			-
20	Methyl 4-(1-methylethyl)-benzoate	12.3		trace	0.06							-
21	Mills scan #372	13.1									trace	+
22	Dihydro-ar-curcumene	13.8							0.19			+
23	Mills scan #446	14.0										+
24	Ionene	14.1	0.02	trace	0.17		trace	0.11	0.14	0.14	0.29	+
25	1,1,5,6-tetramethyl-1,2,3,4-tetrahydro-naphthalene	14.6						0.09			trace	+

7. STOUT ET AL. *Gedanite and Gedano-Succinite*

26	Calamenene	14.7								0.22	+
27	Dimethyl nonanedioate	15.2	trace			trace			0.04		+
28	Methyl dodecanoate	15.2				trace					-
29	Mills Scan #528	15.4	0.06	0.09					0.08		+
30	an azulendi	15.5	0.25	0.30					0.26	trace	+
31	Cadina-1(10),6,8-triene	15.7								0.06	-
32	Methyl fenchyl succinate	17.4	0.05	0.77		0.23			0.75	0.35	+
33	1-hydroxymethyl-1,5,6-trimethyl-1,2,3,4-tetrahydronaphthalene	18.0	0.08			trace		0.33		0.76	+
34	Methyl 1,5,6-trimethyl-1,2,3,4-tetrahydro-1-naphthalenecarboxylate	18.2									+
35	Methyl bornyl succinate	18.3	0.23	1.37		0.63			1.49	0.91	+
36	Mills scan #695	18.5	0.06	0.30		0.06					+
37	Mills scan #710	18.7	trace			trace			trace		+
38	18-nor-13-methylpodocarpatiene	18.9									+
39	19-nor-13-methylpodocarpatiene	19.3									+
40	Methyl pentadecanoate	19.3	trace			trace				0.02	+
41	Methyl hexadecanoate	20.6	0.06	0.09		0.40		0.84	0.48	0.06	+
42	18-norabietatriene	20.7					0.16			0.03	-
43	19-norabietatriene	21.1					0.38			0.04	+
44	Dehydroabietane	21.6								0.07	+
45	Methyl 9-octadecanoate	22.5								0.06	+
46	Methyl octadecanoate	22.8	0.15	0.05		0.15		0.28	0.12	trace	-
47	Heptacosane	22.8	0.15								-
48	Methyl 8,15-isopimaradien-18-oate	23.2	2.39	0.65		0.50	2.13	1.46	1.29	2.62	+
49	Methyl 8,15-pimaradien-18-oate	23.6	1.09	1.24				1.24	0.47	0.33	+
50	Methyl 8-pimaren-18-oate	23.7	0.61	0.29		0.26	0.33	0.18	0.39	0.55	+

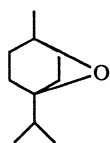
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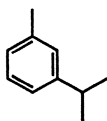
Table II. Constituents of Gedanite, Gedano-Succinite, and Succinite (Cont'd)

No.	CONSTITUENT	RI(min)	100.1365	100.1366	100.1367	R7293	R7296	17339	53944	Vassar	20710	Mills
51	Methyl pimarate	23.8	0.85	0.89	0.34	4.39	2.03	0.63	0.92	1.10	2.22	+
52	Docosane	23.9	trace	trace								-
53	Methyl 8(14)-isopimaren-18-oate	24.1	0.36	0.36								+
54	Methyl isopimarate	24.2	0.34	0.34	0.40	3.26	1.58	0.36	0.39	0.88	5.19	+
55	Methyl dehydroabietate	24.6	0.53	0.50	0.37	1.35	3.46	0.94	1.58	0.88	0.84	+
56	Tricosane	24.9	0.38	0.38								-
57	Methyl abietate	25.1	0.11	0.08						1.16	0.84	+
58	Mills scan #1123	25.8	0.89	0.08	trace			0.13		0.15		+
59	Dimethyl dihydrogathate	25.9	0.89	0.26	0.26			0.59		0.73	0.79	+
60	Dimethyl dihydro- $\Delta^8$ -agathate	26.0	0.15	0.15	0.15			0.23		0.12	1.02	+
61	Methyl 15-hydroxydehydroabietate	26.1	0.46					0.57				+
62	Fenchyl bornyl succinate	26.5		0.11								+
63	Mills scan #1159	26.6									0.35	+
64	Methyl 7-oxodehydroabietate	26.7	1.76	0.12	2.10	3.92	0.31	2.80				+
65	Methyl docosanoate	27.0	0.12	0.12	trace							-
66	Dibornyl succinate	27.3	0.11									+
67	Hexacosane	27.8	0.57	0.57								-
68	Heptacosane	28.8	0.84	0.84								-
69	Methyl tetracosanoate	28.9	0.68	0.68	trace							-
70	Methyl dehydroabietyl succinate	29.8	trace	trace								+

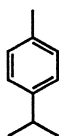
## Appendix



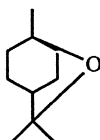
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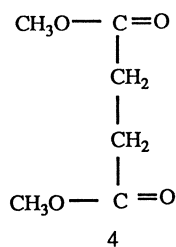
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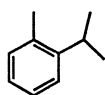
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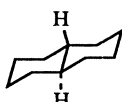
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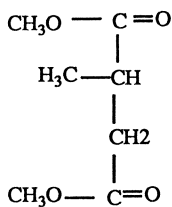
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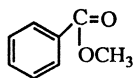
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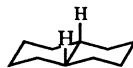
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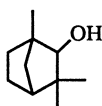
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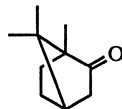
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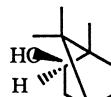
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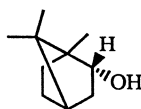
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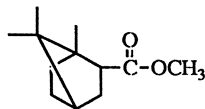
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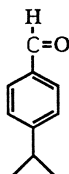
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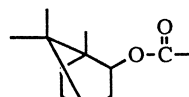
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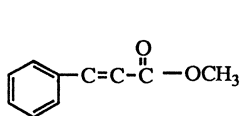
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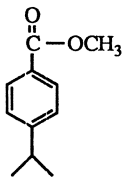
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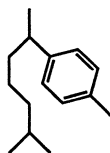
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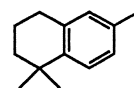
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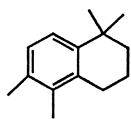


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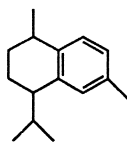


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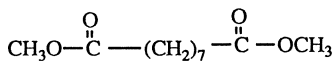
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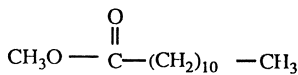
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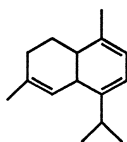
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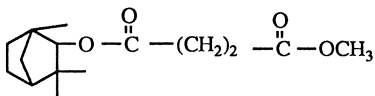
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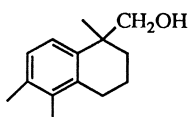
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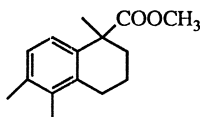
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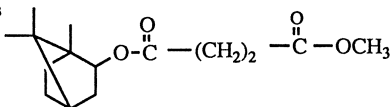
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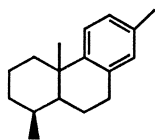
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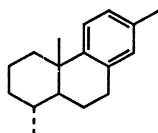
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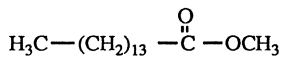
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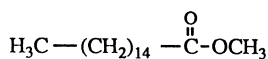
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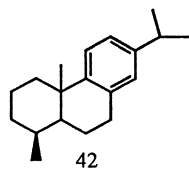
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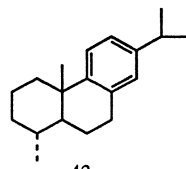
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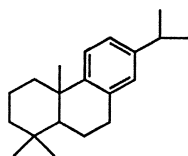
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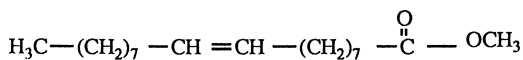
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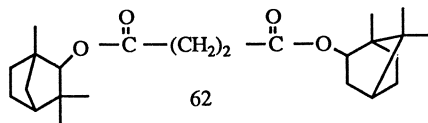
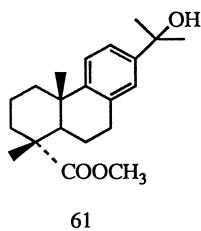
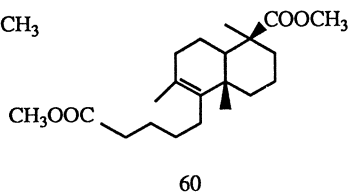
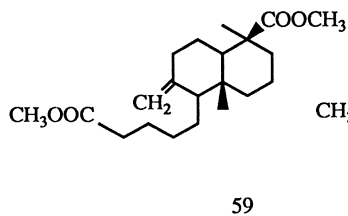
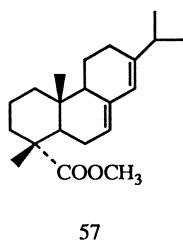
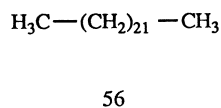
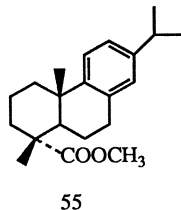
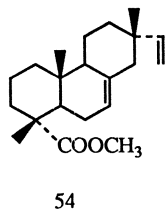
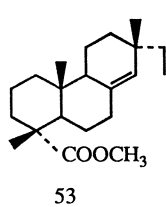
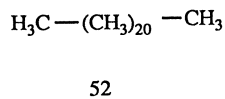
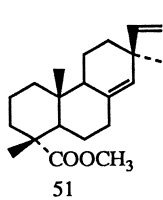
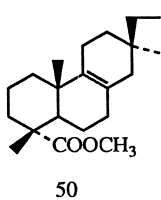
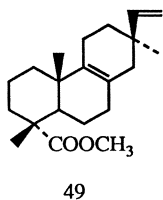
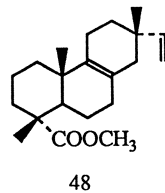
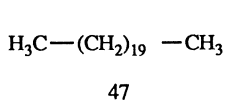
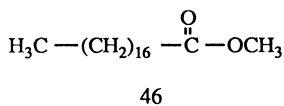
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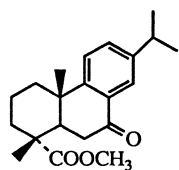


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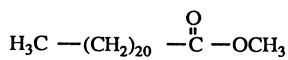


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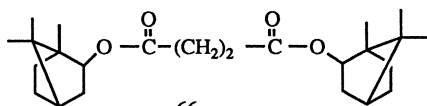
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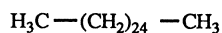
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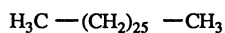
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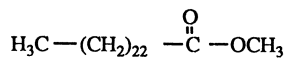
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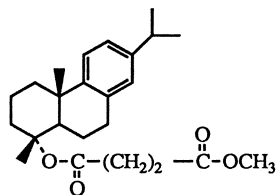
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more, most of these constituents were also found by Mills *et al.* (21) in Baltic succinite. Of the 21 components not found by the British team, most are non-terpenoid: There are five normal alkanes with 21 to 27 carbon atoms, twelve carboxylic acids including the straight-chain fatty acids with 12, 16, 18, 22, and 24 carbon atoms (Mills *et al.* (21) found only the C<sub>16</sub>-acid), and two degradation products (Compounds 6 and 10). The only terpenoid compounds found in our study but not found by Mills *et al.* (21) are the monoterpene 1,4-cineole (Compound 1) and the sesquiterpene cadina-1(10),6,8-triene (Compound 31). It is worth emphasizing that *all* the 18 diterpene-derivatives found in our samples have also been found in succinite. Moreover, while gedanite, gedano-succinite, and succinite are clearly distinguishable by their succinic acid and succinic ester contents, there are no categorical differences in their other constituents. Not only are gedano-succinite and succinite compositionally identical, but all but four of the constituents of gedanite were also found in the other two resins. The exceptions Compounds 21, 34, 39, and 69) are hardly significant, since they occur in amounts that border on the limits of detection. This strongly supports the contention of Savkevich (24,25) that not only gedano-succinite but also gedanite itself have the same botanical origin as succinite.

### Infrared Spectroscopy

The conclusions drawn from the GC-MS work are borne out fully by infrared spectroscopy which provides a more rapid and convenient method of identifying fossil resins. The infrared spectrum of Baltic succinite is well known and has been used extensively for the characterization of fossil resins from the Sambian deposits (30) as well as for the provenience analysis of prehistoric amber artifacts (31,32). It is characterized by a single absorption maximum in the carbon-oxygen single-bond range at  $1160 \pm 5 \text{ cm}^{-1}$  and is preceded by a broad shoulder. This 'Baltic shoulder' has a zero slope in well preserved succinite but assumes increasingly negative slopes as oxidative degradation progresses. Baltic succinite is further distinguished by a small but distinct absorption near  $890 \text{ cm}^{-1}$  that indicates out-of-plane vibrational frequencies of an exocyclic methylene group,  $=\text{CH}_2$  (Beck *et al.* 31). Fourier transform infrared (FTIR) spectra of the nine resins used in this study were taken with a Perkin-Elmer Model 1750 FTIR spectrometer, both before and after extraction with ether. The spectra of the residues are in all major respects identical with those of the whole resins, showing that the latter are representative of the insoluble, polymeric constituents. As expected, the Paris sample 100.1367, which has already been identified as succinite by the GC-MS data, gives a perfect succinite spectrum. The other samples are identifiable as gedanite and gedano-succinite by spectral features well established by work in the Museum of the Earth using a dispersive Perkin-Elmer Model 577 instrument (Kosmowska-Ceranowicz 33). Figure 1 shows the infrared spectra of gedano-succinite from Poland (Mikoszewo (54.20 N 18.58E) and Modla (54.33N 16.48E) near Slupsk), from the Ukraine (Klesów (51.20 N 26.56 E)), and from the extensive German fossil resin deposits in the Bitterfeld area, specifically at the open pit at Goitsche (51.37 N 12.20 E). The Bitterfeld resins have been subject to detailed study, including infrared spectroscopy, by the Polish author (33-36). The spectra resemble those of succinite, with small but diagnostically useful differences: The carbonyl band is more sharply split into two distinct absorptions at  $1700 \text{ cm}^{-1}$  (characteristic of carboxylic acids) and  $1735 \text{ cm}^{-1}$  (characteristic of carboxylic esters). Furthermore, the 'Baltic shoulder' has a slightly positive slope, something never seen in Baltic succinite and perhaps an early indication of the fully developed second maximum in gedanite. The fact that the spectra of gedano-succinite are quite similar to those of true succinite (Figure 2) strengthens the evidence that this resin is very closely related to succinite.

The infrared spectra of ten gedanite samples in the Museum of the Earth are shown

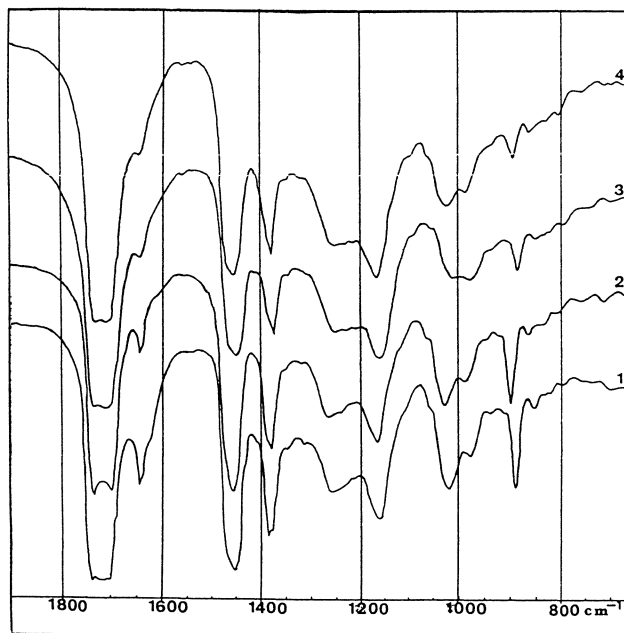


Figure 1. Infrared spectra of gedano-succinite from Mikoszewo, Poland (1), Modla, Poland (2), Klesów, Ukraine (3), and Bitterfeld, Germany (4).

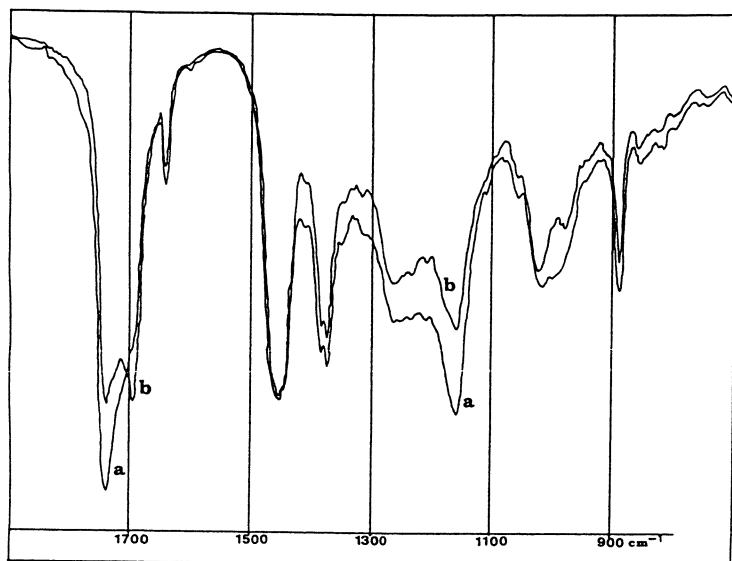


Figure 2. Comparison of infrared spectra of succinite (a) and gedano-succinite (b).

in Figure 3; six specimens come from the Baltic Coast at Mikoszewo and four from Goitsche. They differ sharply from the spectra of gedano-succinite and succinite in that they have two well-defined absorption maxima in the carbon-oxygen single-bond region at about  $1176\text{ cm}^{-1}$  and  $1125\text{ cm}^{-1}$ . Like gedano-succinite, gedanite shows a fairly strong exocyclic methylene band near  $890\text{ cm}^{-1}$ .

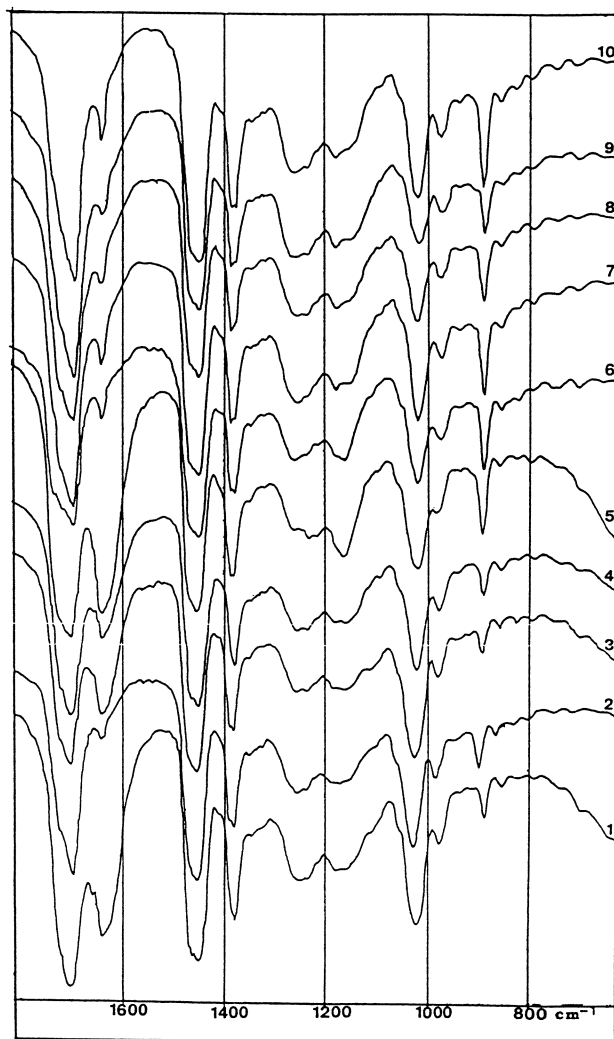


Figure 3. Infrared spectra of gedanite from Bitterfeld, Germany (1-4) and Mikoszewo, Poland (5-10).



A Cretaceous fossil resins from the Khatanga district (72.00 N 104.00 E) in north-central Siberia (Museum of the Earth Inventory No. 17232) has been identified by the Polish author as gedanite by infrared spectroscopy (Figure 4). Sokolowa has also described gedanite from this area (37), but her comment that it is "poorly soluble" is at odds with the substantial solubility of the gedanite samples used in this study. Cretaceous amber from the forest of Chambiers (47.38 N 0.15 E) in the French Department Maine-et-Loire, collected by L. Faillie and donated to the Museum of the Earth by A.W. Skalski (Museum of the Earth Inventory No. 16332) also gives the infrared spectrum of gedanite (Figure 4).

## Conclusions

Compositional analysis by gas chromatography - mass spectrometry shows that specimens labeled 'gedanite' in the mineralogical collections are more often gedano-succinite or even succinite. Succinite is distinguished by a high succinic acid content (more than 3 %) in the form of free acid and of monoterpenol esters. Gedano-succinite contains little free succinic acid but considerable amounts (up to 2.2 %) of monoterpenol succinic esters. Gedanite contains vanishingly small amounts of free succinic acid and no monoterpenol esters. Apart from these differences in succinic acid and esters, the three resins are remarkably similar in their chemical constitution, especially as regards their diterpene resin acid components.

Infrared spectroscopy allows the distinction between succinite and gedano-succinite, although the two resins share their major spectral characteristics. The infrared spectra of gedanite differ radically from those of succinite and gedano-succinite in that they have two distinct absorption maxima in the 1100 - 1300  $\text{cm}^{-1}$  range: Calculations from presently available spectra locate these maxima at  $1168 \pm 6 \text{ cm}^{-1}$  and  $1230 \pm 5 \text{ cm}^{-1}$ . In addition, several of the spectra taken in the Museum of the Earth show a fine structure of both maxima which splits each of them into a poorly resolved doublet.

The results of both analytical techniques are compatible with the hypothesis of Savkevich (24,25) that gedano-succinite and succinite, and perhaps also gedanite, are stages in the diagenetic alteration of a single resin.

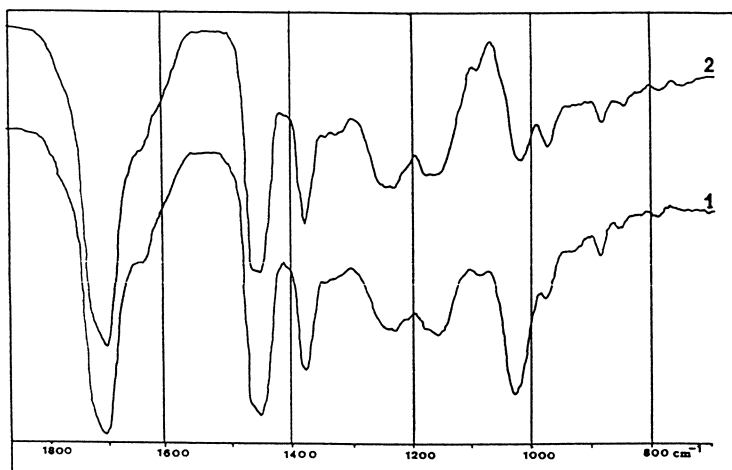


Figure 4. Infrared spectra of gedanite from France (1) and Siberia (2)

## Acknowledgments

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## Chapter 8

# Unusual Resin Chemistry from Upper Carboniferous Pteridosperm Resin Rodlets

P. F. van Bergen<sup>1,2,3</sup>, M. E. Collinson<sup>2</sup>, A. C. Scott<sup>2</sup>, and J. W. de Leeuw<sup>1</sup>

<sup>1</sup>Division of Marine Biogeochemistry, NIOZ, P.O. Box 59,  
1790 AB Den Burg, Texel, The Netherlands

<sup>2</sup>Department of Geology, Royal Holloway, University of London, Egham,  
Surrey TW20 0EX, United Kingdom

Two resin rodlet samples from Upper Carboniferous pteridosperms have been analyzed using Curie-point pyrolysis (610°C)-gas chromatography-mass spectrometry. One sample was obtained from well-characterized Medullosan pteridosperm (seed fern) petiole material, whereas the other was obtained as loose rodlets from a shale residue, probably also derived from Medullosan material. The pyrolysate of the loose resin rodlets is dominated by (alkyl)naphthalenes, alkylbenzenes and phenols. In addition, a homologous series of *n*-alkanes is present which is thought to be derived from occluded material. In contrast, the pyrolysate of the *in situ* rodlets is dominated by phenols, in addition to small amounts of alkylbenzenes and homologous series of alkanes and alkenes. The results from both these samples are unlike any known resin chemistry. Rather than considering these data indicative of a new type of fossil resin, we suggest that a secretion product such as diagenetically altered non-hydrolyzable tannin is the most likely source of this material. However, the possibility that the chemical composition of these resin rodlets is specific for Carboniferous Medullosan pteridosperms cannot be excluded, because these plants are unlike any plant groups living today.

Natural resins are defined as the sticky plant secretions that harden on exposure to air (*1*). The chemical composition of resins varies considerably, however, the majority of the known natural resins are terpenoid in nature. Whereas in Recent resins low-molecular-weight constituents often are the bulk of the material, the majority of fossil resins and resinites are primarily composed of high-molecular-weight compounds.

<sup>3</sup>Current address: Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, United Kingdom

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Based on polymeric constituents, fossil resins and resinites (Note: the term resinite is only used when dealing with material described as macerals rather than fossil resins in general) can be subdivided into three main classes (2). Class I are polyditerpenoid resins with a labdanoid moiety as the monomeric unit (2,3). Precursors of these kinds of resins belong to the gymnosperm order Coniferales (the conifers) and the angiosperm family Leguminosae (2). Class II are resins composed of polysesquiterpenoid moieties. Their basic structural unit is a sesquiterpenoid cadinane skeleton (4). Polysesquiterpenoid resins are as yet only demonstrated in two families of the angiosperms, *viz.* the Dipterocarpaceae and the sub-family Mastixioideae belonging to the Cornaceae (4,5). Class III of polymeric resin is a natural polystyrene which is only reported from the angiosperm genus *Liquidambar*, family Hamamelidaceae (6).

As is clear from the aforementioned, all precursors of polymeric resins known today belong to only two main groups, the angiosperms and the Coniferales (an order of the gymnosperms). Cladistic evidence indicates that the angiosperm line (stem extinct angiosperms) might have diverged from within angiosperms (*Pentoxylon*, Bennettitales, Gnetales & angiosperms) by the late Triassic (7) and several putative "angiosperm-like" fossils are known from the early Mesozoic (8). However, within angiosperms, crown group or "true" angiosperms only began to diversify by the mid-Early Cretaceous as evidenced by fossils of several angiosperm clades (7) and by patterns of occurrence of pollen and leaf fossils (9,10). Conifers have a longer fossil record extending back to the latest Carboniferous (11). However, families similar to those known today only evolved during the Triassic and Jurassic whereas modern genera do not become common until the Cretaceous or even the Tertiary (12). Hence, based on the above it is not surprising that evidence of the three known types of polymeric fossil resins only extends back to the Cretaceous (2).

Palaeobotanical and petrographic studies of materials, as old as Upper Carboniferous, referred to as resin rodlets or resinites are, however, numerous (13). To date, the most likely source for the resin rodlets are Medullosan pteridosperms, a group of seed ferns (13-15) whereas the origin of most of the resinites is still unclear. Chemical data on Upper Carboniferous resin rodlets and resinites are rare. One resinite from the Indiana Paper Coal (Upper Carboniferous; Lower- to Middle-Pennsylvanian), Kansas, USA, obtained by density gradient centrifugation has been studied using flash pyrolysis (16). However, the chemical constituents were thought primarily to be derived from material other than resin (see also **discussion** section). With respect to Carboniferous resin rodlets, only one study reported data obtained from elemental analysis and infrared spectroscopy (IR) (13). Both IR and elemental analysis data showed that the chemical constituents present in the resin rodlets were dissimilar to Eocene resin from New Zealand (13,17). These data, therefore, seem to indicate that the commonly used terms resinite and resin rodlets for Carboniferous material may be misleading because the resinous nature/origin of these materials has, as yet, not been proven.

In order to obtain additional insight into the chemical composition of Carboniferous fossil resins we analyzed two morphologically well-described Upper Carboniferous resin rodlet samples using Curie-point (610°C) pyrolysis-gas chromatography and Curie-point (610°C) pyrolysis-gas chromatography-mass

spectrometry. Flash pyrolysis was used since it is considered to reveal most clearly the molecular composition of polymeric fossil resins (2). Moreover, we believe that studying material of known botanical origin, which is also morphologically well-defined, is essential for understanding fossil resins. The importance of studying fossil resins with a known botanical origin has been clearly demonstrated earlier with respect to the polycadinene type resins (5,18). Until recently, the recognition of polycadinene derived compounds in sediments and oils were, and sometimes still are (19), considered exclusively to be derived from Dipterocarpaceae (18). Such results were even used to interpret the fossil record of Dipterocarpaceae input (18). However, a subsequent study of resin from resin canals of fossil fruits of Mastixioideae (subfamily of the Cornaceae) (5) showed that conclusions based on only dispersed material can be misleading. The importance of botanically well-characterized samples is also discussed in other chapters of this volume.

### Sample Description

Sample 1 represents *in situ* resin rodlets (Figs 1b,c) obtained from individual petioles (Fig. 1a; *Myeloxylon*) from an unidentified species of Medullosan pteridosperm (seed fern). The individual petioles are from a coal ball obtained from the roof of the Springfield No 5 coal at the Eby Pit, part of the Lynville Mine (20). The material is Middle Pennsylvanian (Upper Carboniferous, Westphalian D) in age (21). The sample is High Vol. bituminous C coal rank and has a maturity of  $R_0$  0.49 (20). The resin rodlets are opaque and those studied show no autofluorescence. Resin rodlets similar to those studied have been described in *Medullosa distellica* (22). It was demonstrated that they were formed in secretory canals with a clear epithelial layer (cf. epithelial cells Fig. 1c). However, the author (22) noted that 'their resinous nature is inadequately proven' but that 'they are essentially similar to resin in their mode of occurrence within the plant'.

Sample 2 represents loose resin rodlets (Figs 2a,b) obtained from a light grey floodplain shale within the coal-bearing sequence at Swillington Birckpit southeast of Leeds, Yorkshire, England (Bed 20f unit 9) (23). The sample is of Westphalian B, Upper Carboniferous age, has a High Vol. bituminous C rank and a maturity of  $R_0$  0.62. Similar material from Swillington has been reported to show clear casts of epithelial cells which lined the secretory cavity (24). Our material only shows impressions of these cells on the surface of the rodlets (Fig. 2b). Fractures of similar rodlets revealed the presence of numerous cavities (13,15). Based on the aforementioned morphological features of these rodlets and their known abundance in Medullosan petioles, but rarity in other Carboniferous plants (25), we believe that these rodlets are secreted products from Medullosan pteridosperms.

### Experimental

The coal balls containing the *in situ* material were treated with dilute HCl to release the organic material. The rodlets were hand picked from individual petioles. The loose resin rodlets were released from the shale using cold concentrated HF and picked using a low power binocular microscope.

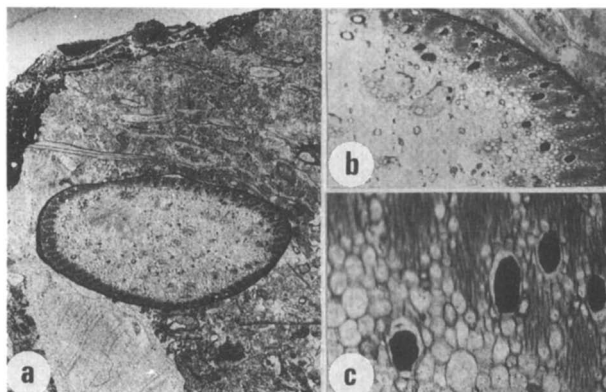


Figure 1. a) Transverse section of *Myeloxylon*, a Medullosan petiole, showing *in situ* resin rodlets (dark bodies) at primarily the edge of petiole x25. b) Detail of a) x10. c) Close up of several *in situ* resin rodlets. Note the presence of two epithelial cells at the right edge of the rodlet at the far most right x50. For further details see text. (Adapted from ref. 20)

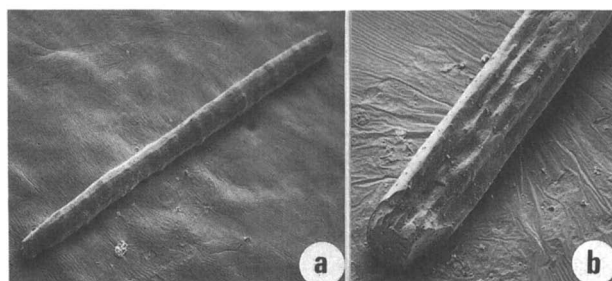


Figure 2. a) Loose resin rodlet isolated from shale x40 (length approx. 1600 $\mu$ m). b) A close up of a loose resin rodlet showing impressions of epithelial cells on external surface x75. For further details see text. (Adapted from ref. 20)

Both samples were extracted using dichloromethane and methanol. The samples were not powdered to allow sample handling.

Curie-point pyrolysis-gas chromatography (Py-GC) analyses were performed with a Hewlett-Packard 5890 gas chromatograph using a FOM-3LX unit for pyrolysis. The Curie temperature was 610°C. The pyrolysis time was 10 seconds. The gas chromatograph, equipped with a cryogenic unit, was programmed from 0°C (5 min) to 300°C (10 min) at a rate of 3°C/min. Separation was achieved using a fused-silica capillary column (25m x 0.32mm) coated with CP Sil-5 (0.4 μm). Helium was used as the carrier gas.

Curie-point pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) analyses were performed using the same equipment and conditions as described above for the Py-GC. The gas chromatograph was connected to a VG 70S mass spectrometer operated at 70eV with a mass range  $m/z$  45-800 and a cycle time of 1.8s.

The C<sub>0</sub>-C<sub>2</sub> alkylphenol pyrolysis products were identified by comparison of their relative retention times and mass spectra with those of authentic standards. All other compounds were identified by comparison of their relative retention times and mass spectra with those reported in the literature (26-28)

## Results

The insoluble residues of both resin rodlet samples were analyzed by Py-GC and Py-GC-MS using wires with a Curie-temperature of 610°C. The TIC trace of the pyrolysate of the *in situ* resin rodlets and the GC trace of the loose resin rodlets are shown in Figure 3. The pyrolysate of the *in situ* sample is dominated by (alkyl)phenols and (alkyl)benzenes (Fig. 3a). In addition, (alkyl)naphthalenes and homologous series of *n*-alkanes and *n*-alk-1-enes are prominently present. In contrast, the GC-trace of the pyrolysate of the loose rodlets is dominated by (alkyl)naphthalenes and *n*-alkanes (Fig. 3b). In addition, (alkyl)benzenes and (alkyl)phenols are relatively abundant.

In order to obtain clearer insight into the macromolecular composition and to perform a detailed comparison between the two samples, single and summed mass chromatograms revealing specific compounds were used.

**Aliphatic Hydrocarbons** The mass chromatograms of  $m/z$  57 of both pyrolysates show an *n*-alkane distribution ranging from C<sub>6</sub> to C<sub>32</sub> (Figs 4a,b). In addition, a homologous series of *n*-alk-1-enes is present.

The *n*-alkane distribution pattern of the pyrolysate of the *in situ* sample reveals a maximum at C<sub>11</sub> after which it gradually decreases (Fig. 4a). In contrast, the distribution pattern in the pyrolysate of the loose rodlets shows a maximum at C<sub>14</sub> with two submaxima at C<sub>10</sub> and C<sub>25</sub> (Fig. 4b). Furthermore, it shows an odd over even predominance in carbon number of the long-chain alkanes, in particular those with 25, 27 and 29 carbon atoms. The mass chromatogram of the loose rodlets also reveals substantial amounts of isoprenoid hydrocarbons, with pristane as the most dominant member.



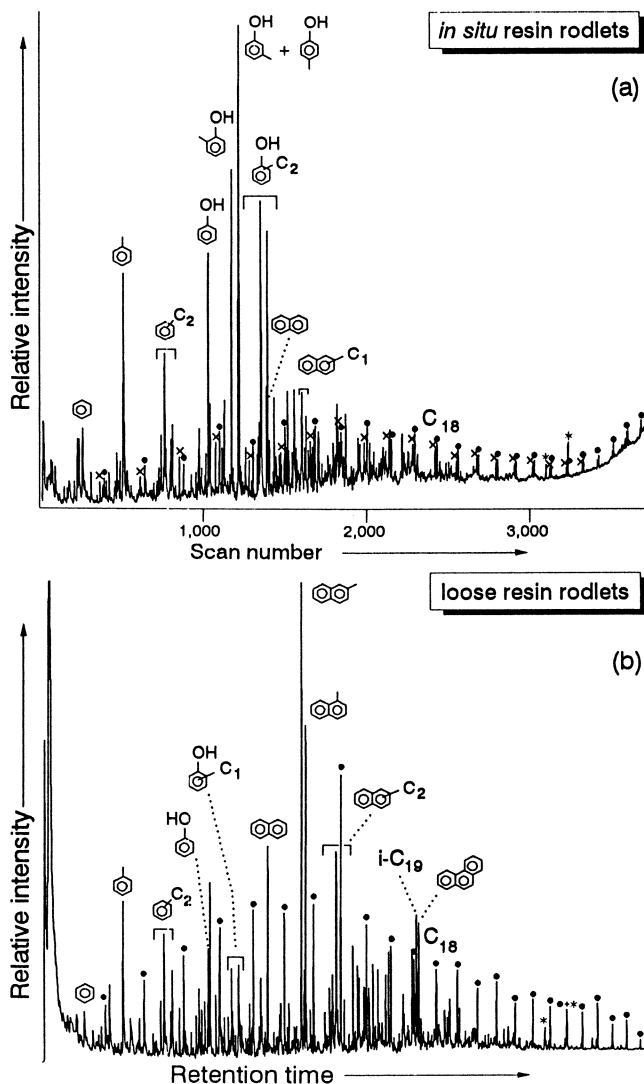


Figure 3. a) TIC trace of the pyrolysate (Curie-temperature 610°C) of *in situ* resin rodlets and b) Py-GC (Curie-temperature 610°C) of loose resin rodlets. Both samples are from Carboniferous Medullosan Pteridosperms. Key: x = *n*-alk-1-enes, • = *n*-alkanes, \* = contaminants, C<sub>18</sub> = octadecane, *i*-C<sub>19</sub> = Pristane. For additional information about the source of the material see text. (Adapted from ref. 20)

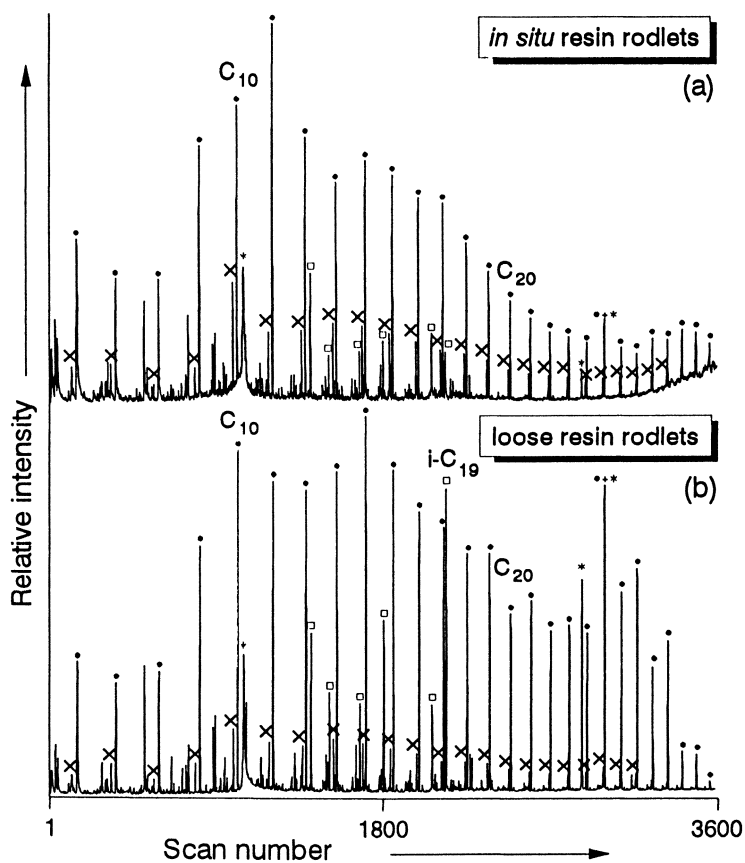


Figure 4. Mass chromatograms ( $m/z$  57) of the pyrolysates of a) *in situ* resin rodlets and b) loose resin rodlets. Key: x =  $n$ -alk-1-enes, • =  $n$ -alkanes, □ = isoprenoid hydrocarbons, \* = contaminants,  $C_{20}$  = icosane,  $i-C_{19}$  = Pristane. For additional information about the source of the material see text.

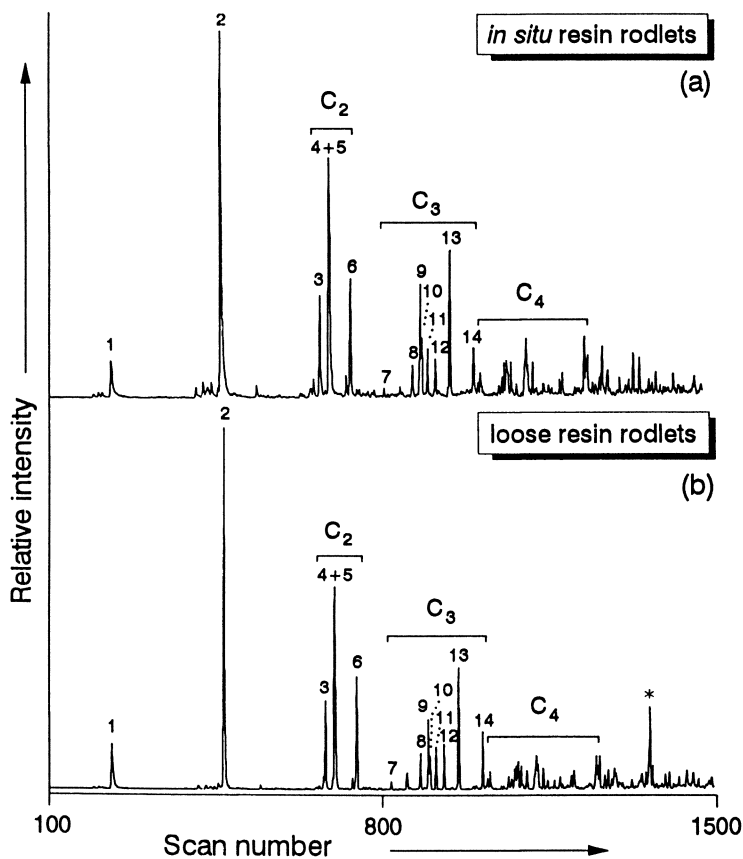


Figure 5. Partial summed mass chromatograms ( $m/z$  78+91+92+105+106+119+120+133+134) of the pyrolysates of a) *in situ* resin rodlets and b) loose resin rodlets illustrating benzene, toluene and the  $C_2$ - $C_4$  alkylated benzenes. Key: 1 = Benzene, 2 = Toluene, 3 = ethylbenzene, 4 = 1,3-dimethylbenzene, 5 = 1,4-dimethylbenzene, 6 = 1,2-dimethylbenzene, 7 = isopropylbenzene, 8 = *n*-propylbenzene, 9 = 1-methyl-3-ethylbenzene, 10 = 1-methyl-4-ethylbenzene, 11 = 1,3,5-trimethylbenzene, 12 = 1-methyl-2-ethylbenzene, 13 = 1,2,4-trimethylbenzene, 14 = 1,2,3-trimethylbenzene, \* = contaminants. For additional information about the source of the material see text.

**Aromatic Hydrocarbons** Both pyrolysates contain substantial amounts of aromatic hydrocarbons. However, the contributions of the different classes detected, (alkyl)-benzenes, (alkyl)naphthalenes and (alkyl)phenanthrenes/anthracenes, vary considerably between the two samples.

Figure 5 shows the summed mass chromatograms ( $m/z$  78+91+92+105+106+119+120+133+134) revealing  $C_0$  to  $C_4$  alkylated benzenes. The distributions in both samples are similar and are dominated by toluene (2).

The distributions of the  $C_0$  to  $C_4$  alkylated naphthalenes present in the pyrolysates are revealed in Figure 6 by their summed mass chromatograms ( $m/z$  128+141+142+155+156+169+170+183+184+197+198). In general, the distribution patterns are similar and dominated by 2-methyl- (2) and 1-methylnaphthalene (3). A small difference noted, is the relatively higher amounts of 1,2,5-trimethylnaphthalene (26) and co-eluting 2,3,8-trimethyl- and 1,2,6-trimethylnaphthalene (23 + 24). The significant difference is the presence of cadalene (1,6-dimethyl-4-isopropyl-naphthalene; 28) in the pyrolysate of the *in situ* rodlets from the coal ball. It should be mentioned that more saturated compounds with a carbon skeleton similar to cadalene (cadinane skeleton), which are often found in pyrolysates of polycadinene type (Class II) resins (4,5), are not detected.

Figure 7 shows the summed mass chromatogram ( $m/z$  178+191+192+205+206+219+220) revealing the  $C_0$  to  $C_3$  alkylated phenanthrenes and anthracenes. Phenanthrene (1) dominates the distribution pattern of the pyrolysate of the loose rodlets (Fig. 7b), whereas anthracene (2) and methylanthracenes (5, 8) are relatively more important in the pyrolysate of the *in situ* rodlets (Fig. 7a).

**Phenols** The distribution patterns of the alkylated phenols as revealed by their summed mass chromatograms ( $m/z$  94+107+108+121+122+135+136) are similar and are dominated by co-eluting 3- (4) and 4-methylphenol (3; Fig. 8). Amongst the  $C_2$  phenols, it is interesting to note that in both pyrolysates the peak reflecting co-eluting 3-ethyl- (10) and 3,5-dimethylphenol (11) is higher than the peak reflecting 4-ethylphenol (9; Fig. 8). The contribution of the former is relatively higher in the pyrolysate of the *in situ* sample.

## Discussion and Wider Implications

Detailed mass chromatographic comparison between the pyrolysates of the two resin rodlet samples indicates that the main macromolecular moieties present are relatively similar in both samples and are dominated by phenol and naphthalene moieties. The main differences noted in the pyrolysates are thought to be primarily due to the relative amounts of the alkylated phenols versus the alkylated naphthalenes. Whereas the phenols are the dominant moieties in the *in situ* rodlets (Fig. 3a), naphthalene moieties dominate in the loose resin rodlets (Fig. 3b). In addition, the pyrolysate of the *in situ* sample also revealed the presence of a highly aliphatic moiety as reflected by the homologous series of *n*-alkanes and *n*-alk-1-enes (Fig. 3a). With respect to the homologous series of *n*-alkanes observed in the pyrolysate of the loose rodlets (Fig. 3b), we believe that most of these compounds are present as such despite the extensive extraction of the sample. This is mainly based on the fact that only relatively

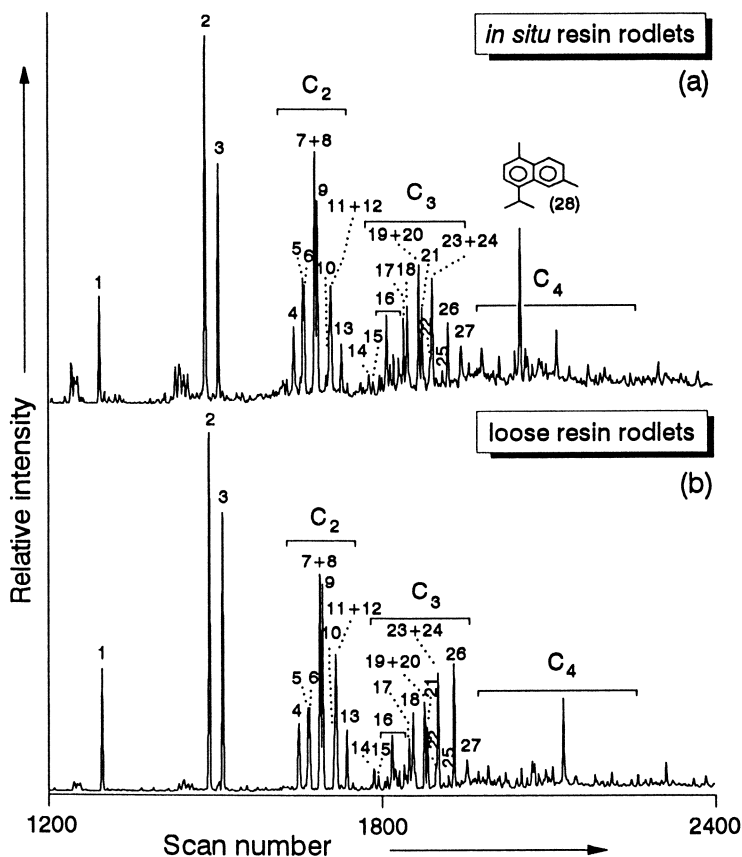


Figure 6. Partial summed mass chromatograms ( $m/z$  128+141+142+155+156+169+170+183+184) of the pyrolysates of a) *in situ* resin rodlets and b) loose resin rodlets illustrating naphthalene and the C<sub>1</sub>-C<sub>4</sub> alkylated naphthalenes. Key: 1 = Naphthalene, 2 = 2-methyl-naphthalene, 3 = 1-methylnaphthalene, 4= 2-ethylnaphthalene, 5 = 2,6-dimethylnaphthalene, 6 = 2,7-dimethylnaphthalene, 7 = 1,3-dimethylnaphthalene, 8 = 1,7-dimethylnaphthalene, 9 = 1,6-dimethylnaphthalene, 10 = 1,4-dimethylnaphthalene, 11 = 2,3-dimethylnaphthalene, 12 = 1,5-dimethylnaphthalene, 13 = 1,2-dimethylnaphthalene, 14 = 2-propylnaphthalene, 15 = 1-propylnaphthalene, 16 = ethyl-methylnaphthalene, 17 = 1,3,7-trimethylnaphthalene, 18 = 1,3,6-trimethylnaphthalene, 19 = 1,4,6-trimethylnaphthalene, 20 = 1,3,5-trimethylnaphthalene, 21 = 2,3,6-trimethylnaphthalene, 22 = 1,2,7-trimethylnaphthalene, 23 = 1,6,7-trimethylnaphthalene, 24 = 1,2,6-trimethylnaphthalene, 25 = 1,2,4-trimethylnaphthalene, 26 = 1,2,5-trimethylnaphthalene, 27 = 1,2,3-trimethylnaphthalene, 28 = 1,6-dimethyl-4-isopropyl-naphthalene. For additional information about the source of the material see text.

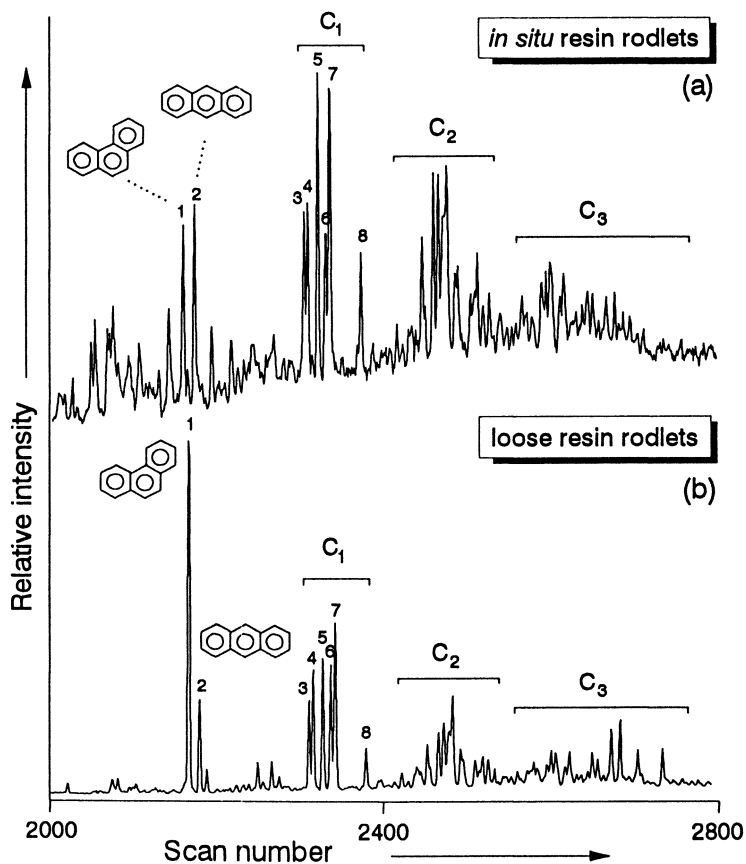


Figure 7. Partial summed mass chromatograms ( $m/z$  178+191+192+205+206+219+220) of the pyrolysates of a) *in situ* resin rodlets and b) loose resin rodlets illustrating phenanthrene and anthracene and the C<sub>1</sub>-C<sub>3</sub> alkylated phenanthrenes / anthracenes. Key: 1 = phenanthrene, 2 = anthracene, 3 = 3-methylphenanthrene, 4 = 2-methylphenanthrene, 5 = 2-methylanthracene, 6 = 1-methylphenanthrene, 7 = 9-/4-methylphenanthrene, 8 = 9-methylanthracene. For additional information about the source of the material see text.

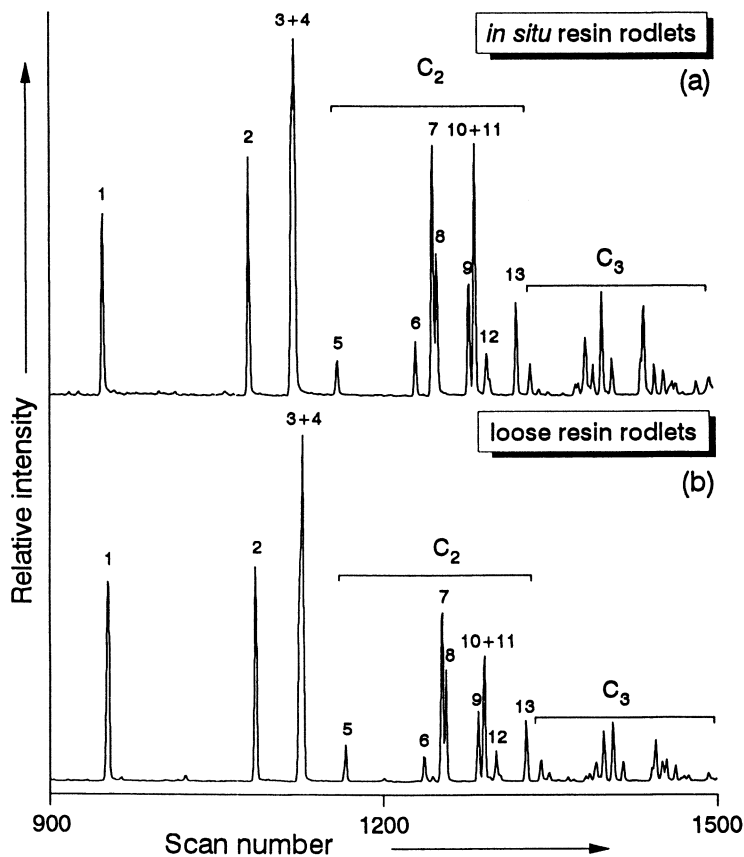


Figure 8. Partial summed mass chromatograms ( $m/z$  94+107+108+121+122+135+136) of the pyrolysates of a) *in situ* resin rodlets and b) loose resin rodlets illustrating phenol the C<sub>1</sub>-C<sub>4</sub> alkylated phenols. Key: 1 = Phenol, 2 = 2-methylphenol, 3 = 4-methylphenol, 4 = 3-methylphenol, 5 = 2,6-dimethylphenol, 6 = 2-ethylphenol, 7 = 2,4-dimethylphenol, 8 = 2,5-dimethylphenol, 9 = 4-ethylphenol, 10 = 3-ethylphenol, 11 = 3,5-dimethylphenol, 12 = 2,3-dimethylphenol, 13 = 3,4-dimethylphenol. For additional information about the source of the material see text.

small amounts of unsaturated counterparts, *n*-alk-1-enes, are detected (Fig. 3b), therefore excluding a highly aliphatic macromolecular moiety. Additional evidence for interpretation of these compounds being 'free', are the significant amounts of pristane (i-C<sub>19</sub>) and phenanthrene which, if observed in pyrolysates, are considered to be present as such. A possible explanation for these compounds may be that they are occluded within the rodlets and that an impenetrable exterior prevents solvent penetration. This idea finds support from other observations of cavities and vesiculate features in rodlets surrounded by a continuous outer zone (13,15). Moreover, it should be mentioned that difficulties removing extractable compounds from resins, in general, has been reported (4,29).

The data presented here are unlike any pyrolysis data reported from resins to date. This, therefore, leads to the question as to how to interpret these results with reference to the resinous nature of these Upper Carboniferous pteridosperm resin rodlets. Four possible interpretations are considered:

- 1) The original material was of a known type of resin, however, it has been diagenetically modified,
- 2) the samples are composed of a unknown extinct type of resin,
- 3) the original material was not resin but another type of secretion or,
- 4) the material is secondary infill formed during diagenesis and coalification processes.

**Modified Resin** To date, the only three types of polymeric fossil resins known are those based on labdanoid moieties (polyditerpenoid), cadinane units (polysesquiterpenoid) or polystyrene units representing the Classes I, II and III (2), respectively. Amongst the pyrolysis products detected only one alkylated benzene and three alkylated naphthalenes can in principal be linked to polymeric structures of known types of resins. 1-Methyl-4-isopropylbenzene (not shown), 1,6-dimethylnaphthalene (9; Fig. 6) and cadalene (28; Fig. 6) have been shown to be indicative of polysesquiterpenoid resins (27,30,31), whereas 1,2,5-trimethylnaphthalene (26; Fig. 6) has been shown to be derived from polyditerpenoid resins (32,33). In particular, abundant occurrences of cadalene amongst C<sub>0</sub> to C<sub>4</sub> naphthalenes in pyrolysates and extracts is, almost exclusively, considered to be due to input from polycadinene type resins even in the absence of other products (18,19,28,31).

The fact that the pyrolysate of the *in situ* rodlets reveals significant amounts of cadalene could suggest that this sample contained polysesquiterpenoid resin. However, flash pyrolysates of polysesquiterpenoid resins normally reveal additional, more saturated, pyrolysis products which have a carbon skeleton similar to cadalene (cadinane skeleton) (4,5). These are not detected. One explanation for their absence could be diagenetic alteration. However, the maturity of the sample is relatively low (R<sub>o</sub> 0.49) and, more importantly, the amount of 1,6-dimethylnaphthalene (the diagenetically more stable compound) to be released (19), is not exceptionally high. This therefore implies that a polysesquiterpenoid resins source is unlikely. Moreover, preliminary analyses of the extract of this sample revealed no bicadinanes, compounds normally associated with polycadinene resins (4). Furthermore, as mentioned in the introduction, polycadinene type resins have only been reported in two angiosperm families (*viz.* Dipterocarpaceae and Mastixioideae a subfamily of the Cornaceae) to



date (4,5). Since the 'true' angiosperms only evolved during the mid-Early Cretaceous (see **introduction**) and polycadinene seems to be restricted to this group of plants, we believe that the *in situ* resins rodlets are not composed of (diagenetically altered) polysesquiterpenoid resin. The origin of the cadalene in the pyrolysate remains as yet unknown.

With respect to the loose resin rodlets only the slightly higher amount of 1,2,5-trimethylnaphthalene could suggest a polyditerpenoid resin type derivation (32,33). Alternatively, macromolecularly bound aromatized 8,14-*seco*-triterpenoids are the precursors of this compound (28). To date, polyditerpenoid have been mainly detected in gymnosperm (fossil) resins, especially in the Coniferales (conifers; 2,3,29,31). The fossil record of conifers does extend back to the latest Upper Carboniferous (11) and, thus, conifers could be the source for these loose resin rodlets rather than Medullosan pteridosperms (seed ferns). Alternatively, the gymnosperm *Cordaites*, which is common in the Upper Carboniferous of Euramerica, may be the source of these materials since cordaitan wood has been reported to be a source of resinite globules in coals (13). However, their overall contribution is considered to be low (13) and the most likely source of the loose rodlets remains the Medullosan pteridosperms. With respect to the chemical data of these loose resin rodlets, we are of the opinion that these rodlets do not contain polyditerpenoid resin.

To date, very little chemical data has been reported on Carboniferous resin rodlets. The chemical evidence revealed by elemental analyses and IR data (13) supports our findings showing that the chemical composition of these rodlets is different from known resins, and suggesting closer similarity with inertinite macerals such as fusinite (13). We, therefore, believe that the chemical results from the *in situ* and loose fossil resin rodlets show no evidence for a close resemblance with any of the polymeric fossil resins known.

**Unknown Extinct Type of Resin** As outlined above, our data do not fit in with any of the known types of polymeric fossil resin and could therefore, in principal, be considered as representing a new type of unknown extinct polymeric fossil resin specific for Medullosan pteridosperms composed of phenol and naphthalene moieties. Whether, in that case, the highly aliphatic macromolecular moiety recognized in the *in situ* sample reflects an actual part of the original resin or is derived from occluded material is not clear. However, it should be mentioned that previous Curie-point pyrolysis data from a resinite fraction from the Upper Carboniferous Indiana paper coal, obtained by density gradient centrifugation, revealed the exclusive presence of a highly aliphatic macromolecule (16). Rather than considering the highly aliphatic macromolecule to reflect the actual resinite, these authors considered impurity of the sample analyzed as one of the possible explanation for its presence. Alternatively, they suggested that, due to fluidization of highly aliphatic macromolecules derived from other materials, such as cuticles, and subsequent migration, aliphatic moieties might have become incorporated within the resinite. It is interesting to note that this latter suggestion was considered to explain the presence of aliphatic moieties in the pyrolysates of buried angiosperm wood of *Rhizophora mangle* (34) with those moieties being derived from material present as cell inclusions. In our case, a third explanation for the aliphatic constituent could be cell wall material of the epithelial

cells surrounding the *in situ* resin material, which has become part of the sample analyzed. For example, if the cell wall material was composed of suberan (20,35), this could account for the aliphatic moieties. However, this latter explanation is deemed unlikely based on the microscopic observations showing that the epithelial cells are thin walled (Fig. 1c) and reveal no evidence of suberization.

Associated cell wall remains could explain the phenols, if the original macromolecular constituent of these walls was lignin-cellulose. This, because phenols are known to be diagenetic products of lignin (36-38). An interesting aspect of this latter possibility is that it could also explain the lower relative amounts of (alkyl)phenols in the pyrolysate of the loose rodlets. Cell wall material would only occur on the outer surface of the sample so that, abrasion of the surface of the loose rodlets from the shale would result in substantially lower amounts of phenols. However, one chemical aspect, which to a certain extent contradicts a contribution of lignin-derived material, is the distribution of the C<sub>2</sub> phenols. In diagenetically altered lignin samples, C<sub>2</sub> phenol distributions show a dominance of the 2,4-dimethyl- and 4-ethylphenol isomers (36,37) whereas in the resin rodlets co-eluting 3-ethyl- and 3,5-dimethylphenol dominate over the 4-ethylphenol isomer (Fig. 8). On the other hand distribution patterns of phenolic compounds, similar to those observed in our samples, have been reported in pyrolysates of Carboniferous coals (27). Assuming that lignin was the main precursor for the phenols observed in these coals this then could still mean that the phenols observed in our pyrolysates are derived from lignin. The different distribution of the C<sub>2</sub> phenols being caused by a different type of diagenetic chemical alteration. Alternatively, the chemical structure of the lignin produced by plants, such as Medullosan pteridosperms, growing during the Carboniferous was different from modern lignins, the structural building blocks of which are methoxyphenols with an alkyl side chain substituted at the C-4 position (39). This latter suggestion should be considered since none of the main groups of modern day plants producing modern lignins, conifers and angiosperms, contributed significantly to the coals in the Carboniferous. Rather than containing methoxyphenols the lignins of the Carboniferous plants could be composed of alkylated phenols. Such an assumption could explain why, as yet, only two papers have reported small amounts of methoxyphenols from Carboniferous material (40,41). However, as with the suggestion of suberan incorporation, lignin contribution to the resin rodlets is deemed unlikely based on the morphological observation showing that the epithelial cell are thin walled.

The pyrolysis data from the resin rodlets may reflect the chemical composition of resins produced by Medullosan pteridosperms (with possibly some attached and/or occluded material). The actual resinous nature of this material is, however, difficult to prove partly because of the absence of extant counterparts. One possibility to circumvent the problem of the recognition of resins is using purely chemical evidence. This would need clear chemical definitions of resins, primarily based on modern material. However, this could create a bias against unknown extinct resins because they might not fit in such an established scheme due to the absence of modern material. The facts that the chemical composition is completely different from any of the polymeric fossil resins known and reveals more similarity with vitrinite, in the case of the *in situ* rodlets, and semifusinite, in the case of the loose rodlets (see also section

**Secondary Infill**), suggest to us, that these samples are not composed of resin (*sensu stricto*). Moreover, the fact that, in particular the *in situ* rodlets, show no autofluorescence seems to imply that the samples are not true resin, since fossil resins and resinites show autofluorescence (42). However, fluorescence is not an unambiguous feature and it shows significant variation (42). Moreover, we would like to emphasize caution with respect to fluorescence of resinites from density gradient centrifugation, because incorporated highly aliphatic moieties, derived from materials such as cuticles, may represent the bulk of such resinites (16). Hence, the autofluorescence detected may not necessarily be due to the presence of the resinous material.

As suggested previously (2) a distinct chemical composition represented by only one 'resin' sample, should not immediately be regarded as a new type of resin. Despite the fact that we analyzed two samples, we are of the opinion that, prior to considering these rodlets as a new type of unknown extinct resin, more Carboniferous resin rodlets should be analyzed, in order to establish a diagnostic chemical signature.

**Secretions other than Resin** Palaeobotanical observations suggest that the ducts containing the analyzed infills are certainly secretory in nature; they have been described as 'elongate secretory canals filled with amorphous contents' (25). Therefore, another possible explanation for the absence of autofluorescence, as well as for the distinct chemical nature of these rodlets, could be that they are secretions other than resin such as gum, tannin or rubber.

Gums, however, are primarily composed of monosaccharide moieties, such as galactose, mannose and rhamnose, which become hard nodules upon dehydration (43). The fossilization potential of such polysaccharide biopolymers is considered low and, as yet, we know of no studies reporting gums in the fossil record (43).

In contrast, non-hydrolyzable tannins (proanthocyanidins), which are primarily composed of flavan-3-ols units (44), are considered to have a much higher preservation potential (43). This is substantiated by solid state  $^{13}\text{C}$  NMR data showing evidence of tannins in brown coals (45). Recent studies using tannin monomers, (catechin = flavan-3-ol), and condensed tannins showed that upon flash pyrolysis only two major compounds were released *viz.* 1,2-benzenediol and 4-methyl-1,2-benzenediol (46-48), compounds which were not detected in our samples. This therefore, seems to exclude tannins. However, upon fossilization 1,2-benzendiol moieties, in for example fossil lignin, are shown to give rise to phenol moieties via dehydroxylation (36,37). Hence, diagenetically altered non-hydrolyzable tannin might be the source of the resin rodlets.

Another secretion product that should be considered is rubber which has been reported in the fossil record (49). Based on solid state NMR data the fossil material was shown to be a *cis*-polyisoprene rubber (49). Upon pyrolysis such a polyisoprene polymer is to be expected to release almost exclusively isoprene moieties, which is in contrast with our pyrolysis data. This, therefore, excludes rubber as a precursor for the rodlets studied here.

**Secondary Infill** A fourth alternative explanation for the chemical composition of these rodlets could be that these materials are secondary infill formed during the

coalification processes via humification and gelification. This possibility must be considered because comparisons between pyrolysates of the resin rodlets and those of whole coals and different coal fractions reveal remarkable similarities (16,27). Not only the total pyrolysates but also the distributions of the different compound classes, such as (alkyl)benzenes (Fig. 5), (alkyl)naphthalenes (Fig. 6), (alkyl)phenols (Fig. 8) and polyaromatics (Fig. 7), show significant similarities when compared with, in particular, pyrolysates of Carboniferous coals of High Vol. Bituminous rank. The pyrolysate of the *in situ* resin rodlet samples is most comparable with those of vitrinites (16,27), whereas the pyrolysate of the loose resin rodlets shows greatest similarities with semifusinite and fusinite fractions (27). In the case of this latter sample, the loose rodlets, it should be noted that they are from a bed containing an appreciable amount of fusian (23). It is therefore quite possible that the loose rodlets are derived from partially charred pteridosperm petioles and hence, have been altered both pre- and post-depositionally. In the case that the loose rodlets are charred it could explain the substantial amounts of aromatics in the pyrolysate, because relatively high amounts of naphthalenes and phenanthrenes, as well as a dominance of the chemically more stable phenanthrenes over anthracenes, are reported from materials effected by palaeofires such as fusinite (27). The observations that the pyrolysate of the rodlets are similar to those of coals may imply that the rodlets analyzed were composed of low-molecular-weight products which became mobilized during coalification and condensed in empty secretory canals. Alternatively, replacement of, or mixing with, the original material might have taken place.

However, with regard to secondary infill, it should be mentioned that the rodlets analyzed are taxonomically characteristic of Medullosan pteridosperm, whereas they are absent in other Carboniferous coal forming plants, such as lycopods. To date, they are only known to occur in specialized ducts surrounded by epithelial cells in specific parts of the plants. Surrounding cells have no infills. Any migration and/or secondary infill of cells would not be so specific. The epithelial cells were not infilled as seen with in the *in situ* rodlets (Fig. 1c) and by the impressions of epithelial cells on the isolated rodlets (Fig. 2b). Furthermore, as pointed out by Scott and Rex (50), most coal balls are formed very early during peat formation prior to gelification. This, therefore, almost completely precludes migration and filling of cells to any significant extent. Hence, based on these morphological observations an origin of the resin rodlets as being formed by secondary infill is highly unlikely.

### Differences between the Resin Rodlets

Assuming that both samples are derived from Medullosan pteridosperms, the differences observed between the pyrolysates may be caused by a variety of aspects. It is clear that the Indiana coal balls formed in mires which may have been subject to some marine influence (21), whereas the Swillington shales were deposited on a 'swampy floodplain' or clastic swamp (23). Hence, the difference in the resulting lithology, caused by those different depositional settings, from which the samples are obtained; *in situ* rodlets from a calcareous permineralization, a coal ball, whereas the loose rodlets are from a shale, may have effected their chemical composition. Moreover, the shale sample might be more physically abraded. In addition, the

Swillington sequence is of Westphalian B age whereas the Indiana sequence is Westphalian D in age, each containing their own specific Medullosan pteridosperms. Alternatively, the slightly higher maturity of the shale sample,  $R_0$  0.62, versus 0.49 for the *in situ* sample, could explain the higher amounts of naphthalenes and polycyclicaromatics when compared with the *in situ* sample because increasing maturity has been shown to be related to increasing aromaticity. Overall the differences between the two samples may reflect a combination of a) different species, b) different ecological setting, c) different enclosing sediment, d) different diagenetic history, e) different pre-depositional history. Prior to distinguishing between these possibilities much more data from, in particular rodlets from rocks of low maturation, is needed.

## Conclusions

Two morphologically well-characterized Upper Carboniferous resin rodlet samples have been analyzed using Curie-point pyrolysis (610°C)-gas chromatography(-mass spectrometry). One sample represented *in situ* resin rodlets from Medullosan pteridosperm petiole material obtained from a coal ball, whereas the others were obtained as loose rodlets from a shale residue, probably also derived from Medullosan material. The pyrolysate of the *in situ* resin rodlets was dominated by alkylated phenols. In contrast, the pyrolysate of the loose rodlets was dominated by alkylated naphthalenes. The use of detailed mass chromatograms revealed that the samples are composed of similar moieties, namely phenols and naphthalenes, with the variations observed in the pyrolysates primarily due to differences in relative abundances of the various compound classes. Differences in lithology and/or maturity are suggested to have effected the chemical composition. The pyrolysis results from both these samples are unlike any known fossil polymeric resin. Rather than considering these data indicative of a new type of fossil resin, we suggest a secretion product such as diagenetically altered non-hydrolyzable tannin to be the most likely source of this material. In addition, the homologous series of *n*-alkanes present in the loose rodlets is believed to be derived from trapped extractable material present in enclosed cavities. With respect to the *n*-alkanes and *n*-alk-1-enes observed in the *in situ* sample, these are suggested to be derived from highly aliphatic moieties originated from material other than these rodlets. The origin of this aliphatic material and the way in which it has become incorporated is as yet unknown. No extant counterparts can be studied since Medullosan pteridosperms became extinct during the Permian, hence, the possibility that the chemical composition of these resin rodlets is specific for resin of Carboniferous Medullosan pteridosperms cannot be fully excluded. However, prior to drawing any final conclusions about the chemical composition of these and other so-called resin rodlets we are of the opinion that more morphologically and botanically well-characterized material should be analyzed. Hence, because a resinous nature/origin of these rodlets has, as yet, not been established we would like to suggest the term 'resin rodlets' for these materials rather than resin rodlets *sensu stricto*.

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## Chapter 9

# Analysis of Fossil Resins from Axel Heiberg Island, Canadian Arctic

Ken B. Anderson<sup>1,3</sup> and Ben A. LePage<sup>2,4</sup>

<sup>1</sup>Amoco Oil Company, P.O. Box 3011, Naperville, IL 60566–7011

<sup>2</sup>Department of Biological Sciences, University of Alberta, Edmonton T6G 2E9 and Canadian Circumpolar Institute, Old St. Stephen's College, University of Alberta, Edmonton, Alberta T6G 2E2, Canada

Ambers are well known and abundant in terrestrial sediments all over the world. However, due largely to the absence of definite morphological characteristics, the precise botanical origin of most amber samples are, at best, often a matter of speculation. This has severely restricted the usefulness of amber in paleobotanical and paleoecological interpretations. The molecular composition and structural characteristics of fossil resins however, may preserve evidence of their botanical origin, which could be of great value in geochemical, paleobotanical and paleoenvironmental studies. The remains of a number of exceptionally well-preserved Taxodiaceae-dominated swamp-forest communities have been discovered in the sediments of the middle Eocene (45 million years old) Buchanan Lake Formation of Axel Heiberg Island, Canadian Arctic Archipelago. Amber collected from these ancient *in situ* forests provides a unique opportunity to characterize these resins chemically and taxonomically. Resinite associated with *Metasequoia* Miki *ex* Hu & Cheng, *Pinus* L. and *Pseudolarix* Gordon has been characterized using Pyrolysis-Gas Chromatography-Mass Spectrometry. This method provides a direct analysis of the molecular structure and composition of the resin. In several cases, both bled and cone-resin samples have been characterized. The results of these analyses are presented and discussed. The implications of these results for the botanical origins of other ambers represented in the fossil record (including succinite) are also discussed.

NOTE: This chapter is Part VI of a series entitled "The Nature and Fate of Natural Resins in the Geosphere." Part V of this series is Chapter 6 of this volume.

<sup>3</sup>Current address: Chemistry Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439

<sup>4</sup>Current address: Department of Geology, University of Pennsylvania, Philadelphia, PA 19104–6316

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Ambers, which are also commonly referred to as resinates or fossil resins, are the "fossilized" resins of vascular plants. These materials are common constituents of fossiliferous deposits, and due to their potential value as biological markers they are currently receiving considerable attention from scientists in a number of earth science disciplines. With the application of modern analytical techniques, a considerable number of fossil resins have now been characterized and considerable detail of the (macro)molecular structures of these materials has been established (1-7). There is, however, very little data available concerning the analysis of fossil resins which can be unambiguously assigned to specific extinct or extant species. This is due to the fact that (i) in most cases the structural characteristics of the fossil resins are not necessarily diagnostic of specific species, and (ii) well-preserved, relatively uncompressed, botanically identifiable macrofossil remains (e.g., wood and seed cones) with recoverable resinite still present within the tissues, are uncommon. In rare instances however, fossil resins occur in association with identifiable plant remains and in such cases, information concerning the structural characteristics of the resins produced by these fossil representatives can be obtained.

Succinite, the most common and abundant form of Baltic Amber, and related Class Ia resinates are an extraordinarily abundant resource which have been mined throughout and before recorded history. By some estimates, reserves of Baltic Amber may be as great as  $4.8 \times 10^8$  kg (8, 9), and this does not include microscopic resinite fragments which undoubtedly add significantly to this figure. The species of tree(s) which produced this exceptional resource, however, is unknown and represents, perhaps arguably, one of the great mysteries of the organic geosciences.

On Axel Heiberg Island, one of the northernmost islands in the Canadian Arctic Archipelago, a series of exquisitely preserved fossil forests of Eocene age (ca. 45 million years) were discovered in sediments of the Buchanan Lake Formation (Figure 1) (10-13). Macrofossil remains containing resinite and unassociated resin "balls" (see sample description given below) are common throughout these deposits. In this paper we describe the analytical results of a number of resinates from this site and discuss the implications of these data for the origin of succinite and related Class Ia resinates which are exceptionally abundant in the Baltic region.

### **Speculation on the Botanical Nature of the "Amber Trees"**

Scientists have been attempting to identify the species of tree responsible for the production of the Baltic Amber for over 150 years. Resin-bearing fossil woods from the Baltic region were first reported as an unidentified species of *Pinus* L. by Aycke in 1835 (14). Göppert (15) subsequently identified this wood as an extinct species of conifer showing affinity with living representatives of the family Pinaceae and named these specimens *Pinites succinifer* Göppert. Since then, interest in the plant remains associated with Baltic Amber has produced several names for these resin-bearing woody fragments (e.g., *Abies bituminosa* Haczewskiego (16), *Pinites succinifer* (17), *Taxoxylum electrochyton* Menge (18), *Pityoxylon succiniferum* (Göppert) Kraus (19), *Picea succinifera* (Göppert) Conwentz (20), *Pinus succinifera* (Göppert) Conwentz (21, 22), *Pinus* (23), *Pinus halepensis* Miller (24), and *Agathis* Salisbury (25-28)), but

little has been added to resolving the true identity of the resin-producing tree(s) and its relationship with living conifers.

On-the-whole, analysis of the anatomical and morphological features of the plant remains associated with Baltic Amber indicate the amber was produced by an extinct member of the Pinaceae. However, removal of succinic acid from Baltic Amber by alkaline hydrolysis has been shown to give a product with an infrared spectrum nearly identical with that of *Agathis australis* (Lambert) Steudel (Family Araucariaceae) resin (25, 29); and conversely, succinylation of *A. australis* resin results in a product with an infra-red spectrum comparable to that of Baltic Amber (27). Based on these analyses, a number of workers (25, 29) have suggested that Baltic Amber may have been derived from the resin of an extinct araucarian species, probably related to *Agathis*. This has been widely interpreted as evidence for phylogenetic affinity between Baltic Amber and resins produced by members of the Araucariaceae, especially *Agathis* (25, 28-31).

These analyses however, demonstrate only that the gross structural characteristics of these products are similar. Py-GC-MS analyses have shown that the macromolecular structures of Class Ia ambers (including succinite) are derived from resins based on polymers of *regular* labdanoid diterpenes, including communic acid, communol, and biformene (7). Various analyses of living (32) and fossil (2, 3, 26, 29) *Agathis* resins indicate that these resins are also based on a *regular* polylabdanoid macromolecular structure, and therefore, give results similar to those obtained for Class Ia ambers. However, of all the resin samples analyzed from living species of *Agathis*, none have been shown to contain succinic acid - a diagnostic component of Class Ia resinites. Furthermore, in addition to *Agathis* a significant number of species belonging to the Cupressaceae (33-35), and Taxodiaceae (including *Metasequoia* Miki ex Hu & Cheng, see 36, 37, and data presented below) also produce resins based on *regular* polylabdanoid macromolecular structures, but lack succinic acid. Because the macromolecular structures of the resins from any of these genera are based on comparable mixtures of *regular* labdanoid diterpenes, spectroscopic analysis of the applicable resins of each of these genera will give results essentially similar to those obtained by spectroscopic analysis of *Agathis* resin or hydrolyzed Baltic Amber. Likewise, succinylation of the resins of any of these resins will give a product broadly similar to Baltic Amber. Therefore, based on results derived from spectroscopic analyses alone it is impossible to support the notion that Baltic Amber is derived from *Agathis* or an *Agathis*-like ancestor.

In addition, it is also important to note when considering the question of the relationship of *Agathis* with the amber producing trees that, unequivocal fossil remains of *Agathis* are restricted to the Southern Hemisphere (38, 39).

Assignment of *Pinites succinifera* to the Pinaceae is based on morphological and anatomical data and its association with *Pinus*-like cones and needles in the "Blue Earth" sediments. The Blue Earth sediments are marine in origin and all of the plant fossils associated with these sediments are allochthonous, having been transported a considerable distance from the site in which the trees originally grew. If organic connections between the wood and *Pinus*-like cones and needles existed, they would have inevitably been broken during transport. Without organic connection, the

association of the amber-bearing wood, *Pinites succinifera* with *Pinus*-like cones and needles, especially in allochthonous deposits is essentially meaningless.

Using anatomical and morphological features, Conwentz (20) assigned the wood associated with the Baltic Amber to *Pinus succinifera*, but indicated the wood possessed morphological features common to both *Pinus* and *Picea* A. Dietrich. In a reinvestigation of the "amber tree" wood, Schubert (22) unequivocally concluded that the amber trees belonged to the genus *Pinus*, based on morphological features of the bark and the presence of calcium oxalate crystals in the wood parenchyma cells. However, among the conifers, calcium oxalate crystals are not unique to the genus *Pinus* and have subsequently been shown to occur in living species of *Ginkgo* L. (maidenhair tree), *Abies* Miller (fir) and *Pseudolarix* Gordon (golden larch) (40).

Hence, although anatomical, morphological, and chemical features of the resinites and resin-bearing woods from the Baltic region are consistent with several genera within the Pinaceae, previous workers have largely assumed that the amber producing tree belonged to an extinct species of *Pinus* (14, 21-24). Furthermore, in attempting to identify the resin-producing tree responsible for deposition of Baltic Amber, scientists have usually assumed that living representatives or descendant species would be copious resin producers. The notion that a species of tree that typically produces little resin can, in a particular environment become a copious resin producer has, largely, not been considered. Thus, rather than systematically analyzing the resin produced by each living species of conifer, at least within the Pinaceae, attempts to identify the amber tree have generally been limited to a few common or economically important species, such as *Pinus palustris* Miller, *P. caribaea* Morolet, and *P. elliottii* Engelman, all of which are able to produce considerable amounts of resin (41). Consequently, representatives of uncommon or relictual genera such as *Keteleeria* Carrière, *Cathaya* Chun & Kuang, and *Pseudolarix* have never been considered as possible sources of the amber.

## Experimental

**Samples.** The collection site, which is informally referred to as the fossil-forest site is located east of the Geodetic Hills at 79°55'N, 89°02'W on eastern Axel Heiberg Island (Figure 2). The sediments are comprised of interbedded sandstone, mudstone, siltstone, and lignite sequences. Autochthonous leaf-litter mats and mummified wood are commonly associated with the coal seams and represent the *in situ* forest-floor remains of poorly drained, low energy meander plain swamps (Figure 3) (11-13, 42, 43). Megafossil diversity and abundance data from a number of litter mats indicate *Metasequoia* (dawn redwood) and *Glyptostrobus* Endlicher (Chinese swamp cypress), both members of the conifer family Taxodiaceae (redwood family), were the dominant vegetational constituents of these swamp forest communities (11, 42).

Extensive late Cenozoic and Quaternary erosion has resulted in widespread exposure of the fossils contained within the leafy litter mats. Unassociated resinite "balls" as large as 4 cm in diameter (Figure 4) and resinite associated with wood (Figure 5), cones and cone scales (Figures 6-8) were collected from a number of leafy litter mats at several localities. It is important to recognize that none of the resinite samples collected and analyzed from Axel Heiberg Island show signs of significant

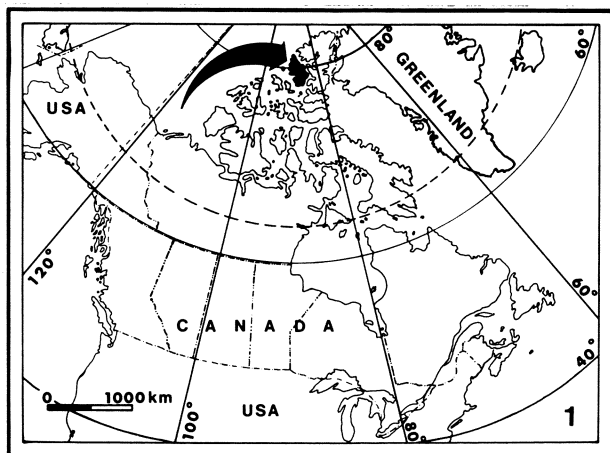


Figure 1. Map of Canada showing the location of Axel Heiberg Island in the Canadian Arctic.

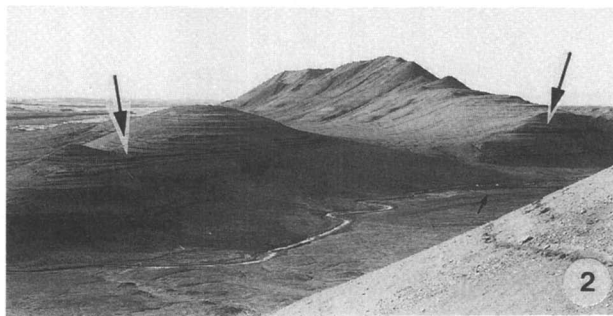


Figure 2. A view of the Axel Heiberg Island site. The black bands (arrows) are coal seams that represent the remains of swamp forest communities. Tents (small arrow) for scale.



Figure 3. A coal seam showing the portion of the seam from which forest-floor litter mats containing well-preserved fossil plants commonly occur. Note the woody debris located just above the pen and the leafy litter just below the wood and near the pen. Pen for scale.

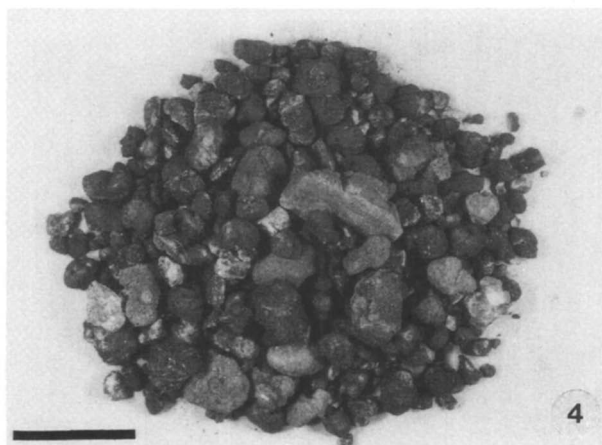


Figure 4. Unassociated fossil resinite "balls". Scale bar = 5.0 cm.

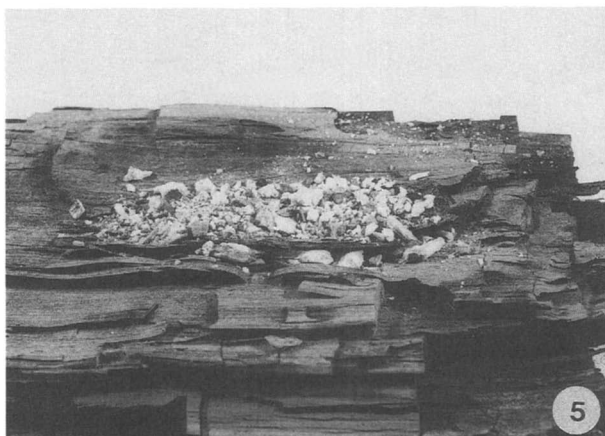


Figure 5. Fossil *Metasequoia* Miki ex Hu & Cheng wood with an exposed resin-filled cyst, X 1.6.

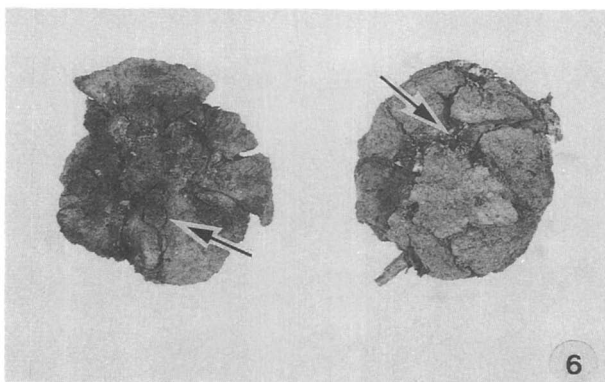


Figure 6. Fossil *Metasequoia* cones showing extruded resin (arrows), X 1.6.

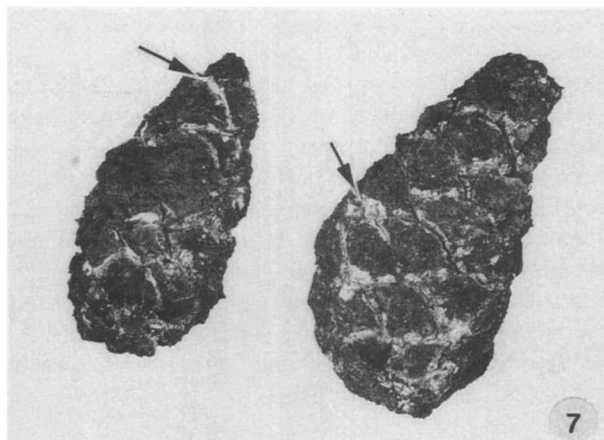


Figure 7. Fossil *Pinus* L. cones showing extruded resin (arrows), X 2.0.

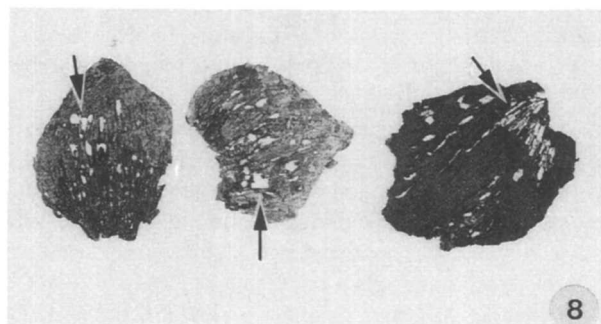


Figure 8. Fossil *Pseudolarix* Gordon cone scales showing resin still contained within the resin canals (arrows), X 2.0.



surface abrasion or any other features that would indicate the material was transported. Consequently, all of the samples discussed herein, including the unassociated resin balls, are considered to be *in situ*.

**Analytical Procedures.** Pyrolysis-gas chromatographic-mass spectrometric analyses were carried out as follows: Resinite (0.5-1mg) was placed in a quartz pyrolysis tube and pyrolysed using a CDS pyroprobe pyrolyser directly coupled to the injection port of an HP 5890 (II) GC. All pyrolyses discussed in this report were carried out at 480°C in the presence of excess tetramethyl ammonium hydroxide (TMAH) (2). It is noteworthy that for these analyses it was necessary to crush the resinites rich in succinic acid in order to ensure complete methylation of the succinic acid. The resulting pyrolysate was then chromatographed using a 60m DB-1701 capillary GC column programmed as follows: Initial temp. = 40°C, initial time = 1.5 min., ramp rate = 4°C/min., final temp. = 280°C, and minimum final time = 18.5 min. The pyrolysate was chromatographed directly into the ion source of an HP 5971 mass selective detector. Peak assignments were based on spectral interpretation, comparison with literature data, and comparison and co-elution with authentic standards whenever possible. Additional details of the techniques used in these analyses have been reported elsewhere (2). Details of the mass spectra of previously unreported compounds are given in Appendix I.

### Results and Discussion

The primary aim of this study was to characterize fossil resins associated with well-defined paleobotanical remains. This objective was derived, in part, from preliminary analysis of unassociated resinites collected at the Axel Heiberg Island site, and forms part of a larger, more general global survey of the structural characteristics of fossil resins (3-5, 7).

Py-GC-MS analysis of the unassociated resinites indicated that most were closely comparable in structural and compositional characteristics, and therefore almost certainly derived from a common botanical source. Representative data for three of these unassociated resinites are illustrated in Figure 9. The most significant find in these data is the presence and abundance of succinic acid (observed as its dimethyl ester) in the pyrolysates of most of these samples (see Figure 9 a, b). Succinic acid is a major constituent of Succinite and related Baltic Ambers, but is only rarely reported in other ambers (44). The presence of succinic acid in these resinites, and the potential for locating other succinic acid-containing resinites in unambiguous association with identifiable macrofossil remains suggested a possibility for identifying a botanical source for Baltic Amber, and was, therefore, of great interest.

Our initial efforts focused on collecting and analyzing resinite samples which could be unambiguously associated with identifiable fossil woods. Ultimately, four samples of wood identified as *Metasequoia* (Hayashi, K., Ehime University, personal communication 1994) with large resinite-filled cysts (see Figure 5) were collected and the resinite analyzed. Py-GC-MS results from two of these samples are illustrated in Figure 10. Data for the remaining samples were closely comparable with those illustrated in Figure 10. The pyrolysates of all four samples, which are typical of

## Appendix I

## Mass spectra of previously unreported compounds

Compound	MS Data and Assignment
<b>I</b>	222 (6), 207 (5), 190 (25), 177 (64), 175 (51), 161 (14), 147 (10), 133 (9), 122 (12), 121 (22), 119 (18), 107 (56), 105 (32), 95 (100), 93 (31), 91 (36), 81 (40), 79 (29), 77 (19), 69 (10), 67 (19), 55 (27), 53 (11), 45 (32), 41 (31). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1-methoxymethyl-1,4a,6-trimethyl [1S, 4aS, 8aS]
<b>II</b>	222 (15), 190 (40), 177 (39), 175 (22), 161 (31), 133 (35), 121 (31), 120 (42), 119 (70), 109 (46), 107 (61), 105 (85), 97 (74), 95 (100), 93 (41), 91 (55), 81 (64), 79 (40), 77 (36), 69 (19), 67 (34), 65 (16), 55 (67), 45 (46), 41 (63). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-1-methoxymethyl-1,4a,6-trimethyl [1S, 4aS, 8aS]
<b>III</b>	236 (17), 221 (23), 204 (8), 191 (43), 189 (62), 175 (8), 161 (21), 149 (18), 148 (15), 147 (18), 135 (42), 133 (29), 121 (84), 119 (47), 109 (100), 107 (43), 105 (34), 95 (75), 93 (36), 91 (45), 83 (19), 81 (28), 79 (32), 77 (23), 69 (14), 67 (31), 65 (11), 59 (13), 57 (11), 55 (42), 53 (17), 45 (51), 41 (47). Naphthalene-1,2,3,4,4a,7,8,8a-octahydro-1-methoxymethyl-1,4a,5,6-tetramethyl [1S, 4aS, 8aS]
<b>IV</b>	234 (22), 219 (4), 202 (10), 189 (15), 108 (41), 173 (30), 161 (18), 159 (35), 147 (19), 146 (28), 145 (47), 133 (82), 132 (100), 131 (29), 120 (25), 119 (73), 117 (25), 107 (53), 105 (53), 98 (15), 97 (10), 95 (19), 93 (24), 91 (65), 81 (18), 79 (32), 77 (35), 67 (15), 65 (14), 55 (37), 53 (17), 45 (51), 41 (40). Naphthalene-1,2,3,4,4a,5,8,8a-octahydro-1-methoxymethyl-1,4a,6-trimethyl-5-methylene [1S, 4aS, 8aS]
<b>V</b>	336 (4), 270 (2), 204 (28), 189 (100), 175 (8), 161 (23), 148 (26), 147 (20), 135 (15), 134 (14), 133 (45), 121 (43), 119 (43), 115 (73), 109 (49), 107 (25), 105 (25), 95 (43), 93 (24), 91 (24), 83 (21), 81 (16), 79 (20), 67 (20), 59 (26), 55 (69), 41 (27). 1-Naphthalenemethanol-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl monomethyl succinyl ester [1S, 4aS, 8aS]
<b>VI</b>	334 (0), 270 (1), 203 (10), 202 (21), 187 (65), 173 (28), 159 (27), 146 (50), 145 (36), 133 (78), 132 (100), 120 (21), 119 (46), 117 (16), 115 (69), 107 (21), 105 (32), 95 (14), 93 (14), 91 (36), 79 (21), 77 (15), 59 (30), 55 (61), 41 (21). 1-Naphthalenemethanol-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl-5-methylene monomethyl succinyl ester [1S, 4aS, 8aS]

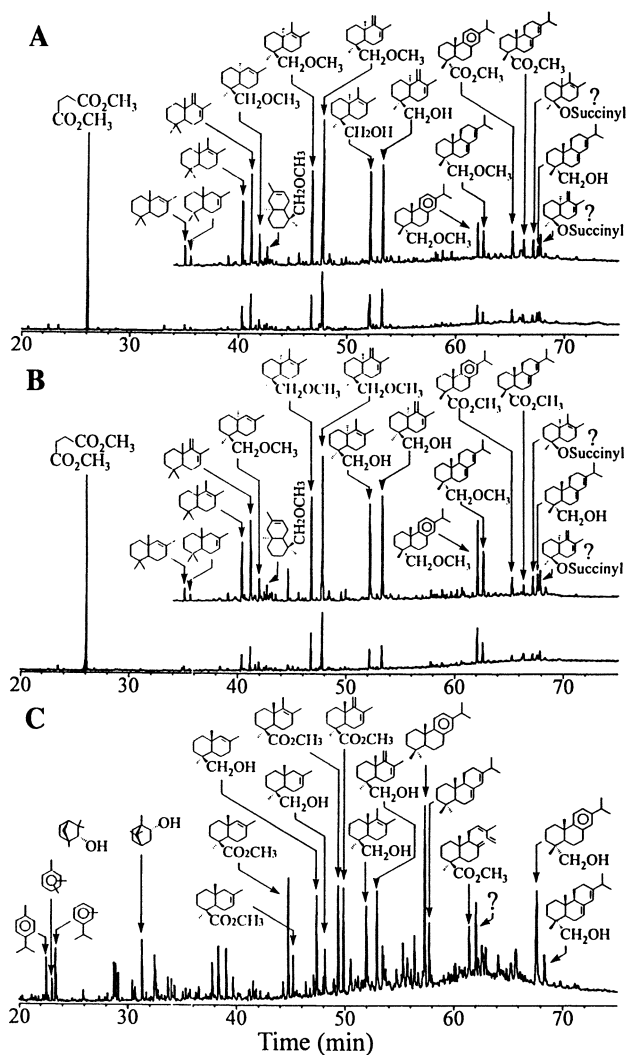


Figure 9. Py-GC-MS total ion chromatograms illustrating the distributions of products observed in pyrolysates of three unassociated resinite "balls". Based on analysis of associated resinites from the same site (see main text) these are identified as follows: A and B unassociated fossil *Pseudolarix* resinite; and C unassociated fossil *Metasequoia* resinite.

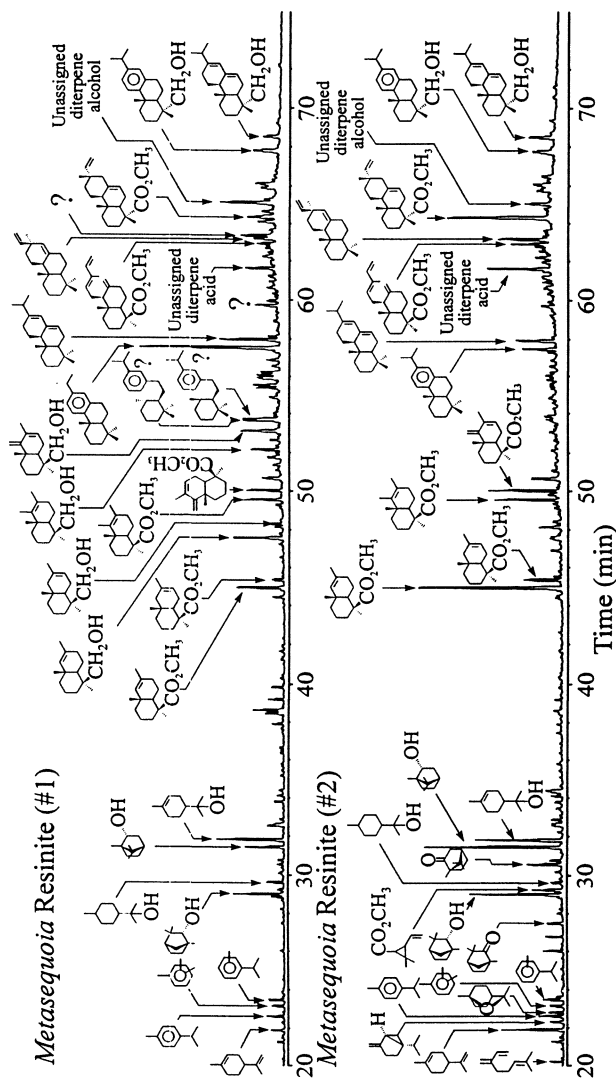


Figure 10. Representative Py-GC-MS total ion chromatograms obtained by analysis of fossil *Metasequoia* wood resinite.

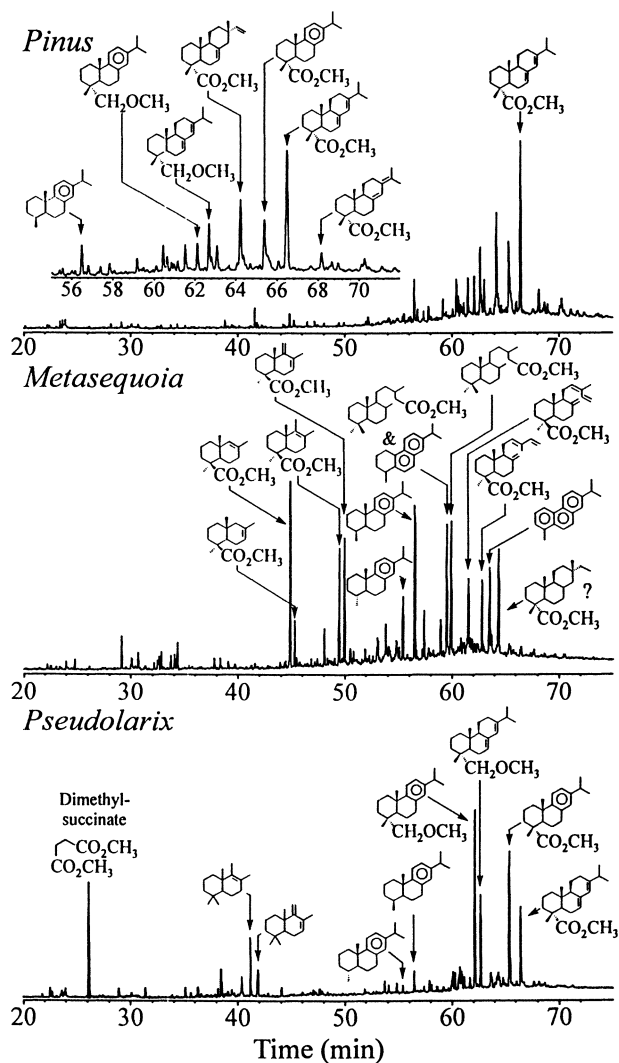


Figure 11. Py-GC-MS total ion chromatograms illustrating the product distributions observed in the pyrolysates of fossil cone scale resinites of: *Pinus*; *Metasequoia*; and *Pseudolarix*.

relatively immature Class Ib resinates, contain characteristic bicyclic products derived from communic acid and communol moieties within their macromolecular structures. A number of readily assignable mono- and diterpenoids which were undoubtedly occluded within the macromolecular network are also observed. Disappointingly, none of these resinates contained any trace of succinic acid. Nevertheless, comparison of these data with those obtained for the unassociated resinite illustrated in Figure 9c suggests this unassociated resinite is likely to have been derived from *Metasequoia*.

Attempts to collect additional bled resinite samples associated with identifiable fossil woods of other species were unsuccessful. However, numerous *Pinus*, and *Metasequoia* cones, and *Pseudolarix* cone scales were collected, and many of these retained small resinous inclusions within the tissues and resin canals (Figures 6-8). As noted by Langenheim (30), it is important to take into account the nature of the resin-producing organ in assessing the results of comparative studies of modern and fossil resins. In the present study, it has been found that the structural and compositional characteristics of each of the cone and cone-scale resinates analyzed was directly comparable to those of either (i) one of the associated or unassociated bled resinates, or (ii) known modern and fossil bled resins from other sites. Hence, by analysis of the known cone and cone-scale resinates and associated bled resinates, it is possible to unambiguously identify the source of each of the unassociated resin "balls", which are undoubtedly derived from bled resins. The results of analysis of resinite samples from *Pinus* and *Metasequoia* cones, and *Pseudolarix* cone scales are illustrated in Figure 11.

Data obtained from the *Pinus* cone resinates indicate the presence of abietane, pimarane, and isopimarane skeleton diterpenoids - typical of many living pine resins and closely comparable with other Class V resinates (2, 3). The results for *Metasequoia* cone resinates show a typical distribution of diterpenoid and diterpenoid-derived products, and characteristic bicyclic acids derived from communic acid monomers (presumably at least partially incorporated into a polymeric structure, although both *cis*- and *trans* communic acid methyl esters are observed in the pyrolysate). These data are consistent with those obtained for the *Metasequoia* bled resinates illustrated in Figure 10 and the unassociated resinite illustrated in Figure 9c, now also assigned to *Metasequoia*. Data for the *Pseudolarix* cone-scale resinates, (Figure 11), indicate succinic acid is a major component of these resinates. These resinates also contains small amounts of bicyclic hydrocarbons derived from labdanoid structures present within the resinite, and significant amounts of abietane skeleton diterpenoids, including methyl ether analogues of abietol and dehydroabietol. These results are consistent with those obtained for a large number of the unassociated resinates collected and analyzed from the Axel Heiberg Island site including those illustrated in Figure 9a and b. These data indicate that the unassociated resinates are likely to have been derived from *Pseudolarix*.

It is noteworthy that *Pseudolarix* resinates are the most abundant of the unassociated resinates found at the Axel Heiberg Island site, despite the fact that macrofossil evidence indicates *Pseudolarix* was a uncommon member of the original vegetational mosaic (43, 45, 46). This suggests that *Pseudolarix*, at least in this environment, was either a copious resin producer, or produced large amounts of resin in response to some environmental stress or unknown pathogenic or entomophilous vector.

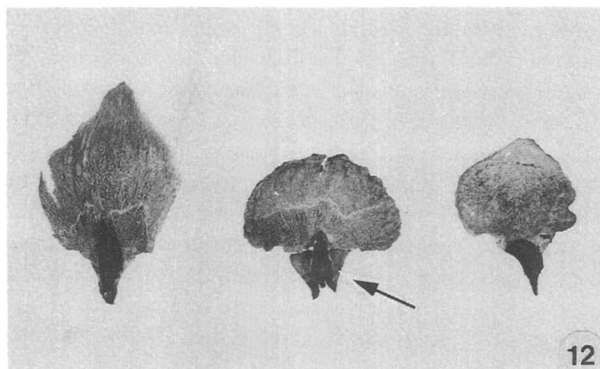


Figure 12. Fossil cone scales of *Pseudolarix amabilis* (Nelson) Rehder, a short-bracted species, X 1.9. Arrow showing short bract.

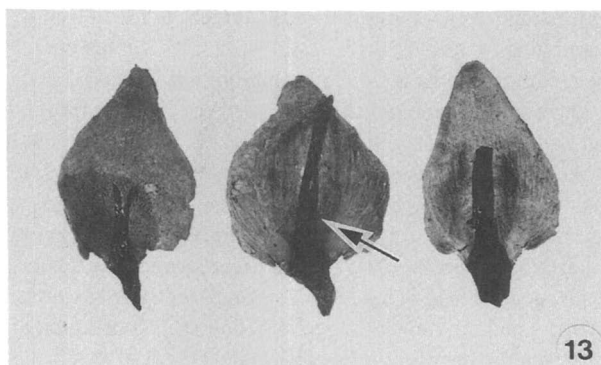


Figure 13. Fossil cone scales of *Pseudolarix wehrlii* Gooch, a long-bracted species, X 2.0. Arrow showing long bract.

At least two distinct species of *Pseudolarix* were contemporaneous in a number of the high-latitude fossil forest communities on Axel Heiberg Island (46). Species discrimination is based on differences in the length and morphology of the bract subtending the cone scale (Figures 12, 13). One species, *Pseudolarix amabilis* (Nelson) Rehder, a short-bracted form, is morphologically and anatomically identical to the monotypic *P. amabilis* (Figure 12). The second species, *P. wehrlii* Gooch, a long-bracted form, appears to represent an extinct lineage, for there are no known living representatives of this species (Figure 13). Based on the relative amount of macrofossils recovered for each species of *Pseudolarix*, the data indicates that *P. amabilis* was more abundant than *P. wehrlii*. For the purposes of comparison with the blend-resinite data, cone-scale resinite from both *P. wehrlii* and *P. amabilis* fossils were collected and analyzed. The results of these analyses are illustrated in Figure 14.

Based on the results illustrated in Figure 14, it is apparent that *P. wehrlii* is likely to have been the source of the unassociated *Pseudolarix* resinates. Although resinates of both *P. amabilis* and *P. wehrlii* contain succinic acid, it is clear that this acid is only a minor component in the resinite of fossil *P. amabilis*, whereas it is the predominant product in the *P. wehrlii* resinite. Moreover, the two abietane skeleton methyl ethers (observed in significant abundance in the unassociated *Pseudolarix* resinates) are major components in the pyrolysate of the *P. wehrlii* resinite, but are very minor components or are absent in the pyrolysate of *P. amabilis* resinite.

Given the presence of succinic acid and characteristic labdanoid-derived bicyclic products in the pyrolysates of these *Pseudolarix* resinates, comparison of these ambers with succinite is inevitable. In order to address the question of the relationship, if any, between these *Pseudolarix* derived resinates, succinite, and related Baltic Ambers, a number of comparative analyses were undertaken. It was apparent from the preliminary comparative analyses that significant differences exist between the *Pseudolarix* resinates characterized here and typical Class Ia resinates. These differences were initially observed as inconsistencies between the chromatographic behavior of products identified by mass spectrometry as characteristic bicyclic alcohols and methyl ethers. These inconsistencies suggest differences between the polyabdanoid macromolecular structure of these *Pseudolarix* resinates and normal Class Ia resinates. Therefore, in order to establish the precise nature of these products, and hence establish the precise structural characteristics of the macromolecular structures of the *Pseudolarix* resinates, a series of co-pyrolysis experiments were completed to compare the behavior of the *Pseudolarix* derived products with analogous products from other, well characterized, resinates.

Partial results from these analyses are illustrated in Figure 15. These data compare the chromatographic behavior and distributions of  $C_{15}$  bicyclic alcohols observed in the pyrolysate of unassociated *Pseudolarix* resinates with those observed in experiments in which *Pseudolarix* resinite was co-pyrolysed with representative Class Ia, Ib and Ic resinates. The results indicate that the  $C_{15}$  alcohols seen in the pyrolysates of the *Pseudolarix* resinates are identical with those derived from Class Ic resinates. These characteristic alcohols are known to be derived from ozol, and are epimeric with the analogous communol derived  $C_{15}$  alcohols observed in Class Ia and Ib resinates.



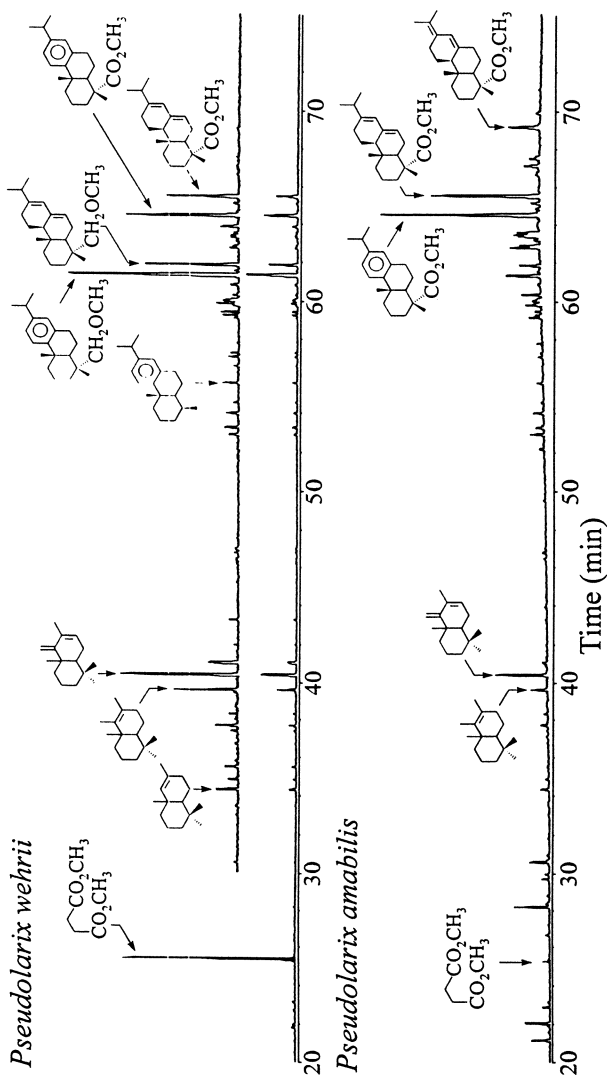


Figure 14. Comparison of the distributions of products observed in Py-GC-MS analysis of cone scale resinites of fossil *Pseudolarix amabilis* and *Pseudolarix wehrlii*.

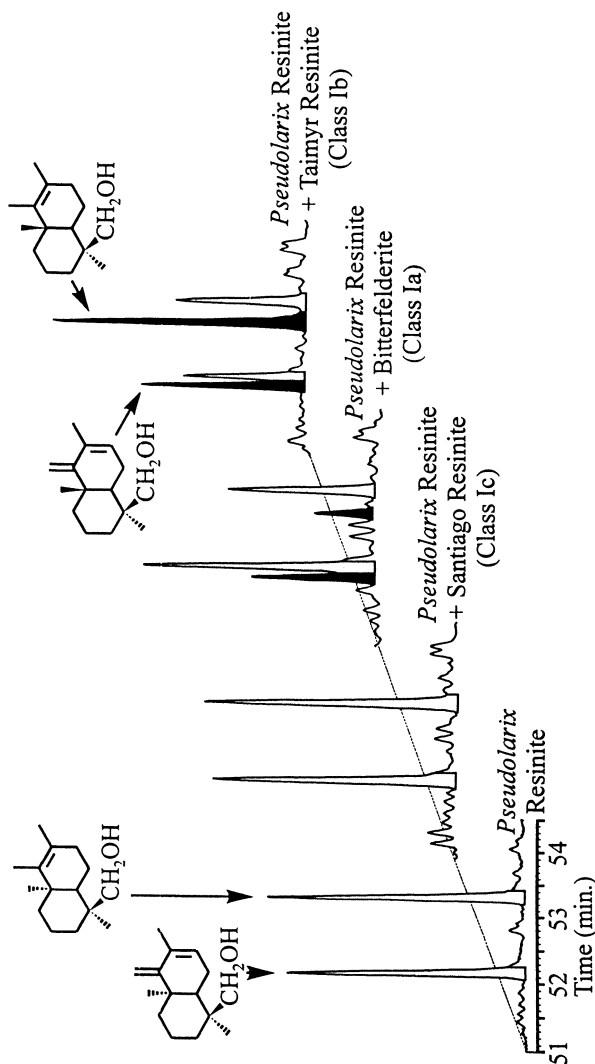


Figure 15. Partial Py-GC-MS chromatograms illustrating the distribution of  $C_{15}$  bicyclic alcohols in the pyrolysates (and co-pyrolysates) of fossil *Pseudolarix* resinite and representative Class Ia, Ib and Ic resinites from other sites.

Similar results are obtained from analysis of the distributions of C<sub>14</sub> alcohols and analogous C<sub>14</sub> and C<sub>15</sub> bicyclic methyl ethers. (Characteristic bicyclic hydrocarbons derived from *regular* and *enantio* biformene precursors are enantiomeric and hence are not resolvable on normal chromatographic phases.) This result clearly differentiates these *Pseudolarix* resinites from Succinite and other Class Ia resinites which are derived from polylabdanoid structures based on *regular* labdanoid diterpenes, and hence precludes *Pseudolarix* as a direct source for Baltic Amber.

This observation inevitably raises the question: "*What relationship, if any, exists between the Pseudolarix resinites characterized in this study, and succinite and other Class Ia ambers*". The fact that both the *Pseudolarix* resinites described here and Class Ia resinites, are derived from polylabdanoid resins, and both incorporate significant amounts of succinic acid, strongly suggests that some relationship is likely. Although the precise nature of this relationship cannot be determined based on the limited data presently available, a number of possibilities exist, and the relative merits of each are worthy of discussion:

1. It is possible that one of the two known fossil species of *Pseudolarix* existed throughout the circumpolar regions during the Paleogene (ca. 65-22 million years) and that distinct ecotypic populations existed; the Axel Heiberg Island population producing the *enantio* polylabdanoid resin and the Baltic population producing a *regular* Class Ia resin.

To the present authors, this scenario seems unlikely for two reasons. First, there are no reports of ecotypic populations of a species, or even closely related species, producing qualitatively different resins. Although distinct populations of a species may produce distinguishable resins (30) the differences are quantitative rather than qualitative. That is, rather than significant differences in the macromolecular structure, the structural and compositional characteristics of the resins are limited to differences in the relative abundances of specific components or incorporation of components with small structural modifications such as double bond isomers or differing degrees of oxidation. Secondly, the "Blue Earth" deposits have been estimated to be early Oligocene to late Eocene (ca. 35-40 million years) in age (31). *Pseudolarix* did not appear in the Baltic region until the latest Oligocene/early Miocene (ca. 22 million years) (45), more than 10 million years after deposition of the "Blue Earth" and the associated succinite and related Class Ia ambers.

2. A third species of *Pseudolarix*, endemic to the Baltic region, evolved and produced a *regular* polylabdanoid resin that incorporated succinic acid into its macromolecular structure.

This scenario is not supported by the known fossil record. Of the nearly seventy reports of *Pseudolarix* fossils in the literature only two distinct species have been recognized (45, 46). It seems unlikely that a large population of a third species of *Pseudolarix* could have existed in and around the Baltic region for millions of years without leaving evidence of its occurrence.

3. Succinite and related Class Ia resins were produced by an unknown species of vascular plant which has subsequently evolved and given rise to one or more living representatives that no longer have the ability to produce resins that incorporate succinic acid into their macromolecular structure.

The suggestion that Baltic Amber may be derived from an extinct species of *Agathis* is based on this concept. A specific discussion of difficulties with the *Agathis*/araucarian origin of Baltic Amber has already been provided above. In general however, in the absence of additional data from species specific resinites, it is not possible to determine whether any living species producing resins based on regular polylabdanoid structures ever had ancestral species that produced resins incorporating succinic acid. It is possible that evolutionary pressures caused this characteristic to be lost, but at least in the cases of the resinites characterized in this study, the basic structural characteristics of the *Pseudolarix*, *Pinus*, and *Metasequoia* resinites appear to have been conserved, at least, over the last 45 million years.

4. That polylabdanoid resinites incorporating succinic acid into their macromolecular structure independently evolved twice. One, now extinct, lineage produced *regular* polylabdanoid resins that incorporated succinic acid and evolved in Northern Europe, while a second independent lineage, which includes *Pseudolarix*, produced *enantio* polylabdanoid resins that incorporated succinic acid and evolved in North America or Asia.

Labdane diterpenoids are relatively complex molecules, which require considerable biosynthetic "dexterity" in their natural preparation. It is, therefore, highly unlikely that resins based on polylabdanoid macromolecular structures have evolved twice. There is a greater possibility that the characteristic of incorporation of succinic acid may have evolved independently in two distinct lineages, but even this possibility seems remote.

5. *Pseudolarix* and the species which produced the Baltic Amber are derived from a common ancestor which probably produced a non-specific polylabdanoid resin (i.e., including both *regular* and *enantio* labdanoids) and incorporated succinic acid into the macromolecular structure of the resin.

This interpretation seems to be the most reasonable, at least in the opinion of these authors. Given that both the Baltic Amber tree and *Pseudolarix* existed in the high latitudes of the Northern Hemisphere at about the same time, and that both produced resins based on polylabdanoid macromolecular structures, and that both incorporated succinic acid into these structures it seems reasonable to suggest that a phylogenetic relationship between these taxa exists.

Given the very small number of resinites, especially pre-Tertiary resinites, which have been characterized at a molecular level, it is not surprising that examples of an appropriate "precursor" resinite (non-specific polylabdanoid incorporating succinic acid) have not been identified in the fossil record, although at least one example of a fossil resin incorporating both *regular* and *enantio* labdanoids is known (7).

## Conclusions

The compositional and structural characteristics of fossil *Pinus*, *Metasequoia*, and *Pseudolarix* resins collected from the middle Eocene deposits on Axel Heiberg Island, have been characterized using Py-GC-MS. Bled and cone resinites from *Metasequoia* were found to be typical of Class Ib resinites, and those of *Pinus* were found to be typical of Class V resinites and closely comparable with living pine resins.

Cone scale resinites from *Pseudolarix amabilis* and *P. wehrii*, and unassociated resinites also assigned to *Pseudolarix*, however, were found to be a previously unknown form of Class I (polylabdanoid) resinite. These samples were shown by Py-GC-MS to be based on a polylabdanoid macromolecular structure incorporating substantial amounts of succinic acid. However, unlike succinite and related Baltic Ambers (which can be described in an identical manner), which are derived from resins incorporating communolic acid, communol, and analogous *regular* labdanoids, the labdanoid precursors incorporated into the polymeric structures of these samples are based on ozol and related *enantio* labdanoids.

The presence of succinic acid and the fact that both succinite and *Pseudolarix* resinites are derived from polylabdanoid resins, strongly suggests that a phylogenetic relationship exists between *Pseudolarix* and the species of tree(s) which originally produced succinite. However, the precise nature of this relationship cannot be unambiguously determined based on the data presently available. Given the differences in the configurations of the labdanoid diterpenes present in the polymeric structures of these two resinites, it is unlikely that succinite was produced by *Pseudolarix*. Nevertheless, it is possible that *Pseudolarix* and the tree(s) which produced the original source resin for Baltic Amber were related, possibly through a common ancestor. Additional analysis of contemporary and fossil resins, especially those associated with well-defined paleobotanical remains may help to further resolve this question.

## Acknowledgments

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## Chapter 10

# Resin from Africa and South America: Criteria for Distinguishing Between Fossilized and Recent Resin Based on NMR Spectroscopy

Joseph B. Lambert<sup>1</sup>, Suzanne C. Johnson<sup>1</sup>, and George O. Poinar, Jr.<sup>2</sup>

<sup>1</sup>Department of Chemistry, Northwestern University, Evanston, IL 60208

<sup>2</sup>Department of Entomology and Parasitology, College of Natural Resources, University of California, Berkeley, CA 94729

The carbon-13 nuclear magnetic resonance spectra of resins from Colombia (South America) and Kenya (Africa) are essentially identical to that of modern *Hymenaea*, when spectra are recorded with either normal or interrupted decoupling. The spectrum of a sample of copal from the Congo in Africa also is nearly identical to that of *Hymenaea*. In contrast, a resin from Tanzania, Africa, exhibits spectral properties associated with fossilization, such as reduced exomethylene resonances. These observations are consistent with a recent date and little decomposition, i.e., immaturity of the Colombian, Kenyan, and Congolese samples.

Resinous material exuded by a variety of plants may become fossilized over geological time under appropriate conditions of temperature and pressure (1). The starting organic molecules are thought generally to be terpenes, and the fossilized material, which may have been polymerized, cross-linked, and oxidized, is often referred to as amber or resinite, although numerous mineralogical names (succinite, rumanite, burmite) have been applied to specific examples that have unique geographical or paleobotanical origins. The chemical characterization of fossilized resin has been carried out by mass spectrometry (2), infrared spectroscopy (3), and high resolution solid state carbon-13 nuclear magnetic resonance (NMR) spectroscopy (4). The NMR method has been applied on a worldwide basis and now includes characterization of fossil resins from the Caribbean (5), Mexico (6), North America (7), and the Pacific (8), as well as Europe

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(4). As with infrared spectroscopy (9), NMR has been used to identify the provenance of amber found archaeologically (10). Modern resins, sampled directly from a plant or from the ground around a plant, also have been characterized by NMR spectroscopy. Often a specific botanical source gives a unique spectrum (8).

For the past decade resinous material from South America and Africa containing biological inclusions has been sold in North America. Recently large amounts of fossiliferous material from Colombia, South America, have appeared and are being sold as either (semi-fossilized) copal or (fossilized) amber. In the present study we have recorded the carbon-13 NMR spectra of samples of these resins from Africa and Colombia and compared them with the spectra of modern resins and of fossilized resins from Mexico and elsewhere. These studies enable us to make a positive identification of the botanical origin in some cases and to draw conclusions that are relevant to the age or maturity of the material.

## Materials and Methods

Three separate samples of Colombian amber were examined, all collected by M. Nisbet and A. Nisbet of Crestone, Colorado, from the state of Santandor. The sample from Kenya was obtained by A. Graffin of Geological Enterprises, Ardmore, Oklahoma, directly from the ground in an area near Mombassa. The Tanzanian sample was provided without further information about location of find.

The copals from the Philippines and the Congo were provided by C. W. Beck, Vassar College, Poughkeepsie, New York. The Filipino sample, called Manila copal, was from Pangasinan. Sample 8-23 was provided by D. Bright, who acquired the piece because it contained a bark beetle (*Scolytidae*: Coleoptera) that he wanted to examine. The source in Manitoba, Canada, was alleged to be from Upper Cretaceous deposits, but its appearance differed from typical Canadian amber.

The Mexican and modern *Hymenaea coubaril* samples were obtained by one of the authors (G. O. P., Jr.). The Mexican sample was from the Totolapa mine La Vuelta.

Carbon-13 NMR spectra were obtained on approximately 70 mg of powdered material with magic angle spinning and cross polarization by procedures already described (4). Some of the spectra also were recorded under conditions of interrupted decoupling, which selects carbons with particular relaxation times (carbons without attached hydrogens and rapidly moving methyl or possibly methylene carbons) with the exclusion of many of the carbons. Spectra were recorded on a Varian VXR-300 spectrometer.

## Discussion

The key portion of the carbon-13 NMR spectrum between  $\delta$  100 and 160 contains the resonances of unsaturated carbons (alkenes and aromatics). For amber it often has four resonances at about  $\delta$  108, 125, 140, and 148. The first and last peaks are strongly diagnostic of an exomethylene group,  $>C=CH_2$ , of which  $>C=$  resonates at about  $\delta$  148 and  $=CH_2$  at about  $\delta$  108. The middle two resonances usually derive from double bond carbons attached only to one other carbon ( $RCH=$ ), as might be found for a double bond within a ring (endocyclic). Certain fossil resins exhibit neither of the exomethylene resonances. The absence may imply that the original terpenes lacked

this functionality. Thus, whereas exomethylene groups are commonly found in labdane structures, they are lacking in abietane structures (4). Alternatively, the exomethylene group may be removed chemically by diagenetic processes during burial. We have observed such decreases with time for resins from the Dominican Republic (5) and from New Zealand (8). Thus modern or relatively young resins exhibit strong exomethylene resonances, normally exceeding in intensity those of the endocyclic and related carbons. Over time, the exomethylene resonances degrade in intensity. For Baltic amber (4) they are roughly half the intensity of the endocyclic resonances. For Mexican amber (6) the exomethylene peaks are quite small, indicative of considerable age or conditions conducive to degradation.

The exomethylene resonances exhibited by the Colombian samples (Figure 1, top and bottom) are quite large. Such observations are indicative of very immature resins and contrast with the spectra of clearly fossilized or mature resins from Mexico or the Baltic Sea. For comparison, Figure 2 (top) gives the spectrum of an authentic sample of modern *Hymenaea coubaril* with well developed exomethylene resonances, and Figure 3 (top) gives the spectrum of a highly fossilized Mexican sample from Totolapa with quite small exomethylene resonances. The exomethylene peaks of the Kenyan sample (Figure 4, top) are somewhat smaller than those of the Columbian sample, indicating slightly higher maturity. The Tanzanian sample (Figure 4, bottom) is normal for fossilized resin (much reduced exomethylene resonances) and is thought to be of Pliocene origin. Although the African samples resemble *Hymenaea*, it is also possible they derive from *Copaifera* sources, for which we presently do not have spectra of modern samples.

The spectra with interrupted decoupling give an alternative diagnostic of these observations. As seen in Figure 1 (middle) for Colombian resin, the only remaining alkene resonances come from carbons without hydrogens. Thus the  $=\text{CH}_2$  resonance at  $\delta$  108 is gone but the  $>\text{C}=\text{C}$  resonance at  $\delta$  148 remains (the resonance at  $\delta$  140 must come from a double bond that carries one substituent). The large size of the  $\delta$  148 peak in the Colombian sample contrasts with the quite small size in Mexican resin (Figure 3, bottom), and is indicative of a very immature sample. The size of the resonance for the Colombian sample is quite comparable with that from authentic *Hymenaea* (Figure 2, bottom). The Kenyan sample closely follows the pattern of the Colombian samples (Figure 4, middle).

It is useful to look for patterns in the saturated portion of the spectra as well ( $\delta$  0-90). These resonances for authentic *Hymenaea* map almost perfectly onto the spectrum for the Colombian resins (compare Figures 1, top, and 2, top), from the little peak at  $\delta$  70 and the large peak at  $\delta$  40 to four peaks in the region  $\delta$  15-30 and the sharp peak with a shoulder at  $\delta$  48. With interrupted decoupling the spectra (Figure 1, middle, and Figure 2, bottom) practically can be laid on top of each other (including the carbonyl resonances at  $\delta$  180-190). This is extremely strong evidence that the Colombia resins are essentially identical to modern *Hymenaea*. The Kenya sample (Figure 4, top and middle) follows this same pattern, except that it lacks the small, sharp resonance at  $\delta$  34 (just to the right of the largest peak in the spectrum in Figure 1), so that the saturated portion of the spectrum with interrupted decoupling has three rather than four peaks. The spectrum of the Mexican resin with interrupted decoupling (Figure 3, bottom) is quite different from those of the modern resins.

Can the Colombian samples be termed copals? This term, originally derived from the Aztec "copalli" ("tree resin") (11), normally refers to semi-fossilized or young

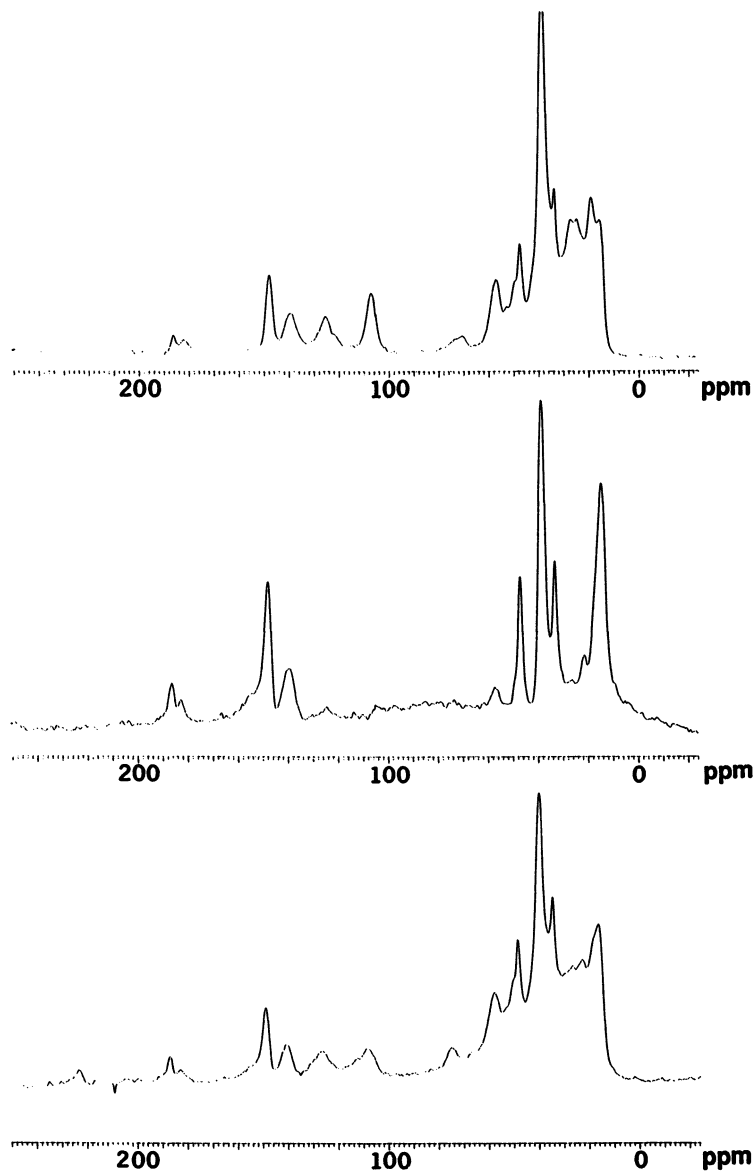


Figure 1. Carbon-13 NMR spectra of resin from Colombia, South America, (top and bottom) with normal decoupling, (middle) same sample as at top with interrupted decoupling.

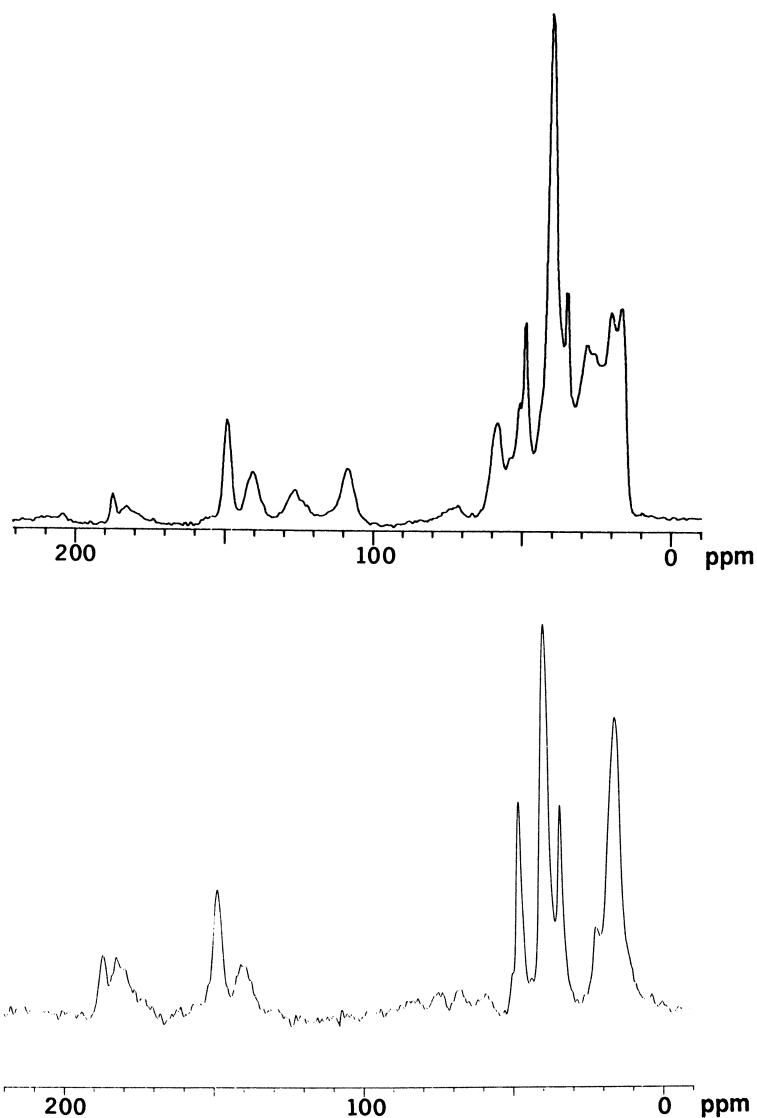


Figure 2. Carbon-13 NMR spectra of *Hymenaea coubaril* (top) with normal decoupling, (bottom) with interrupted decoupling.

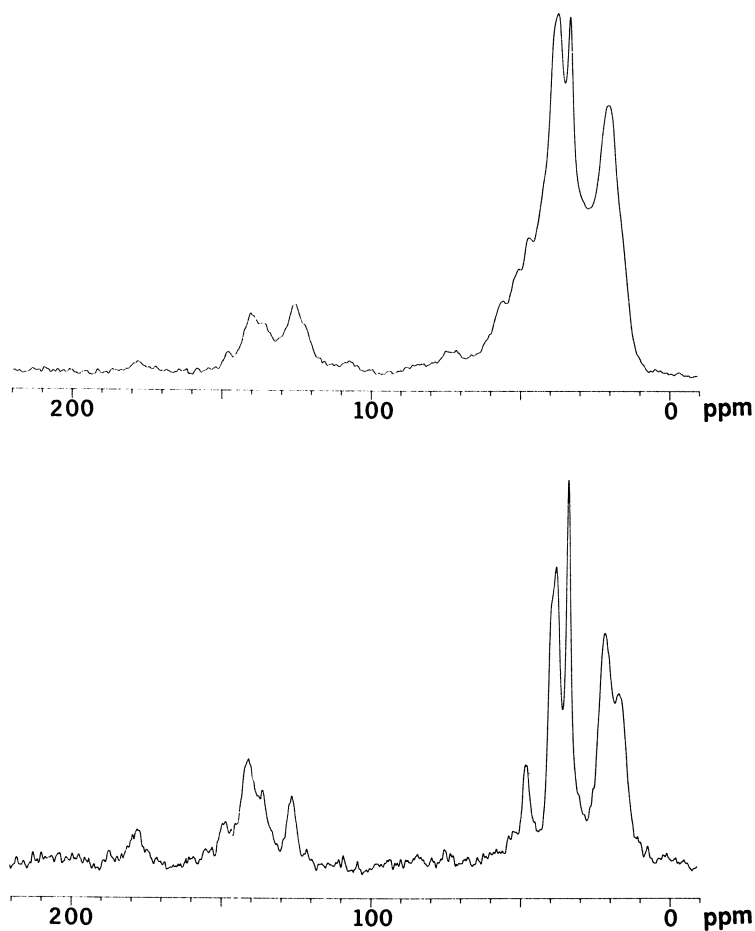


Figure 3. Carbon-13 NMR spectra of amber from Totolapa, Mexico, (top) with normal decoupling, (bottom) with interrupted decoupling.

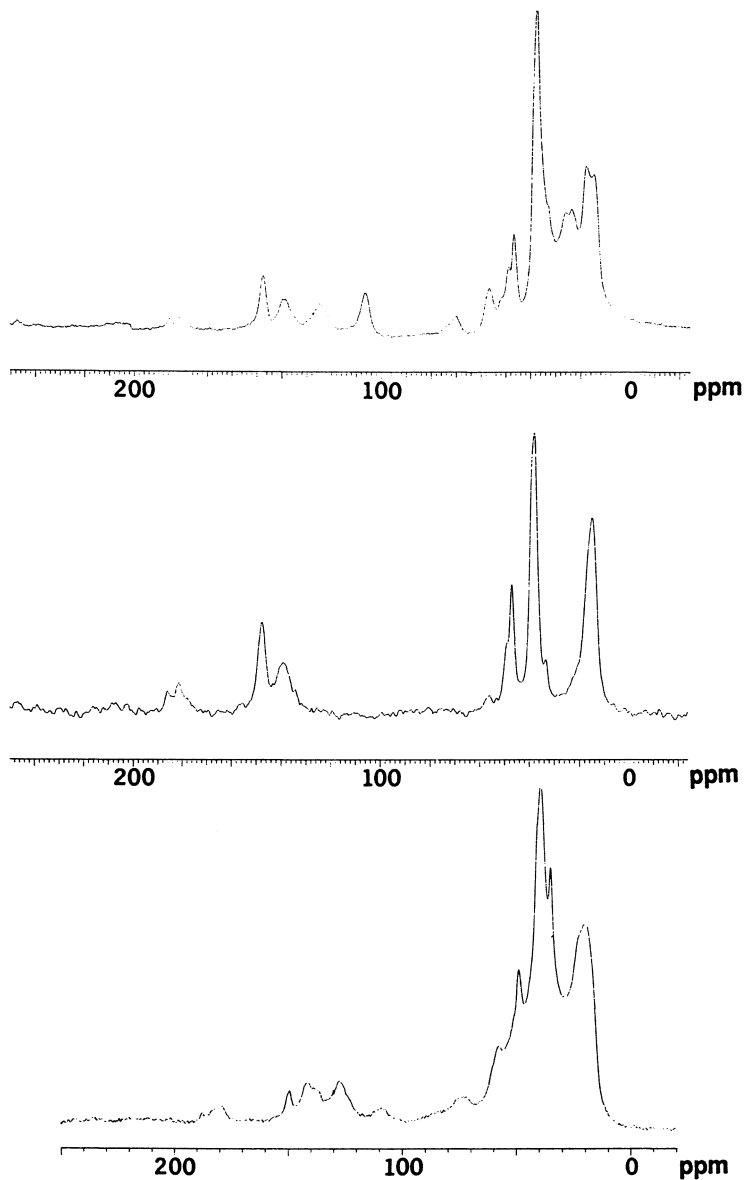


Figure 4. Carbon-13 NMR spectra of resin (top) from Kenya with normal decoupling, (middle) from Kenya (same sample) with interrupted decoupling, and (bottom) from Tanzania with normal decoupling.

amber. We have obtained the spectra of two such materials. The spectrum of the sample from the Congo (Figure 5, top) resembles in every peak both the spectrum of *Hymenaea* (Figure 2) and those of the Colombian samples (Figure 1). The Manila sample (Figure 5, bottom) exhibits the expected enhanced exomethylene resonances of a recent material but has a quite distinct saturated region, indicative of a different botanical source, presumably *Agathis*.

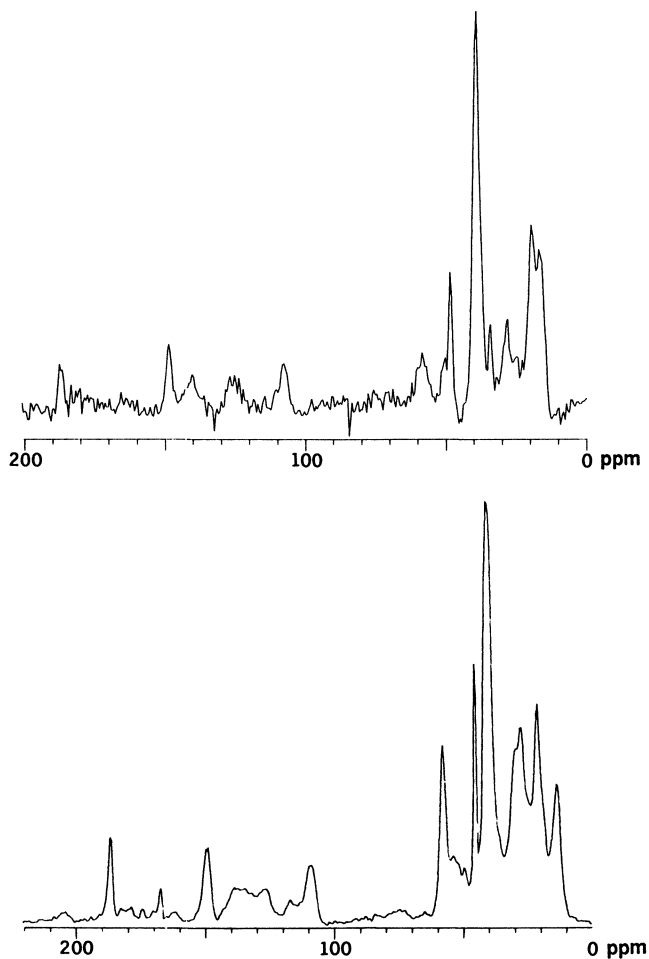


Figure 5. Carbon-13 NMR spectra from the Congo (top) and from the Philippines (bottom) with normal decoupling.

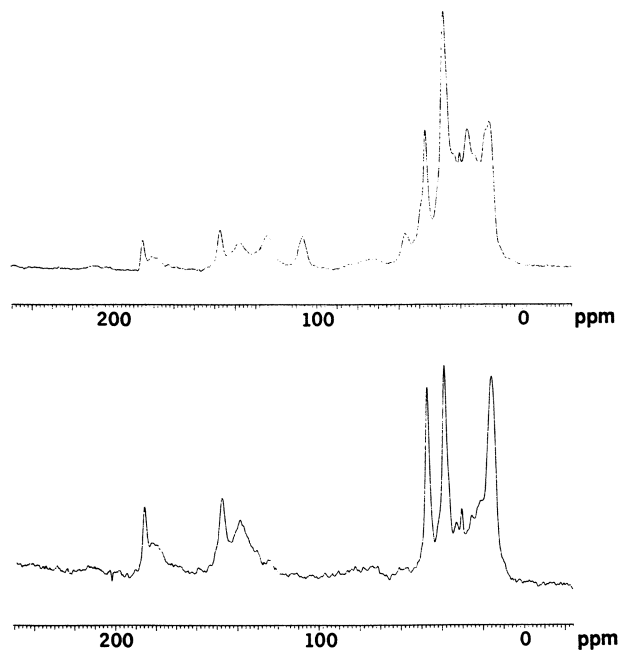


Figure 6. Carbon-13 NMR spectra of sample 8-23 from Manitoba, Canada, (top) with normal decoupling, (bottom) with interrupted decoupling.

Poinar (12) defined copal as "a recently deposited resin that cannot be molded by hand, has a melting point under 150°C, a surface that partially dissolves (becomes sticky) when acetone is applied and is relatively soft (can be scratched with a fingernail)." Thus copal and amber differ primarily in the extent of fossilization, which depends on age and burial conditions. The Colombian and Kenyan materials were soluble to surface application of acetone, had melting points under 150°C, and could be scratched with a fingernail. Thus the physical observations are in agreement with the NMR spectra that these materials are relatively immature and may be classified as copals. The period of transition from copal to amber has not been fully established, and it probably varies with the type of resin and the conditions of burial (heat, pressure) that control the processes of polymerization, crosslinking, and so on.

We present the spectrum of sample 8-23 (Figure 6) as another example of a supposedly ancient resin (Cretaceous Canadian amber in this case) that in fact is recent. The spectra with normal and interrupted decoupling are quite similar to those of the Kenyan sample (Figure 4), suggesting that 8-23 in fact is a modern or immature resin or copal derived from a *Hymenaea* source. It emphasizes that materials thought with all good intention to be fossilized resin may not be, so that the application of physical or spectroscopic tests are necessary for any case of particular significance or novelty.



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## Chapter 11

# The Age of Dominican Amber

David A. Grimaldi

Department of Entomology, American Museum of Natural History,  
Central Park West at 79th Street, New York, NY 10024–5192

Despite the scientific and lay popularity of fossiliferous amber from the Dominican Republic, there is confusion over its age(s). The commonly cited Eocene age (ca. 40 million years old [Ma]) for this amber comes from a study of the maturation of exomethylene resonance using  $^{13}\text{C}$  NMR. The data and conclusions of that study are critiqued here. The best evidence for ages of Dominican resinites comes from  $^{14}\text{C}$  dating (of very young eastern deposits) and, for the much older amber, from stratigraphy based on previous studies of Foraminifera and calcareous nannofossils. At least some of the eastern deposits are probably only several hundred to several thousand years old, and those from the northern mountains yield "true" amber [e.g., crosslinked, highly polymerized resinite] that varies in age from lower Miocene to mid Oligocene (23–30 Ma). In addition, the phylogenetic position of insects preserved in Dominican and Baltic ambers shows Dominican taxa to be relatively younger than the 40 Ma Baltic fossils. All evidence indicates that Dominican amber is considerably younger than the highly popularized age of 40 Ma.

Dominican amber has been admired and used by the Tainos and other indigenous peoples even before Columbus' historic landing there. Sanderson and Farr (1) reported that an abundance of Dominican amber was known to contain fossilized insects and other organisms, although this had been known for several decades before due to work by Pompilio Brouwer, one of the foremost geologists in the Dominican Republic. In the 1980's there began a global availability of fossiliferous pieces, and this spawned (to this day) a variety of papers on rare and unusual fossils in amber, such as snails (2), sun scorpions (3), a mushroom (4); and even vertebrate remains, such as mammalian hair (5) and small frogs (6). The remains of small frogs, *Anolis* and gecko lizards, bird feathers, and hair, aroused special interest, since these were among the oldest fossils of terrestrial vertebrates from the Caribbean. Unfortunately, reports on

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the age of the Dominican amber varied from lower Miocene (7)[ca. 23 Ma], Oligocene (1, litt. cit. J.W. Durham), and even late Eocene [ca. 40 Ma] (8, 9).

The scientific romance with amber fossils has recently intensified, largely as a result of two discoveries. One is the remarkable preservation of insect and plant tissues preserved in amber, revealed with electron microscopy (10-14 [the last reference addresses consistency and differences of preservation between Dominican and Baltic amber fossils]). The second development is the polymerase chain reaction (PCR), which amplifies molecular amounts of DNA into quantities that can be sequenced. Once the technique had been applied to some mid-Miocene (ca. 17 Ma) plant fossils in clay (15, 16), the race was on for sequencing DNA fossilized in amber. The first two reports on DNA sequences involved a termite, *Mastotermes electrodominicus* (17) and a stingless bee, *Proplebeia dominicana* (18), both in Dominican amber. The age of the bee was cited as being 40 Ma (upper Eocene); the termite report deferred to the age stratigraphically best documented for the Dominican amber: lower Miocene, perhaps upper Oligocene.

Repeated citation of the upper Eocene age of Dominican amber derives entirely from a study by Lambert, Frye, and Poinar (19) on  $^{13}\text{C}$  Nuclear Magnetic Resonance Spectroscopy (NMR). I will review the data and interpretations of that study first; then review what has been published on Dominican stratigraphy, much of it overlooked. Accurate dating has important implications for biology, since the age(s) of Dominican fossils are used for studying biogeography and continental drift, evolutionary relationships, and the rates of DNA evolution. For example, Hedges et al (20), estimated divergence times of various living reptiles and amphibians from the Caribbean, based on immunological distances of the albumin protein. The age of 40 Ma for Dominican amber vertebrates (based on ref. 19) they stated was evidence unresponsive of their hypothesis on the origins of this Caribbean fauna. A younger age of the amber, which is proposed here, would be more supportive of their hypothesis.

In lieu of new data, definitive ages can't be assigned to all of the Dominican amber mines. Incredibly, despite the scientific popularity of Dominican amber, little critique of dating hypotheses has been made. An evaluation of all the available data is needed at this point.

### Amber Localities In The Dominican Republic

Large deposits of fossiliferous amber are quite localized in the Dominican Republic, as discussed by Martinez and Schlee (21). The main group of amber mines is found between 10 and 30 km NE of Santiago, in an area approximately 400 km<sup>2</sup>. The deposits occur in a region of extensive uplift in the Cordillera Septentrional, some 500-1000 m in altitude, usually on the faces of steep ridges. Deposits of considerably younger material are found at Cotui (60 km SE) and Bayaguana and vicinity (Comatillo, Sierra de Agua), some 130 km SE of the main group of mines. Those two regions will be not discussed here, but

mentioned where appropriate below. Figure 1 shows a map of 11 main mines in this area, and 2 mines to the northwest. Included on the map are numbered sites, referring to stratigraphic samples in de Zoeten's thesis (22).

Some mines yield distinctive amber, such as blue amber from Los Cacaos. But none of the mines yield amber consistently different from the others. For example, the La Toca group of mines (in the La Cumbre region of ridges) is known for yielding amber that is dark red; however, it also yields amber as yellow as that from Palo Alto. Thus, discerning provenance of pieces on the basis of color is a method of great uncertainty. The only certainty is that the material from the eastern localities mentioned above is always much lighter in color, softer than the true amber from near Santiago, and it reacts visibly with organic solvents.

### Lambert et al. Study

The study by Lambert et al. (19) analyzed material from four "western" mines [which are actually in the northern mountains, or Cordillera Septentrional] (Palo Alto, La Aguita, La Toca, and Tamboril), as well as from one "mid" (Cotui), and two eastern "mining sites" (Bayaguana, El Valle: in the Cordillera Oriental). Samples were reported as being collected directly from the mines by a party including G.O. Poinar, as well as some obtained from Mr. Jacob Brodzinsky, who is a noted dealer of the fossils receiving material from local dealers and miners around the country. Interestingly, Brodzinsky had not kept records of amber provenance until after 1985 (J. Brodzinsky, personal communication, Nov. 1994), which is the year of publication of the NMR study. Also, provenance of material provided by miners and local dealers is one of probability, not certainty. Which of the samples derived from Brodzinsky and which from specific mines collected by Poinar and associates was not specified in the original report (19).

They examined the intensity of exomethylene resonance in the NMR spectra from the above seven Dominican sites, in a sample of modern *Hymenaea* resin (the genus of tree having formed the Dominican amber), and in a sample of Lower Cretaceous amber (ca. 125 Ma) from Lebanon. Since the exo-methylene group is obvious in modern resins, they used as a working hypothesis the following statement (pg. 48): "If loss of the exo-methylene resonances can be taken as a measure of fossilization and diagenesis, then this spectral aspect might provide a technique for relative dating of ambers." They assumed that the decay of the group would be linear with time. To calibrate the linear decay, they used Baroni-Urbani and Saunder's (7) stratigraphically-based 23 Ma age for the material from the Palo Alto mine, and correctly assumed that the soft, incompletely polymerized material from Cotui was only older than modern *Hymenaea* resin. A plot of their results is shown in Figure 2. An extrapolation of the plot, using their estimates, places the La Toca material at 40 Ma (upper Eocene): "If we assume that it took 20-23 million years for the exo-methylene resonances to be reduced from what they are in recent resin to the Palo Alto

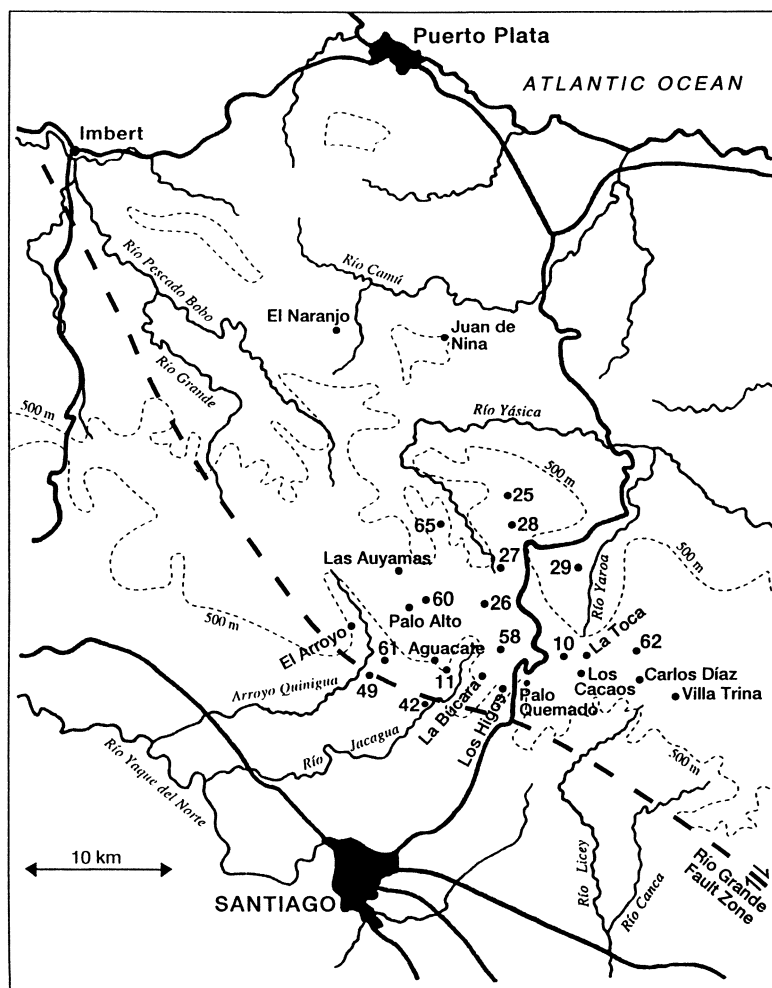


Figure 1. Map of the central region of the Cordillera Septentrional of the northern Dominican Republic. Names and locations of amber mines are indicated (as adapted from ref. 21); numbers refer to sites in ref. 22 where microfossils were sampled for stratigraphic analysis.

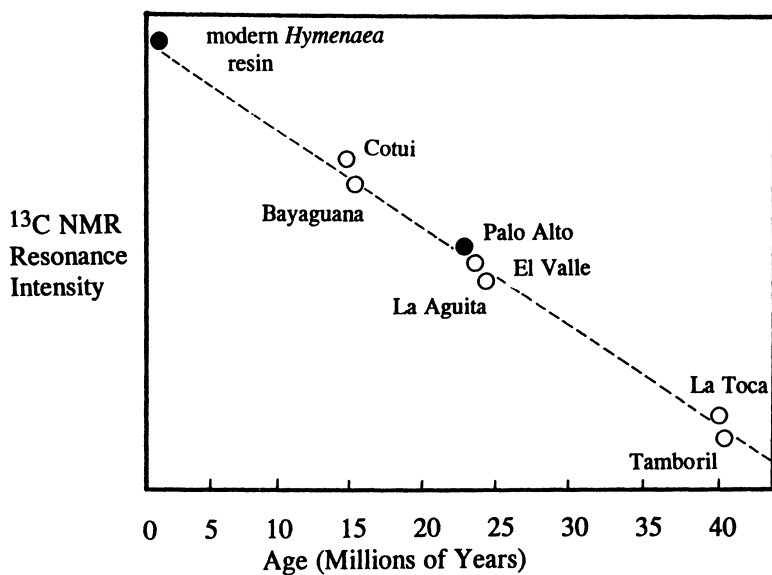


Figure 2. Correlation between exomethylene resonance (based on peak height in  $^{13}\text{C}$  NMR) and presumed age of amber samples (adapted from ref. 19). Dark circles are the samples with ages stratigraphically-determined; open circles are samples dated based on exomethylene maturation.

condition, then it can be extrapolated that the Tambouril and La Toca ambers are 10-20 my older..." (pg. 50). In contrast, the youngest deposits (Cotui and Bayaguana) were estimated to be 15-17 Ma. These results agreed with their observations on the hardness and color of the material: that from La Toca being hardest and dark red, the material from Cotui being very soft and very light yellow. As discussed above, no perfectly consistent differences have been found among the northern mines on the basis of amber color and hardness.

Numbers of replicates are not mentioned in that study (nor are peak heights quantified), but even if we assume that the differences in NMR spectra from the different mining sites are statistically significant, there are some startling problems with their results. For one, they mentioned that no exomethylene group was observed in Mexican amber (an NMR spectrum was not shown), and complete or virtual lack of an exomethylene group at the  $\delta$ 110-150 region of the NMR in Mexican amber is shown elsewhere (23). Mexican amber is stratigraphically dated as 5-15 Ma *younger* than the Lambert et al. ages estimated for Dominican material from Tamboril and La Toca, which they report as having obvious exomethylene peaks (their Figures 5T, Q). The difference between Mexican and Dominican material certainly cannot be due to botanical origin, since both were formed from extinct species of *Hymenaea* trees, as based on infrared spectroscopy with corroborative evidence from characteristic plant inclusions (24, 25). The stratigraphic age of the Mexican amber is firmly established, based on foraminiferal and palynological studies by Frost and Langenheim (26). Another inconsistency is the finding of no difference between Mexican amber that varies in color from yellow to deep red (23), whereas such a color difference in Dominican amber purportedly reflects the degree of exomethylene resonance (19).

Another serious contradictory result is their estimate of the material from Cotui and Bayaguana being 15-17 Ma. Two  $^{14}\text{C}$  datings of Dominican "copal" from these regions revealed dates of approximately 300 years (27, 28). Thus, at least some of the hardened resins from the eastern deposits are younger than they estimated, by more than four orders of magnitude. The  $^{14}\text{C}$  dates correspond very closely with the physical nature of the material: it easily melts near a hot flame and reacts visibly with organic solvents. Material millions of years old would show much more crosslinking.

Lambert et al. (19) measured *maturation* of the exomethylene group, which is a product of sample age, diagenesis (e.g., amount of geothermal energy at and during deposition), and possibly botanical origin. In this case, we cannot assume that the same *Hymenaea* species gave rise to all the Dominican deposits, since the resinite from the eastern mines is certainly derived from the living species, *Hymenaea coubaril* (widespread throughout the Caribbean, Central America, and South America), or *H. torrei* (restricted, at least now, to Cuba). More importantly, it is impossible to separate the effects of maturation based on age and those based on diagenesis (e.g., thermal history), which the authors admit (pg. 51): "Alternative explanations for the gradation in the resonance intensities include differences in paleobiological origins of the samples and

differences in the conditions of fossilization and burial." Despite this admission, dates were still estimated using exomethylene maturation. At the 1994 ACS symposium, Lambert agreed that maturation and age were not equivalent, but the maturation-based dates of Dominican amber have been very widely used by others (2-6, 9, 18).

### Stratigraphic Data

The main group of mines yielding true amber from the Dominican Republic lie just north of the Rio Grande Fault Zone (RGFZ), 12 km northeast of Santiago. They occur in extensively laminated sand, silt, and clays with a great deal of micaceous and carbonaceous material, interbedded with sandstone layers 2.5 m thick or less (8). This is the La Toca Formation. (Poinar [9] applies the name "Altamira faces [sic] of the El Mamey Formation;" the "Altamira" formation was a name informally proposed by Eberle et al. [8], which was not based on microfossil correlation). The La Toca Formation consists of 300 m of basal conglomerate, then 500 m of thin to medium bedded, alternating layers of sandstone and shale, capped by 300 m of thick bedded sandstone. The two overlying sedimentary layers are lower Oligocene to lower middle Miocene in age. The dates are based on identifications of Foraminifera and calcareous nannofossils (by S. Monechi, Univ. Florence, Italy), as reported by de Zoeten (22). Amber occurs in these two overlying sedimentary layers. Stratigraphic studies of the La Toca Formation is complicated by high angle fault deformities north of the RGFZ where the amber deposits occur. Here, strata usually strike north to northwesterly and dip easterly. The deformity can account for disparate geological datings of the amber deposits; yet, with the exception of unpublished data by Cepek and Eberle, the stratigraphy is unquestionably Oligo-Miocene. For example, stratigraphic ages of six localities from the central group of amber mines north of Santiago were reported by Harms (29: pg. 3) in a footnote that cited unpublished nannoplankton data of P. Cepek (Univ. Hannover, Germany). Strata that were mentioned range from Lower Miocene to Upper Eocene. Interestingly, Cepek assigned a slightly *older* age to the Palo Alto mine (upper Middle to Upper Oligocene) than is reported in another study (7).

Indeed, microfossils from the sediments of only one amber mine have been directly examined, which is the Palo Alto mine (7). This was based on two samples taken from the main amber workings of the mine, as well as a sample taken from a small amber site "1-2 km south" of the mine. They assigned an age to these samples as lower part of the Early Miocene. The study by de Zoeten (22) analyzed microfossils from 12 sites in the amber-producing region. Although none of the samples come directly from amber mines, they are all from the sedimentary layers of the La Toca Formation sampled close to amber mines (numbered sites indicated in Figure 1). The reported geological ages are early Miocene (sites 10, 11, 58), late Oligocene (sites 25, 26, 27), and mid Oligocene (sites 28, 29). Sites 58, 60, 61, 62, and 65 are reported by P. Cepek



(pers. comm. to de Zoeten), on the basis of unpublished microfossil data, to be anywhere from late Eocene to early mid Miocene, and, so, are indeterminate at this point. Table I summarizes the microfossil taxa found in the La Toca Formation by all investigators. The calcareous nannofossils are definitively early-middle Miocene, occurring in zones NN4-NN5 (ca. 16-18 Ma) and are distinctively different in age than the planktonic foraminiferans. Planktonic forams in Table I that indicate the Miocene period are *Catapsydrax*, *Globorotalia*, and *Globigerinoides*; Oligocene forams are *Globigerina ciperoensis*, *G. sellii*, and *Globorotalia opima* (the last in the oldest foram in the list, from the middle Oligocene [Manuel Itaralde, personal communication]). The best available evidence indicates that the sedimentary layers of the La Toca Formation, which contains the amber, are early Miocene (ca. 23 Ma) to mid Oligocene (30 Ma).

**Table I. Microfossils from the La Toca Formation**

**Calcareous Nannofossils:**

*Sphenolithus heteromorphus*  
*Discoaster druggi*

**Planktonic Foraminifera:**

*Catapsydrax dissimilis*  
*Globorotalia mayeri*  
*Globigerina venezuelana*  
*Globigerina ciperoensis*  
*Globigerina sellii*  
*Globigerina* spp.  
*Globigerinoides primordius*  
*Globorotalia opima nana*  
*Globorotalia opima opima*

**Benthic Foraminifera:**

*Anomalina* sp.  
*Anomalinoides pseudogrosserugosus*  
*Bolivina* spp.  
*Cassidulina subglobosa*  
*Cassidulina reflex* or *caribaea*  
*Cibicidoides* sp.  
*Epinoides?* sp.  
*Gyroïdina* sp.  
*Lenticulina* sp.  
*Lagena* sp.  
*Melonis* sp.  
*Oridorsalis* cf. *umonatus*  
*Osangularis* sp.  
*Pleurostomella* sp.  
*Pullenia bulloides*  
*Siphonina tenuicainata*  
*Siphonodorsaria* sp.  
*Spiroculina elongata*  
*Uvigerina* sp.

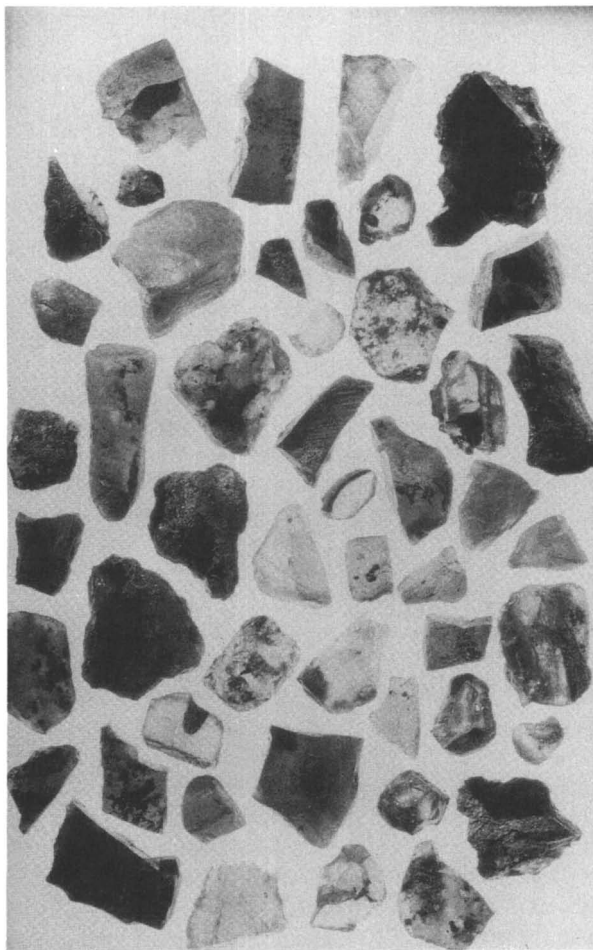
SOURCE: Adapted from refs. 7 and 22.

It must be stressed that these deposits are marine, so the amber has been redeposited from its original terrestrial origin. Thus, the stratigraphic dates are considered a minimum, and this has been emphasized in assignment of an

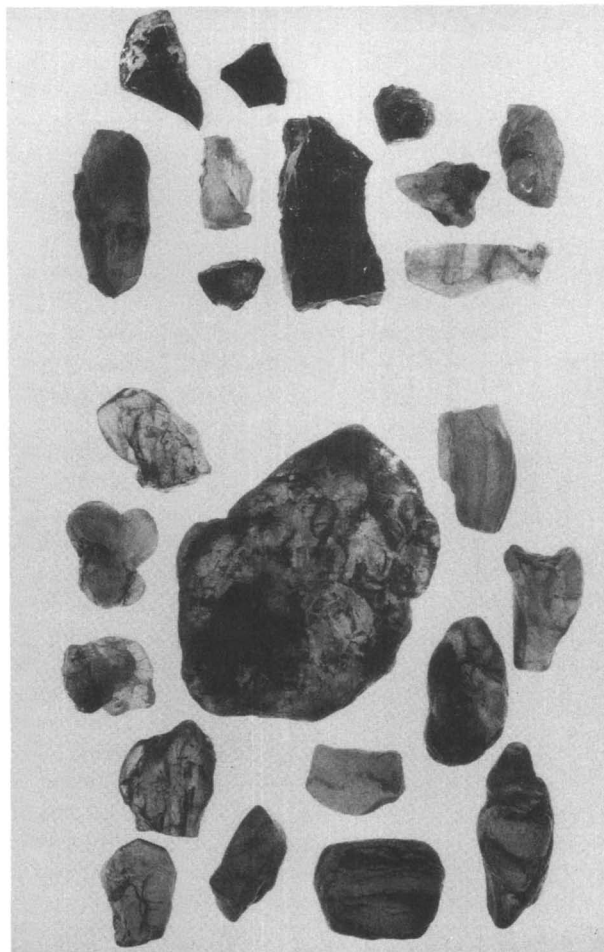
Eocene age to Dominican amber (for example, ref. 9). One possible way of determining how much older is the amber compared to its sedimentary matrix is reflected in the wear of the amber pieces. The assumption is that amber older than its matrix should be highly reworked: globose to spherical pieces with a surface smoothed by years of abrasion in aquatic sediments. However, considerable wear can occur within weeks of being washed out of the original sediments, and then not worn at all after millions of years of burial. The shapes of amber pieces recovered by myself directly from the La Toca mines are angular with irregular surfaces (Figure 3). Compare these to pieces from another Tertiary deposit, from the Paleocene of Sakhalin Island (Pacific coast off Russia). Excavated Sakhalin amber is also irregular and angular; pieces that are picked up considerably downriver from the deposit are rounded and somewhat polished (Figure 4). Thus, extensive reworking of amber in abrasive sediments has obvious signs, which do not show in Dominican amber. Also, in two mines the amber was deposited as a result of low energy water action (30), which also suggests little reworking. The preponderance of aquatic organisms in the amber attests to the proximity of fresh water near the amber forest (the insects include dytiscid, helodid, haliplid, hydrophilid, limnichid, noterid beetles; ceratopogonid, chironomid, culicid, dolichopodid, and ephyrid flies; 8 families of Trichoptera; 2 families of mayflies; and several rarer groups). Streams meandering through the amber forest probably fed a nearby delta, on the shores of which amber and rotting logs (later becoming the lignite) were stranded and eventually buried. Orogeny then thrust the former coastline into cordilleras.

### Relative Dating Based On The Inclusions

A highly informative line of evidence for *relative* dating that is essentially ignored are the inclusions of insects and other organisms preserved in the amber. Since the organisms were preserved at the time of amber formation, the "evolutionary position" of the fossilized inclusions would directly reflect an age of amber formation. "Evolutionary position" is examined using a phylogeny, or cladogram. In a cladogram, species and other taxa are arranged based on shared derived features (synapomorphies), such that the most primitive taxa have the fewest synapomorphies and the most recently derived taxa have the most. The best example of this are studies on the phylogeny of vertebrate classes and mammal orders and the sequence of their appearance in the fossil record (31, 32). Mammals have an excellent fossil record and, as such, one would expect to see the most primitive orders on a cladogram appearing first in the fossil record, and the more derived orders appearing later, which is what is observed. A caveat of this method is that some relatively young strata could harbor older, relict taxa, some of which even exist today as "living fossils." With a large base of comparisons between two fossil deposits, though, age differences should be reflected in the phylogenetic positions of the fossils.



**Figure 3.** Pieces of crude amber taken directly from the La Toca mines (see Figure 1) in 1991 by D. Grimaldi (cf. Figure 4).



**Figure 4.** Pieces of crude Tertiary amber taken directly from lignitic deposits of Sakhalin Island (eastern Russia)(above), compared to pieces that have been washed downstream (below). Note the natural polish of the reworked pieces. Specimens courtesy of Paleontological Institute, Moscow.

**Table II**  
**Comparison of Acalyprate and Other Diptera in Baltic and Dominican Amber**

FAMILY	BALTIC AMBER*	DOMINICAN AMBER**
Acroceridae	<i>Eulonchiella</i> <sup>+</sup>	
	<i>Glaesoncodes</i> <sup>+</sup>	
	<i>Prophilopota</i> <sup>+</sup>	<i>Ogcodes</i>
	<i>Villalites</i> <sup>+</sup>	
Anthomyzidae	<i>Protoanthomyza</i> <sup>+</sup>	<i>Anthomyza</i>
	<i>Xenanthomyza</i> <sup>+</sup>	
Asteiidae	<i>Succinasteia</i> <sup>+</sup>	<i>Asteia</i>
		<i>Loewimyia</i>
Aulacigastridae	<i>Protaulacigaster</i> <sup>+</sup>	<i>Aulacigaster</i>
Bombyliidae	<i>Glaesamictus</i> <sup>+</sup>	
	<i>Paracorsomyza</i> <sup>+</sup>	<i>Glbellula</i>
	<i>Proglabellula</i> <sup>+</sup>	<i>Mythicomomyia</i>
Carnidae	<i>Meoneurites</i> <sup>+</sup>	<i>Carnus</i>
Chloropidae	<i>Protoscinella</i> <sup>+</sup>	<i>Epichlorops</i>
		<i>Tricimba</i>
		<i>Clusiodes</i>
Clusiidae	<i>Electroclusiodes</i> <sup>+</sup>	<i>Clusiodes</i>
Conopidae	<i>Paleomyopa</i> <sup>+</sup>	<i>Stylogaster</i>
Cypselosomatidae	<i>Cypselosomatites</i> <sup>+</sup>	<i>Latheticomyia</i>
Drosophilidae	<i>Electrophortica</i> <sup>+</sup>	<i>Chymomyza</i>
		<i>Drosophila</i>
		<i>Hyalistata</i>
		<i>Miomyia</i> <sup>+</sup>
		<i>Neotanygastrella</i>
		<i>Protochymomyza</i> <sup>+</sup>
		<i>Scaptomyza</i>
		<i>Stegana</i>
		<i>Raineria</i>
		<i>Phyllomyza</i>
Micropezidae	<i>Electrobata</i> <sup>+</sup>	<i>Pholeomyia</i>
Milichiidae	<i>Pseudodesmometopa</i> <sup>+</sup>	<i>Odinia</i>
Odiniidae	<i>Protodinia</i> <sup>+</sup>	

\*Based mostly on Hennig (ref. 35)

\*\*Based on various published records and mostly unpublished identifications by D. Grimaldi (specimens in American Museum of Natural History).

+ denotes an extinct genus.

Fortunately, there is a very good basis of comparison for Dominican amber insects: insects preserved in Baltic amber. Although much of Baltic amber is washed up on the shores of the Baltic Sea, primary deposits of this amber in the "blau Erde" of the Samland Peninsula on the Baltic Sea are of Lower Oligocene - Upper Eocene age (33, 34). Family by family comparisons of the phylogenetic position of Baltic amber flies studied by Willi Hennig, and Dominican amber ones studied primarily by myself provide a test of the hypothesis that, if younger, the Dominican amber flies should be more recently derived than ones in Baltic amber. (Comparisons were made only for families occurring in both ambers; there are several families of flies occurring in only one of each of these ambers). Only two of the 24 genera of flies studied in the Dominican amber belong to extinct genera, whereas the Baltic amber acalyptrates are all primitive with respect to a particular living genus, and thus relegated to extinct genera (Table II). Some might claim that this is an artifact based on taxonomic bias, since Hennig is the author of most of the extinct Baltic amber genera. However, Hennig formulated the phylogenetic method, on which most of modern systematics is founded, and he was an excellent morphologist: his generic concepts generally have withstood scrutiny. Flies are not the only group of insects (just the best studied) which show this pattern; the various bees, ants, and myriad other insects in Dominican amber are definitively more modern than those in Baltic amber. Mexican amber is not as available and is less well studied than Dominican amber. However, the few cyclorrhaphan fly genera reported in the upper Oligocene amber from Mexico are modern genera also found in Dominican amber (i.e., *Drosophila*, *Neotanygastrella*, *Phyllomyza*, *Periscelis*).

While the Dominican amber fauna contains a mix of relatively modern and primitive elements, it does contain a much higher proportion than does Baltic amber of recently derived taxa. This doesn't provide a means for measuring the actual age of Dominican amber, but it is an additional source of information which indicates that Dominican amber is younger than the 40 Ma Baltic amber.

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## Chapter 12

# The Petrology of Resinite in American Coals

John C. Crelling

Department of Geology, Southern Illinois University,  
Carbondale, IL 62901

Resinite macerals are ubiquitous, though minor, components in most American coals below medium-volatile bituminous rank. They are usually absent in coals of higher rank. Although resinite macerals usually make up less than 3% of most U.S. coals, they are particularly abundant in coal of the Wasatch Plateau in Utah where they can account for as much as 15% of the macerals present. Resinite macerals have two common modes of occurrence. In most Appalachian and midwestern U. S. coal seams resinates occur as primary (present at the time of deposition) ovoid bodies with a long axis ranging from 25 to 200 micrometers. While primary ovoid bodies of resinite are also found in western U.S. coals of Cretaceous/Tertiary age, much resinite in these coals occurs as secondary cleat and void fillings. This secondary resinite shows an intrusive relationship to the host coal and often shows flow texture and carries xenoliths of coal in resinite veinlets. Fluorescence microscopy reveals that only the primary resinite ovoids commonly show "oxidation" or "reaction rims" that suggest a surface alteration. Fluorescence spectral analysis can usually distinguish resinite from other macerals and in most cases it can also distinguish between different resinates.

Resinite macerals are ubiquitous, though minor, components in most American coals below medium-volatile bituminous rank. They are derived from resins in the original plants that were the precursors of the coal. Resinite macerals have two common modes of occurrence. In most Appalachian and midwestern U.S. coal seams resinates occur as primary (present at the time of deposition) ovoid bodies with a long axis ranging from 25 to 300 micrometers. While primary ovoid bodies of resinite are also found in western U.S. coals of Cretaceous/Tertiary age, much resinite in these coals occurs as secondary cleat and void fillings.

Although resinite macerals occur in almost all U.S. coals, they are particularly abundant in some western U.S. coals and much of the published petrographic literature deals with resinates from this area (1-10). It is also observed that the resinite in the

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western U. S. coals of Cretaceous and Tertiary age tends to commonly occur in cleats and fissures where it is observable megascopically.

Similar cleat and fissure-filling resinite in coals from England has been reported (11-13). The authors concluded that the increased temperature and pressure of coalification in the bituminous range was sufficient to gently mobilize some of the resinite macerals to coalesce into globules and veins without increasing the reflectance or causing vesiculation of the resinite. On the basis of infrared spectral properties and carbonization behavior Murchison (14) was able to divide the resinite macerals into two types -- one type occurring only in coals of sub-bituminous rank or lower and the other type occurring in bituminous coals. He also noted that much of the resinite in bituminous coals occurred as interconnected globules and veins and he concluded that this was of secondary origin (15).

Fusinized or charred resinites observed petrographically in a rod-like form (rodlets) have been reported in Illinois coals (16) and in other areas (17-18).

### Occurrence

Resinite macerals are found in coals from all of the major coalfields in the United States and they are particularly abundant in the coals of the Wasatch Plateau in Utah. Although no national petrographic data base exists for American coals, the Penn State Coal Data Base is the best collection of information on a representative selection of U. S. coals. With the help of Mr. David Glick from the Coal and Organic Petrology Laboratories at The Pennsylvania State University, data on the occurrence of resinite was compiled. The data included were restricted by sample type (either whole seam or run of mine samples), by rank (vitrinite reflectance less than 1.0 %), and by sample availability. For summaries by seam only the results of seams having more than one sample were included. In Figure 1 the data for the mean resinite content in white light by province is shown. All provinces have less than 1% resinite and the mean for all provinces based on 219 samples is about 0.5 %. In Figure 2 the data for the mean resinite content in blue light by province is shown. All provinces have higher amounts than in white light and the mean for all provinces based on 65 samples is about 1.25% which is 150% higher. The phenomenon of the detection of higher amounts in blue light is common to all liptinite macerals and is well known and widely reported. Even though the data base is limited by both number of seams and number of samples per seam, it is still instructive to break the data down by seam (Figure 3), where it is easily seen that the seams from the Rocky Mountain Province are the richest in resinite.

In addition to the Penn State Data Base, the Illinois State Geological Survey (ISGS) has an important collection of petrographic data on coals of the Illinois Basin. Analyses of hundreds of coal samples from all parts of the Illinois Basin show a resinite content of from less than one percent to more than four percent by volume (19). With the help of Dr. Richard Harvey of the ISGS, data on the occurrence of resinite was compiled for the three most commercially important seams in the basin. This data presented in Figure 4 shows that both the Herrin No. 6 (125 samples) and the Springfield No. 5 (62 samples) seams average more than 1% resinite in blue light and the Colchester No. 2 seam (36 samples) averages less than 0.5%. Even though these data show that resinite is only a minor component of most U. S. coals, it is interesting to note that this amount represents a large resource. For example, when one considers that the state of Illinois has the largest demonstrated reserves of bituminous coal in the U.S.,

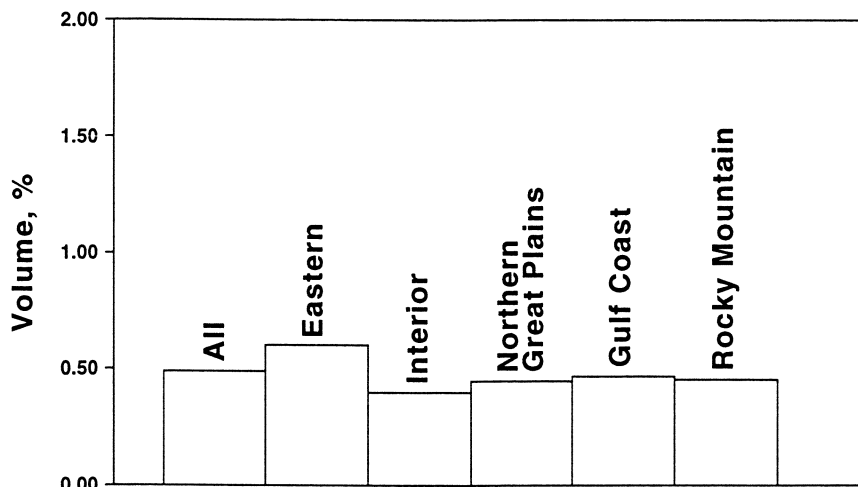


Figure 1. Average occurrence of resinite macerals by coal province by white light petrography. The data are from the Penn State Data Base and are restricted by sample type (either whole seam or run of mine samples) and by rank (vitrinite reflectance less than 1.0 %).

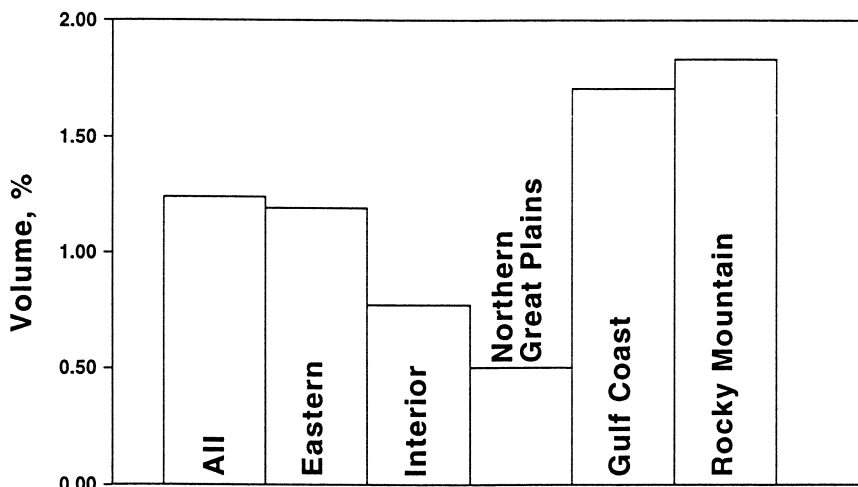


Figure 2. Average occurrence of resinite macerals by coal province by blue light petrography. The data are from the Penn State Data Base and are restricted by sample type (either whole seam or run of mine samples) and by rank (vitrinite reflectance less than 1.0 %). Note that the resinite content in all provinces is higher than in white light.

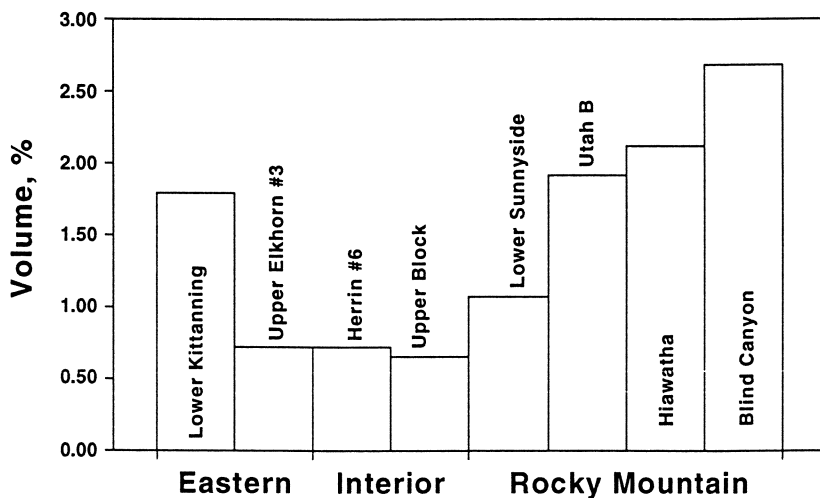


Figure 3. Average occurrence of resinite macerals by seam by blue light petrography. The data are from the Penn State Data Base and are restricted by sample type (either whole seam or run of mine samples) and by rank (vitrinite reflectance less than 1.0 %). Note that the resinite content is highest in the Rocky Mountain Province.

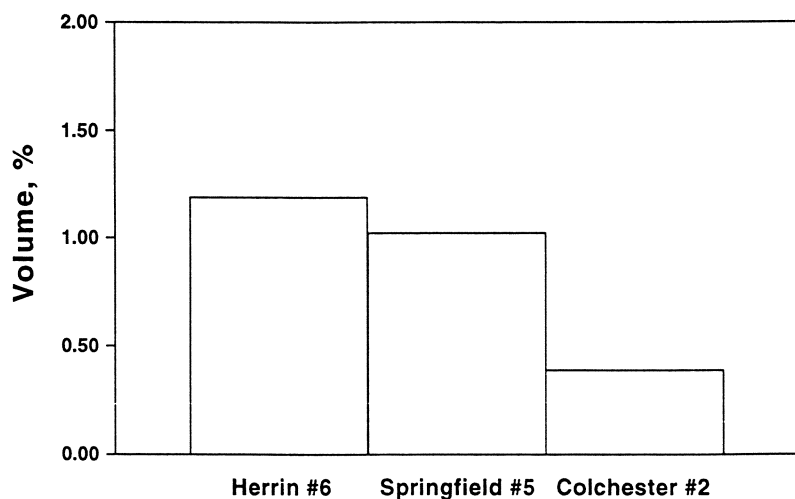


Figure 4. Occurrence of resinite macerals by seam by blue light petrography. The data are from the Illinois State Geological Survey and are restricted by sample type (either whole seam or run of mine samples) and by rank (vitrinite reflectance less than 1.0 %).

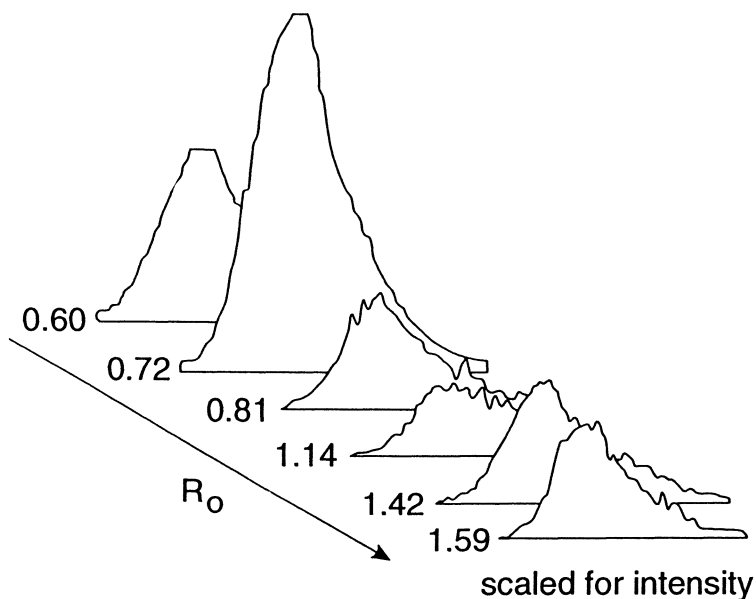


Figure 5. Fluorescence spectra of resinites from the Lower Kittanning seam at different ranks (vitrinite reflectance 0.60% -1.59%). All spectra were determined under the same conditions and are scaled for peak intensity. Note the intensity maximum at 0.72 % reflectance and the decrease at higher ranks.

about 65 billion tons, it is a simple calculation to determine that there is a great reserve of resinite in the state.

Although the resinite in Illinois has not been exploited commercially, resinite has been exploited with success in the western U.S., especially in the Wasatch Plateau coalfields of Utah. Recent work by Miller and his colleagues at the University of Utah (supported by the USDOE) has shown successful pilot-plant proof-of-concept testing in the recovery of resinite. He points out that the resinite from the Wasatch Plateau has been used commercially "in the ink, adhesive, rubber, varnish, enamel, paint and coatings, and thermoplastics industries." and of the solvent refined resinite "This product, at the present time, has a market value of at least \$1.00/kg as a chemical commodity ..." (20, p. 420).

### **Petrography**

**Primary Resinite.** Because resin, the precursor of the maceral resinite, occurs in the original plant material as a fluid secretion, it has no distinct fossil plant (phyteral) morphology such as the linear aspect of cutinite or the bilateral symmetry of sporinite. The dominant form of resinite is a structureless oblate spheroid often described more simply as a blob, bleb, lump, lens, ovoid, globule etc. In coal samples where woody cell structure has been preserved, resinite can commonly be seen in place as a cell filling (Plate 1 A&B). This kind of occurrence clearly represents its original botanical affinity. However, it is also common to find the resinite occurring in the matrix of the coal freed from enclosing cell walls (Plate 1 C&D, Plate 2 A-D, Plate 3 A-D). The boundaries of the resinite in the matrix can be both regular (Plate 1 C&D, Plate 2 A&B) and irregular (Plate 2 C&D Plate 3 A&B) as well as sharp (Plate 1 C&D, Plate 2 A-D) and diffuse (Plate 3 A&C).

Resinite is very resistant to weathering and biochemical degradation and can therefore be concentrated by such processes. In fact, it is often observed that the coal matrix has compacted around isolated resinite bodies. Because the resinite described above is incorporated into the peat precursor of the coal in the initial stages of formation, these occurrences of resinite are considered primary.

**Reflected Light.** In reflected white light resinite appears to be gray with a reflectance darker than the vitrinite in the same sample but usually slightly brighter than the sporinite and cutinite. Sometimes larger particles of resinite are translucent with an orange tint. (Plate 1 C&D). While primary resinites are usually free of noticeable inclusions, pyrite crystals can occasionally be associated with them (Plate 2 A&B). Also it is common for resinites to have a darker outer rim with a brighter inner core. (Plate 1 C&D). This zoning is usually ascribed to weathering or oxidation.

**Fluorescent Light.** In fluorescence microscopy resinites usually fluoresce very well. Two types of illumination are used, ultra-violet with a narrow range of excitation around 365 nm used for spectral analysis and blue light with a broader excitation range peaking around 400 nm for survey viewing. The intensity of the fluorescence is usually highest in the lower end of the bituminous range (0.5-0.75 % vitrinite reflectance) and it decreases with increasing rank. Figure 5 shows a typical fluorescence intensity variation for a rank series of samples from the Lower Kittanning seam. It is different from most other seams in that fluorescence could be detected above a vitrinite reflectance of 1.40%.

NOTE: Plate 1 appears on page 225; Plate 2 appears on page 226; Plate 3 appears on page 227.

The fluorescence color is usually a bright yellow in the high- volatile bituminous rank range, although it can range from bluish green to reddish orange. In any given coal the fluorescence color shifts toward the red end of the spectrum with increasing rank. At any given rank the resinite macerals in a sample will usually have spectra of similar shape although the position of the peaks can vary. In Appalachian and midwestern U.S. coals this variation is most often restricted, but in western U. S. coals the variation can be extreme, as shown in Figure 6. Three of the factors that account for this variation are the original resinite chemistry, the degree of weathering or oxidation the macerals have experienced, and the degree of coalification (rank).

Studies of the spectra of resinite macerals of coals from Utah, Wyoming, New Mexico, and Colorado (7-10, 22-24) and the Canadian Rockies (25) have shown that the resinite macerals can be statistically divided into four or five groups. However, when the spectra are plotted on the C. I. E. chromaticity diagram based on chromaticity parameters, the distribution is continuous. An example for spectra taken in both ultraviolet and blue light from a Hiawatha seam sample is given in Figure 7.

In addition to the petrographic features detailed above, the primary resinite macerals of western U. S. coals and particularly those of the Wasatch Plateau in Utah very commonly show other features such as brittle fracture (Plate 4 D), multiple-layer zoning (Plate 3 D), flow textures (Plate 3 C, Plate 4 C), multi-phase textures (Plate 3 B&D), and minor vesiculation.

**Secondary Resinite.** As much as 50-60 % of the resinite in western coals can occur in cleat and fracture fillings and is, thus, a post-depositional or secondary occurrence. This resinite has been mobilized to flow into cleats and fractures at some point in the geological coalification history of the coal. The source of the resinite must be the same as that for the primary resinite, the resins in the original plant material. No evidence for a source outside of the coal seams has ever been reported.

**Reflected Light.** In reflected white light the secondary resinite appears similar to primary resinite except for its planar (vein-like) nature and obvious inclusions of coal fragments. These xenoliths were picked up during the intrusive flow of the resinite. The reflectance of these macerals is the same dark gray of the primary resinite macerals. This and their brittle fracture distinguishes them from the void-like blackness of exsudatinites, another void filling secondary maceral believed to be an oil-like exudation generated during coalification (26-29).

**Fluorescent Light.** In fluorescence microscopy the secondary resinites also fluoresce very well. The fluorescence color varies from bluish green to reddish orange. The planar cleat filling form is usually very evident. The boundaries of the fillings with the coal mass can be clean and sharp or brecciated. In addition to coal xenoliths (Plate 4 A&B), other evidence of flow can be seen in swirl textures (Plate 3 C, Plate 4 C) and multi-layer zoning (Plate 3 D). The multi-layer zoning suggests multiple episodes of resinite emplacement and this suggestion is supported by cross-cutting relationships of the fillings or veins. An outstanding feature of all of the fillings is the abundance of opaque inclusions which range in size from fragments showing plant cell structure to dust (Plate 3 A-D, Plate 4 C&D). It is this micron to sub-micron material that defines most of the zoning and flow textures.

NOTE: Plate 4 appears on page 228.

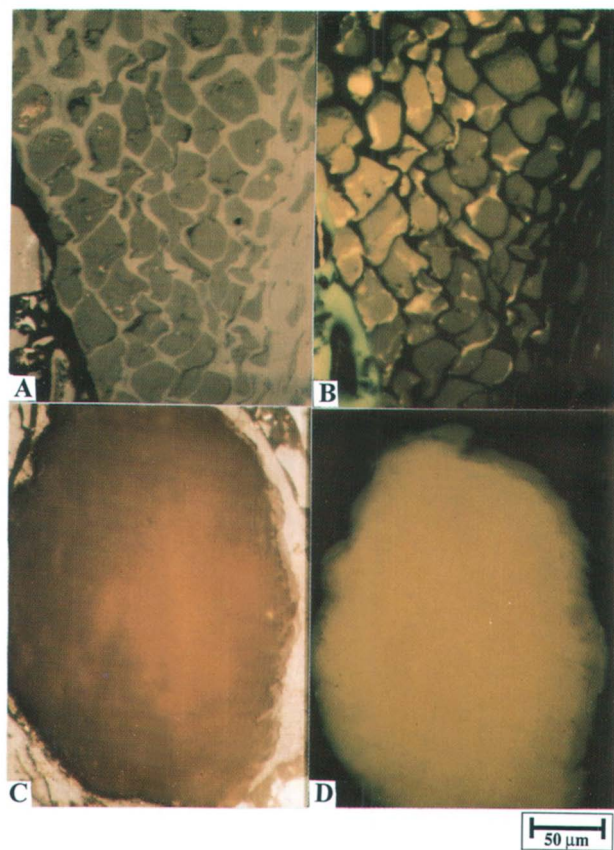


Plate 1. Photomicrographs of resinite macerals: A. Resinite macerals filling cell structures in vitrinite, derived from the original occurrence in the plant precursors (Elkhorn No. 3 seam - white light); B. Same field as A in blue light showing the fluorescence of the resinite; C. Typical resinite ovoid with internal reflections and orange color (Herrin No. 6 seam - white light); D. same field as C in blue light. Note the darker outer rim.



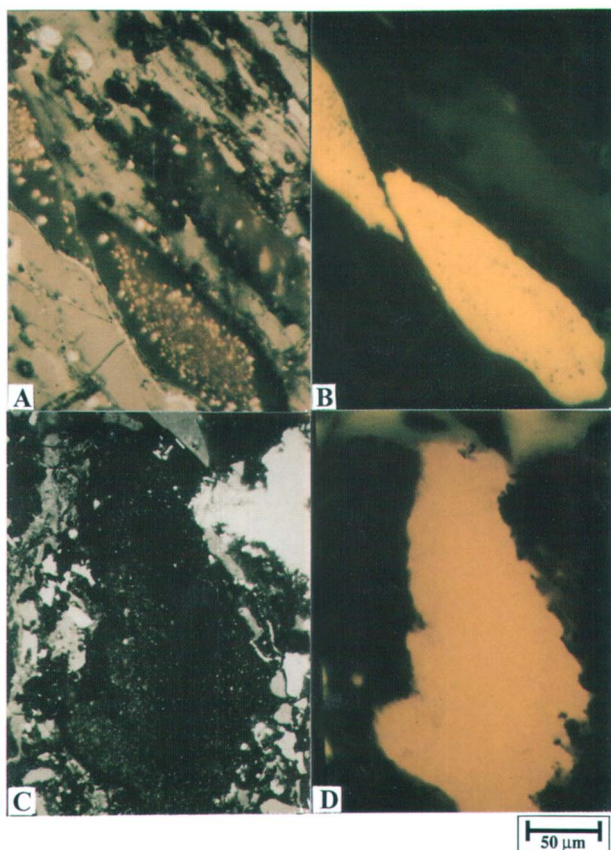


Plate 2. Photomicrographs of resinite macerals: A. Resinite lenses with bright pyrite crystals concentrated in center (Herrin No. 6 seam - white light); B. Same field as A in blue light showing the fluorescence of the resinite and non-fluorescent pyrite; C. Resinite ovoid with irregular boundaries (Herrin No. 6 seam - white light); D. same field as C in blue light.

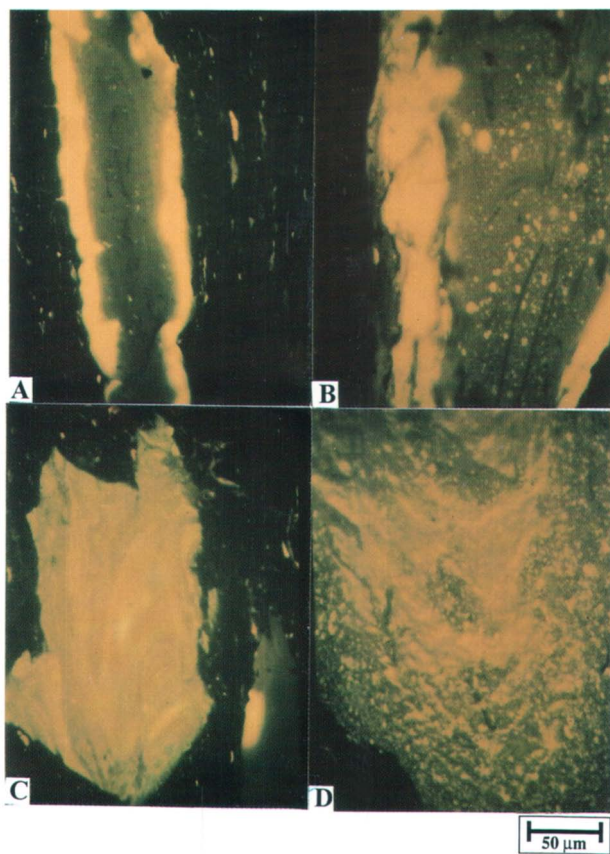


Plate 3. Photomicrographs of resinite macerals: A. Resinite lens showing zoning with a bright rim and dark core (Hiawatha seam - blue light); B. Portion of a resinite lens showing zoning with a bright rim and dark core which contains non-fluorescent inclusions and yellow droplets (Hiawatha seam - blue light); C. Irregular resinite body showing both zoning and flow texture (Hiawatha seam - blue light); D. Resinite mass showing multi-layer zoning.

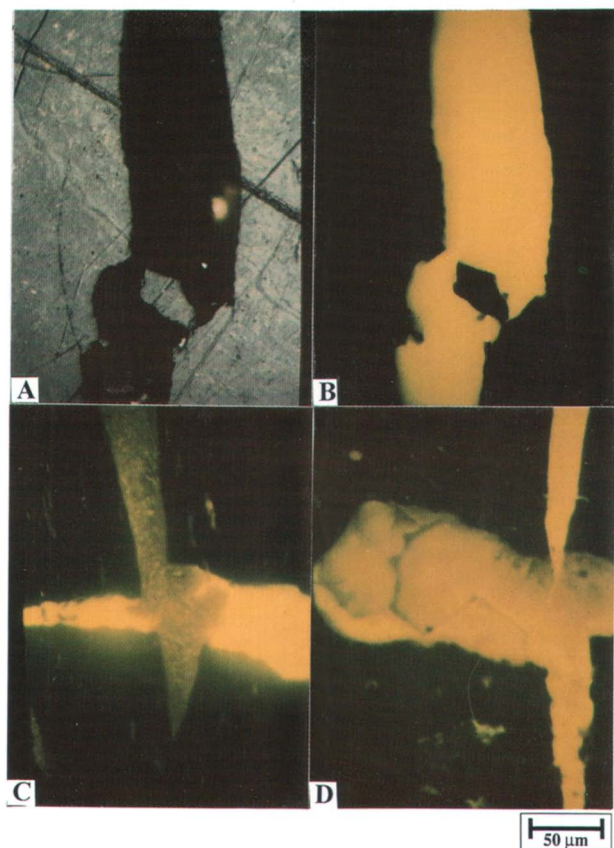


Plate 4. Photomicrographs of resinite macerals: A. Resinite cleat filling including a coal xenolith (Hiawatha seam - white light); B. Same field as A in blue light; C. Cross-cutting resinite veinlets. Note the flow of the darker veinlet into the brighter one (Hiawatha seam - blue light); D. Cross-cutting resinite veinlets. Note the brittle fracture of larger veinlet (Hiawatha seam - blue light).

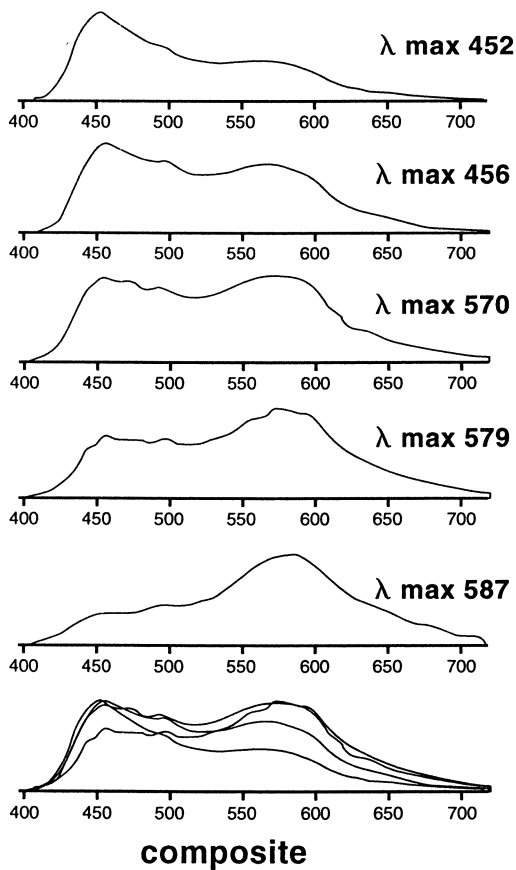


Figure 6. Fluorescence spectra of resinites from the Hiawatha seam (Wasatch Plateau) scaled to peak intensity. All spectra were taken under the same conditions from different resinite macerals in the same sample. The shift in the peaks represent a color change from blue to orange.

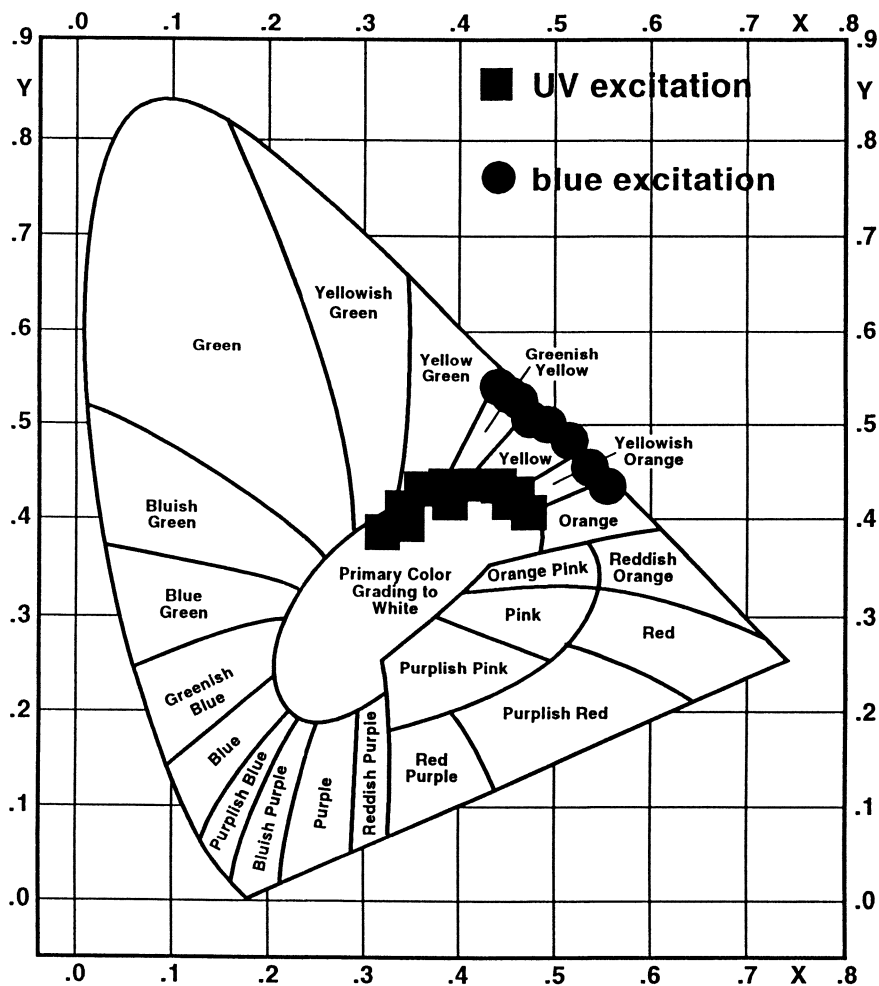


Figure 7. Chromaticity (C.I.E., 1931) plot in both ultra-violet and blue light of resinite spectra from the Hiawatha seam, Utah. Note the continuous distribution.

### Summary

Resinite macerals are ubiquitous, though minor, components in most American coals below medium-volatile bituminous rank. Resinite macerals have two common modes of occurrence. In most Appalachian and mid-western U. S. coal seams of Carboniferous age resinites occur as primary (present at the time of deposition) ovoid bodies. In coals from the western U. S. of Cretaceous/Tertiary age, much of the resinite occurs as secondary cleat and fissure fillings. This secondary resinite shows an intrusive relationship to the host coal and often shows flow texture and carries xenoliths of coal in resinite veinlets.

### Acknowledgments

The author is pleased to acknowledge the assistance of Mr. David Glick from the Coal and Organic Petrology Laboratories at The Pennsylvania State University and Dr. Richard Harvey of the Illinois State Geological Survey with the data bases. The assistance of Dr. David Bensley of the Coal Characterization Laboratory in the Department of Geology at Southern Illinois University with the photomicrographs and fluorescence spectra is also gratefully acknowledged.

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## Chapter 13

# Trace Amino Acid Composition of Natural Resins

## Elucidating the Nature of Resinous Artists' Materials

S. M. Halpine

Scientific Research Department, National Gallery of Art,  
6th Street and Constitution Avenue, N.W., Washington, DC 20565

Amino acid analysis may be useful in characterizing resinous and other nonproteinaceous artists' materials. During an investigation of ancient Egyptian paint binders, an atypical amino acid composition was consistently found in several samples. Reference materials were analyzed, including papyrus and a number of gums, starches, drying oils, and natural resins. Several of these materials showed characteristic protein amino acid profiles. Moroccan sandarac (*Tetraclinis articulata*) resin showed a composition close to that seen in some of the Egyptian samples. Sandarac may have been available in ancient Egypt as the source grows on the North African coast. Results of other amino acid analyses indicate the presence of dammar resin (from several genera of *Dipterocarpaceae*) in oil paint samples. These results were confirmed by triterpenoid analysis using GC-MS. Certainly, the presence of trace amino acids should be considered in order to avoid false interpretations during high-sensitivity analysis of paint samples.

The amino acid analysis system set up within the Scientific Research Department at the National Gallery of Art, Washington, is routinely used to characterize proteinaceous paint binders (1, 2). Egg yolk, egg white, casein, and animal glue (gelatin or collagen), the most commonly used proteinaceous paint binders, can be identified using this analytical method. Determining the type of binders applied to an artifact helps the conservator select the optimal conservation treatment for the object. The nature of the coatings is also of art historical interest.

After analyzing hundreds of samples taken from objects undergoing conservation

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treatment, most containing known protein binders, several consistent but atypical amino acid composition profiles emerged. These atypical compositions did not match the composition of the four main proteinaceous binders and generally showed low levels of amino acids (approximately 30 [1] to 500 nanograms). To determine the source of these unidentified amino acid compositions, nonproteinaceous artists' materials were analyzed as reference standards. The findings were quite surprising. Several nonproteinaceous materials such as gums and resins yielded somewhat characteristic amino acid compositions. The total yield of amino acids was between 0.5 and 6 micrograms per 100 milligrams of resin (5-60 ppm), depending on the type of resin. This chapter will examine the preliminary results of samples taken from a nineteenth-century American painting, several ancient Egyptian painted objects, and a nugget of resin found in an Egyptian tomb. The discussion will focus on the results showing trace amino acids in resin and paint samples, and the relevance of these amino acids as possible indicators of the botanical origin of the resins.

### Background

**Resins as Artists' Materials.** When used as artists' materials, resins are usually applied in thin films on the surface of art objects (3). These films may be either transparent varnishes or pigmented inks and paint layers (4-9). Artists, conservators, and art dealers use resin varnishes to enhance the color saturation and to offer some degree of surface protection to paintings and sculptures (10-13). Similarly, resins added to printing inks or paint media offer faster drying times, better handling properties, and a lustrous appearance not obtained from oil binders alone (6, 14). The proportionally large surface area of the resin films, however, leads to an accelerated breakdown of the characteristic terpenoid components (10, 12, 15-18). From an analytical point of view, the degradation and polymerization of components in aged resin films often make it difficult to identify the botanical source of resins (3, 12, 15-18). Although ancient resin samples preserved under anaerobic conditions or in bulk form have produced interesting findings (3, 17, 19-21), aged resin films often can only be characterized by gas chromatography-mass spectrometry (GC-MS) on the basis of degradation products from the main di- and triterpenoid components (17, 18, 22). For example, in a study of dammar resin, commonly used as a painting varnish, the majority of the triterpenoid and all of the sesquiterpenoid fractions disappear during aging in a fadeometer (Atlas Electric Devices Co.) (10, 15, 23). (The source of dammar resin will be addressed at a later point.) Art historical information that might be garnered from the botanical sources of the resinous materials found on artifacts is consequently limited. This limitation is unfortunate since the botanical source of materials original to a cultural artifact may indicate the resources available to that culture, the extent of trade with other geographical regions, and may place an artifact in a cultural period consistent with the introduction of otherwise unavailable materials.

**Amino Acids in Resins.** The relative permanence of amino acids present in oxidized films may provide a tool for characterizing aged resinous materials. For example, results of four thousand year-old Egyptian animal-glue paint samples matched the composition of unaged collagen (Table I, columns 1 and 2). The effect of aging on amino acids in resins was also considered, and the results of both naturally and artificially aged resin samples compared favorably with the results of unaged materials. The preservation of the amino acids in resins is not fully understood but may be due to the anhydrous environment of resinous matrices (see amino acid analysis of amber-trapped insects in the chapter by Wang et al. in this volume).

The trace amino acids found in natural resins may, in part, arise during the resin duct formation. For example, lysigenous resin duct formation involves the breakdown of resin cells (24, 25). This cell breakdown may account for the levels of hydroxyproline found in several of the resins since a main cell wall protein, extensin, is made up of 25% 4-hydroxyproline (26, 27). The hydroxyl modification of the proline-rich protein occurs in the cytoplasm and extensin is then secreted into the cell wall by membranous organelles (27, 28). Similarly the plant structural breakdown during gummosis, or gum formation (29, 30), may explain the high percentage of hydroxyproline found in gum arabic and gum tragacanth.

Conifers, such as the Moroccan sandarac (*Tetraclinis articulata*), are known to have schizogenous duct formation, with intact cells usually lining the secretory cavities (25). Resinous trees from the *Dipterocarpaceae* family were also reported to have schizogenous duct formation (31), which would account for the lack of hydroxyproline in dammar resin. A clearer understanding of the origin of the various amino acids must await further investigation, however.

Whereas genetic factors appear to control the terpenoid components in resins, as discussed by Langenheim in the first chapter of this volume, the role these factors play in determining the amino acid composition is still unclear. The amino acids stored in plant cells can vary according to abiotic factors such as the availability of water (32). If the amino acid moiety in resins is due to cell breakdown during lysogeny, then seasonal and geographical variations in amino acid composition cannot be ruled without further study.

Although only protein amino acids have been monitored for this study, the plant kingdom is a source of hundreds of nonprotein amino acids (27, 33). Some of the more unusual amino acids may be of value in identifying particular plant types (Bell, E. A., King's College, personal communication, 1992, and 34, 35). Similarly, no attempt has yet been made to isolate the free amino acids in these samples. The results presented here are derived from sample hydrolysates; the configuration of the amino acids *in situ*, whether in proteins, peptides, or as free amino acids, cannot be specified.

**Botanical Nomenclature and Artists' Materials.** The problems associated with the common names of resins are addressed by Langenheim (see Chapter 1). Unfortunately, the usual confusion regarding common names is somewhat amplified for

artists' materials. Artists and art conservators often had to depend on small, sometimes unscrupulous suppliers that in turn may have depended on several layers of intermediaries in the distribution of the raw materials (36). Langenheim's recommendation to study authenticated samples from botanical collectors should be followed whenever possible. This study has been limited to artists' materials, not authenticated samples, and the botanical name of the corresponding resin-producing tree has been included when known (17).

Dammar resin exemplifies the problems of determining a botanic source for artists' resins. The word "dammar" is derived from the Malaysian word denoting any type of resin (37). Dammar generally refers to resin produced by trees from the subfamily *Dipterocarpoideae* in South East Asia. Although this subfamily comprises approximately 15 genera and over 500 species, according to one estimate (3, 38), the resin used in Europe and America probably comes from only a few of these genera, namely *Shorea* and *Hopea* (3). Nevertheless, the botanical origin of dammar resin usually cannot be established by even the most reputable suppliers.

### Sample Preparation and Extraction

The amino acid analysis system used here is based on the Pico-Tag method developed by Waters Corporation (39-40). It consists of a gas-phase acid hydrolysis, precolumn derivatization with phenylisothiocyanate (PITC), and separation on a reverse-phase C-18 column using a Hewlett-Packard 1090M high-performance liquid chromatograph (HPLC) (1).

**One Hour Extraction of Paint Samples.** Very small samples of paint or other coatings from cultural artifacts are taken for analysis. The sample sizes range from approximately 0.1 to 4 micrograms (or approximately 0.03 to 2 micrograms combined total of amino acids, based on an internal calibration standard [1]). Before analysis, each sample is extracted with water for 1 hour at room temperature (2). This produces a water-soluble supernatant fraction and a water-insoluble precipitate fraction, thereby separating media components based on their solubility and isolating inorganic pigments and insoluble contaminants in the precipitate. Both fractions are analyzed, resulting in two chromatograms per sample. This method has been successfully used to characterize small paint samples for conservation-related inquiries (2). (For the sake of brevity, the results of both fractions have been combined in Figures 1B and 7A.)

**Nonproteinaceous Reference Standards.** The analysis of nonproteinaceous reference materials was conducted both derivatized with PITC and underivatized to rule out any interference from nonproteinaceous chromatographic peaks (2). The reference materials included unaged nonproteinaceous binders such as gum arabic and gum tragacanth; the gum resins frankincense and myrrh (Harvard University Botanical Museum); resins such as dammar, mastic, Moroccan sandarac, Manila copal, Congo copal, and rosin; drying oils such as linseed (cold pressed and

Table I. Normalized Percent Composition of Amino Acids in Samples of Collagen and Resins.

Amino Acid	1 Collagen Protein	2 Egyptian Animal- Glue Paint Sample	3 Dammar Resin ( <i>Dipterocar- paceae</i> )	4 American 19th- Century Paint Sample	5 Mastic Resin ( <i>Pistacia lentiscus</i> )	6 Egyptian Mastic Resin ( <i>P. atlantica</i> or <i>terebinthus</i> )	7 Moroccan Sandarac Resin ( <i>Tetraclinis articulata</i> )	8 Egyptian Group A Paint Binder
Aspartate	5	9	7	10	20	24	9	9
Glutamate	7	10	9	11	8	20	11	11
Hydroxyproline	10	10	1	*	16	35	*	*
Serine	4	9	9	12	16	13	13	15
Glycine	33	38	22	22	22	22	22	22
Histidine	1	3	1	1	4	9	2	2
Arginine	5	4	3	4	8	2	6	3
Threonine	2	4	6	5	12	11	4	5
Alanine	11	17	6	5	18	15	7	7
Proline	12	13	5	3	18	13	3	4
Tyrosine	*	3	4	3	10	7	6	5
Valine	2	7	5	5	12	20	6	4
Methionine	1	1	3	3	4	4	1	2
Isoleucine	1	4	5	5	8	9	7	4
Leucine	3	7	8	7	10	9	9	5
Phenylalanine	2	3	3	3	6	4	3	4
Lysine	3	3	3	3	4	2	3	2

**Table I.** Results of amino acid analysis from resin and paint samples, expressed as normalized percent composition. The collagenous samples (columns 1 and 2) have been normalized with hydroxyproline equal to 10, and the resinous samples (columns 3-8) have been normalized with glycine equal to 22 (bold typeface). Unaged resin samples have undergone a liquid-liquid extraction before analysis (columns 5 and 7). Aged resin and artifact samples have undergone a 1 hour water extraction and results of supernatant and precipitate fractions were combined unless noted otherwise (columns 2-4, 6, and 8). Tryptophan and cysteine content cannot be determined using this amino acid analysis system.

\* Below 0.5%.

**Column 1.** Collagen protein, no extraction, from Sigma Chemical Company. **Column 2.** Paint sample from ancient Egyptian painted wooden sculpture, containing an animal glue binder with a composition similar to modern collagen (excavation no. II-B-3; supernatant fraction; Figures 4 and 5). The increased percentage of amino acids such as valine, tyrosine, and leucine in the paint sample (column 2) indicates a possible additional source of amino acids, from either an intentional (e.g. another binder) or an unintentional mixture (e.g. contaminated water).

**Column 3.** Dammar resin (*Dipterocarpaceae* family), aged as film, from Kremer Pigmente. **Column 4.** Paint sample from 19th-century painting, *Ark of the Covenant*, by Erastus S. Field, containing a trace amino acid composition similar to that of dammar resin. (Figures 1 and 2; dammar resin composition reproduced from reference 2.)

**Column 5.** Mastic resin (*Pistacia lentiscus*), unaged, from Kremer Pigmente. **Column 6.** Nugget of mastic resin found in ancient Egyptian tomb (probably *Pistacia atlantica* [19] or *P. terebinthus*, var. *atlantica* [20]; supernatant fraction; Figure 3.)

**Column 7.** Moroccan sandarac resin, unaged, from Kremer Pigmente. **Column 8.** Paint sample from ancient Egyptian limestone sculpture, containing a trace amino acid composition similar to that of sandarac resin (excavation no. 35-11-24; figures 6 and 7).

boiled), poppy seed, and walnut; Zin shofu or Japanese wheat starch; modern papyrus leaf (supplied by Sturman, S., National Gallery of Art, 1991); garlic and onion extracts; and residues from fingerprints. (For other sources and vendors see Ref. 2; for botanical names associated with the resins, see Langenheim's Chapter 1 and Ref. 17.) In order to approximate ancient methods of starch preparation, wheat starch was prepared by hand using a modified Martin or doughball method (Miller, B. F., The Cleveland Museum of Art, personal communication, 1992, and 41). Wheat grains from four different lots were supplied by the National Small Grains Collection (USDA-ARS, Aberdeen, Idaho): emmer wheat (*Triticum dicocum*) grown in both Iran and Saudi Arabia, and durum wheat (*T. aestivum durum*) grown in Algeria and Tunisia.

**Liquid-Liquid Extraction of Amino Acids from Unaged Resins.** Samples of unaged resin used as reference standards required a liquid-liquid extraction for optimum yield of the amino acid components. For example, only 10% of the internal standard amino acid, norleucine, was recovered from a sample of unaged dammar resin analyzed without any sample preparation before hydrolysis (Figure 1A). The low recovery of the internal standard indicates that unaged resinous materials act somewhat like a sponge when exposed to heat and strong acid, thereby entrapping the internal standard and other amino acids. This sponge effect prevents derivatization and/or the release of the derivatized analytes into solution. On the other hand, aged dammar resin samples (natural and accelerated light aged) yield higher levels of native amino acids and, with 75% recovery of norleucine, the "sponge effect" on the internal standard is minimized (Figure 1B). This decreased recovery of amino acids from fresh resin samples is not well understood. It may be due to hydrophobic interactions between the aqueous internal standard, hydrolysis acid, and derivatization reagents and the nonpolar components of unaged resin. During aging, resins tend to become more polar (12, 15, 16, 42), which may explain the increased recovery of amino acids from aged resin samples. Furthermore, the native amino acids may be bound to resinous components or otherwise inaccessible during analysis.

To extract the proteinaceous components, approximately 12 milligrams of each resin were first dissolved in either ethanol or toluene and heated in a water bath. Next, 500 microliters of ethyl acetate followed by 500 microliters of HPLC-grade water with 0.1 % trifluoroacetic acid (TFA) were added to the samples and mixed thoroughly. After phase separation, the aqueous lower fraction was removed and dried down for amino acid analysis. Except for rosin and boiled oils, all the materials showed a somewhat characteristic protein amino acid profile.

### Amino Acid Analysis of Paint Samples: Results and Discussion

**Binder Analysis of a Nineteenth-Century American Painting.** *The Ark of the Covenant* was painted between 1865 and 1880 by the American artist, Erastus Salisbury Field (Figure 2). The painting was being studied to better understand the

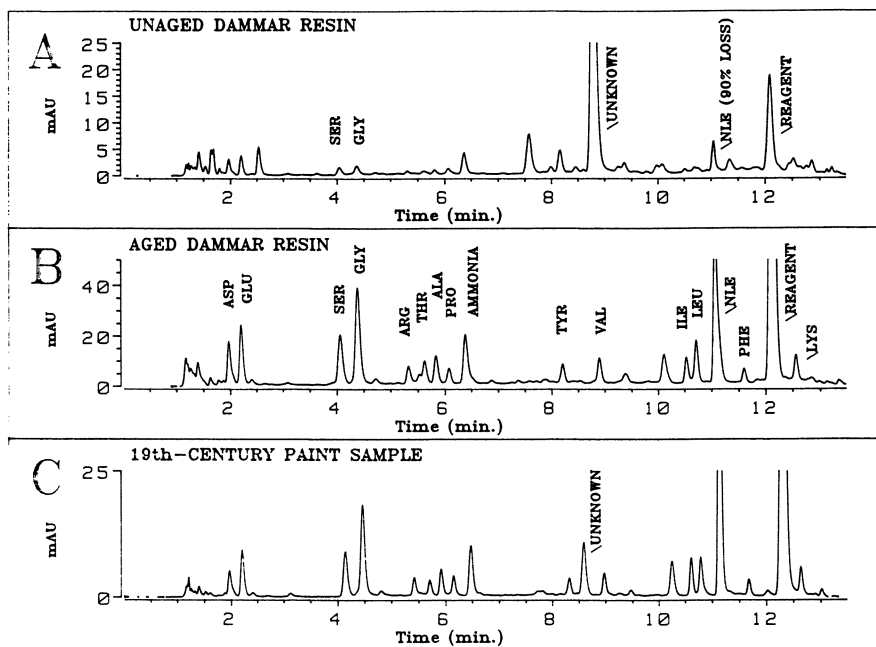
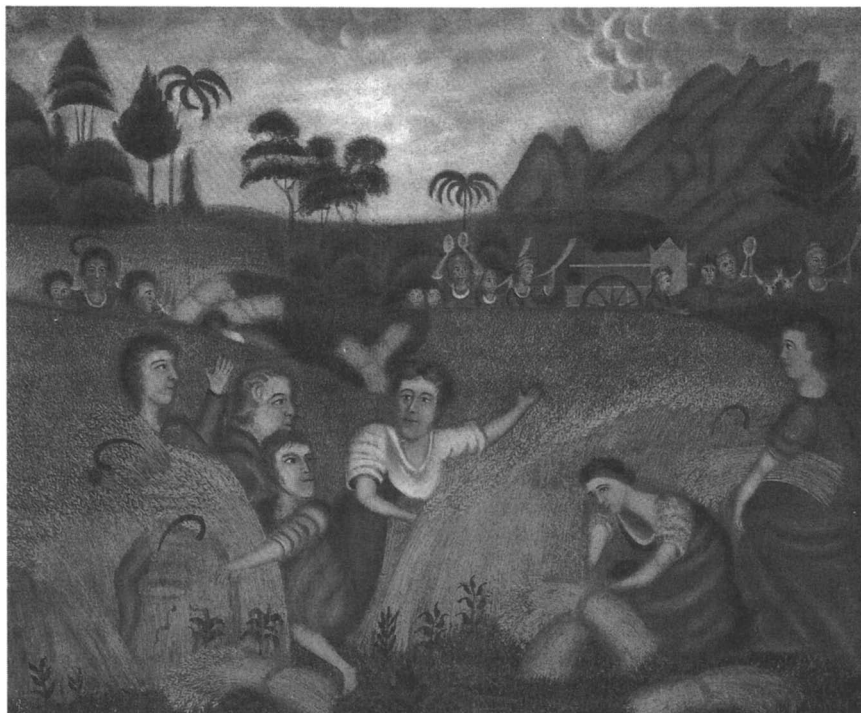


Figure 1. Chromatograms showing amino acid analysis results of dammar resin samples. (A) Unaged dammar resin, no extraction, with less than 10% recovery of norleucine internal standard; (B) dammar resin film aged 1400 hours in a fadeometer with 75% recovery of internal standard (combined supernatant and precipitate fractions of water extraction; no organic solvent extraction); and (C) dammar-containing paint sample from *Ark of the Covenant*, by 19th-century American painter, Erastus Salisbury Field (supernatant fraction; Figure 2). (For normalized percent composition of 1B and 1C, see Table I, columns 3 and 4. Aged dammar resin 1B supplied by de la Rie, E. R., while at the Metropolitan Museum of Art, New York, NY; 2% Tinuvin 292, a UV absorber, was added to resin before aging. Dammar resins in 1A and 1B purchased from Kremer Pigmente.) Figure 1B reproduced with permission from reference 2. Copyright 1995 National Gallery of Art.





**Figure 2.** *Ark of the Covenant* by Erastus Salisbury Field (American, 1805–1900); oil painting on fabric, c. 1865–1880; 20 x 24 1/8 in. (0.508 x 0.613 m); accession no. 1956.13.3. Gift of Edgar William and Bernice Chrysler Garbisch, National Gallery of Art, Washington, DC. Damar resin was found associated with paint samples. (Figure 1C and Table I, columns 3 and 4.) Reproduced with permission from the National Gallery of Art.

artist's methods and materials. The type of paint binder used by the artist was not known; the paint had a dry, pasty appearance. Therefore small paint samples were taken by the conservator and submitted for amino acid analysis and for fatty acid/resin analysis by GC-MS.

The amino acid analysis results of the samples did not indicate a proteinaceous binder (43). The composition did, however, match the trace amino acid composition of several dammar resin samples (Figure 1B and 1C; Table I, columns 3 and 4). The composition was distinct from that of mastic, another widely used triterpenoid resin, which generally contains the amino acid hydroxyproline (Figure 3B and Table I, column 5). Dammar resin was introduced in Europe in 1829 and since 1844 has been used extensively as a varnish and as an occasional paint additive (3, 14, 23). Resin analysis of this sample by GC-MS confirmed the presence of the triterpenoid components associated with dammar resin. Palmitate, stearate, and azelate components consistent with a drying oil paint binder were also found (44). Whether the dammar resin was mixed with the drying oil binder or applied as varnish by the artist, a dealer, or a conservator has not yet been determined. Nonetheless, amino acid analysis results correctly indicated the source of the resin present in the paint sample.

Chemotaxonomic studies have been carried out on the terpenoid fractions of the genus *Shorea* (45, 46) and several other Dipterocarpaceae genera (17, 38, 47-52) using GC-MS and other analytical methods. However, as mentioned previously, the sesquiterpenoid and triterpenoid fraction in dammar resin films largely disappear during light aging (10, 15, 23). Moreover, there is as yet no correlation between the composition of the terpenoid and the amino acid moieties. Further study using authenticated samples will clarify whether the above-mentioned trace amino acid profile for "dammar" resin characterizes the resin exuded from trees in the subfamily, a few genera or specific species.

**Study of Egyptian Paint Binders.** In order to characterize the coatings and paint binders used on ancient Egyptian painted objects, a collaborative investigation began between the Museum of Fine Arts, Boston, and the National Gallery of Art, Washington. GC-MS analyses of sugars, fatty acids, and resins present in the samples were conducted by Richard Newman at the Museum of Fine Arts, while the author characterized the proteinaceous components using amino acid analysis (53). The combined results from all analyses of the Egyptian paint binders will be presented at a later date.

The Egyptian artifacts were collected during expeditions sponsored, in part, by the Museum of Fine Arts and were transferred directly from the sites to the museum (Newman, R., Boston Museum of Fine Arts, personal communication, 1991). Samples were taken for analysis from painted objects made of wood and limestone in the MFA collection. The objects date from the Old Kingdom, approximately 2500 BC, through the Middle and New Kingdoms, up to the Greco-Roman period of 300 BC. The samples are believed to be from the original paint

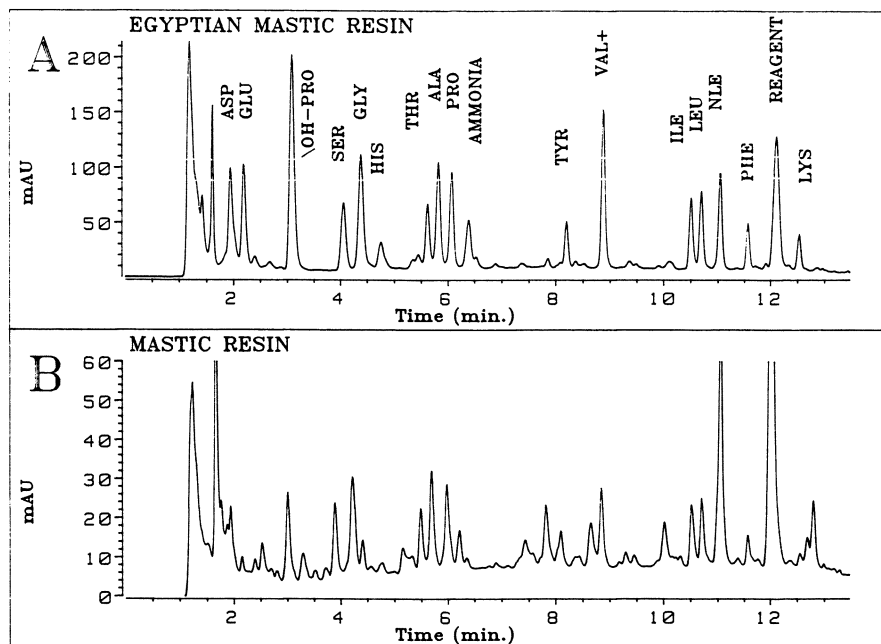


Figure 3. Chromatograms showing amino acid analysis results of mastic resins. (A) Nugget of Egyptian mastic resin (probably *Pistacia terebinthus* var. *atlantica*; supernatant fraction); and (B) mastic resin, unaged (*P. lentiscus*; after liquid-liquid extraction; supplied by Kremer Pigmente). Note hydroxyproline peak at 3 minutes; for normalized percent composition see Table I, columns 5 and 6.

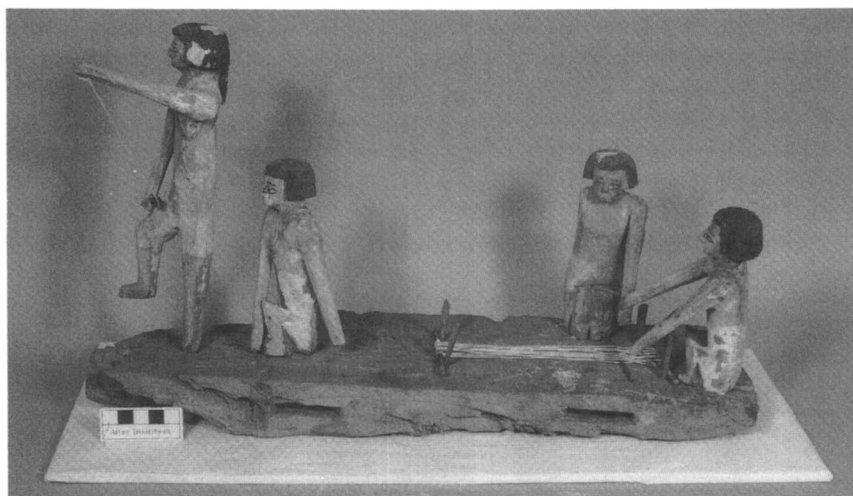
layers, since there is little evidence of early conservation treatment on the objects and recent treatment areas were avoided during sampling.

**Collagen-Containing Samples.** Some Egyptian paint samples clearly contained animal glue, a common artists' material made from animal hide or bone. An amino acid composition matching that of collagen was found in a paint sample taken from a sculptural grouping of Egyptian weavers dating from the Middle Kingdom (c. 2065-1785 BC) (Figure 4). In the analytical results (Figure 5 and Table I, columns 1 and 2), note the 7 to 10% hydroxyproline and the approximately 1:7 ratio of serine-to-glycine for both the Egyptian sample and in the collagen reference sample.

These results indicate that the amino acids in the samples had not been significantly altered in the intervening 3 to 4 thousand years, especially entombed in the dry Egyptian climate. In fact, Wyckoff (54) found that most protein amino acids survived in dinosaur bone samples of approximately 65 million years of age. Therefore rapid changes in amino acid composition would not be expected to occur during a period of a few thousand years in such an arid environment.

**Group A Samples.** In addition to collagen-containing samples, a consistent but unidentified amino acid composition appeared in several Egyptian paint samples. These samples were collectively called Group A samples. Paint from a limestone fragment found in Giza dating from the Old Kingdom (Figure 6), the same period as the pyramids (c. 2778-2300 BC), is representative of the Group A composition (Figure 7A and Table I, column 8). The Group A samples lack hydroxyproline, show a 1:2 ratio of serine-to-glycine, and have a high alanine content compared to threonine and proline levels (between 3 and 6 minutes on the chromatograms): the composition does not correspond to the any of the four most commonly used proteinaceous paint binders. The Group A binder was found in paint samples taken from both wood and limestone substrates and throughout the time periods examined (Old, Middle, and New Kingdoms and the Late Period including the Greco-Roman Period). GC-MS results from these samples have to date been inconclusive.

**Mastic Resin Sample.** We also were able to analyze a bulk sample of Egyptian resin, taken from a lump of mastic found in an El Bersheh tomb dating from the Middle Kingdom, or approximately four thousand years ago. Using GC-MS, Newman identified this resin as mastic, probably "Chios turpentine" from the tree *Pistacia atlantica* (19) or *P. terebinthus*, var. *atlantica* (20) (Newman, R., Boston Museum of Fine Arts, personal communication, 1991). Amino acid analysis of the resin showed a composition similar to that of unaged mastic from a related species (*P. lentiscus*). (Figure 3 and Table I, columns 5 and 6.) More significantly, the composition of the Egyptian mastic resin, which includes a sizable hydroxyproline peak, did not match that of the Group A samples.



**Figure 4.** Wooden model of weavers, Egyptian, Middle Kingdom; approximately 10 in. high (25.3 cm); accession no. 21.891; in the collection of the Museum of Fine Arts, Boston. This object is from a group of painted wooden figures with an animal glue paint binder (analytical results from a similar object are presented in Figure 5A and Table I, column 2). Reproduced with permission from the Museum of Fine Arts, Boston.

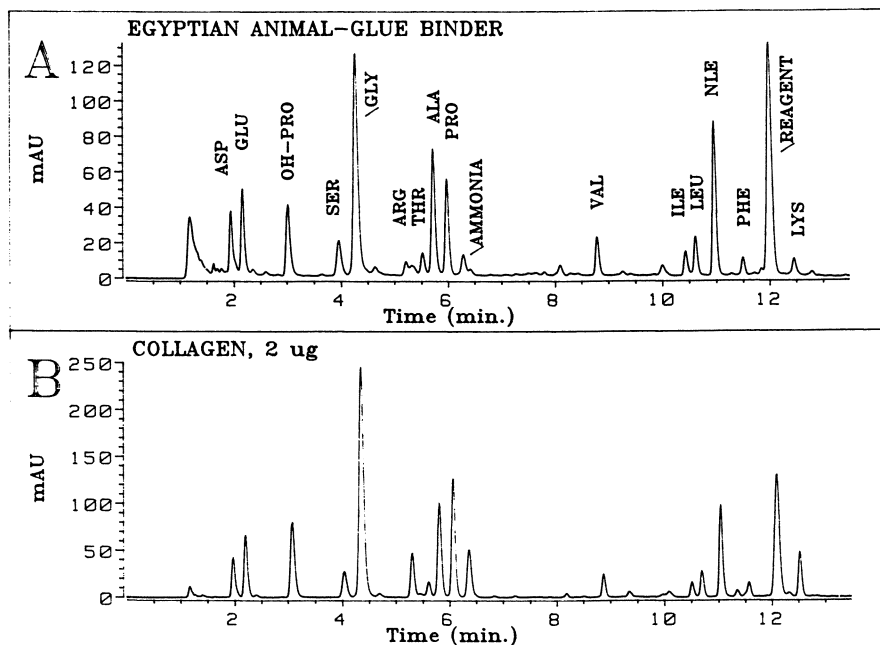
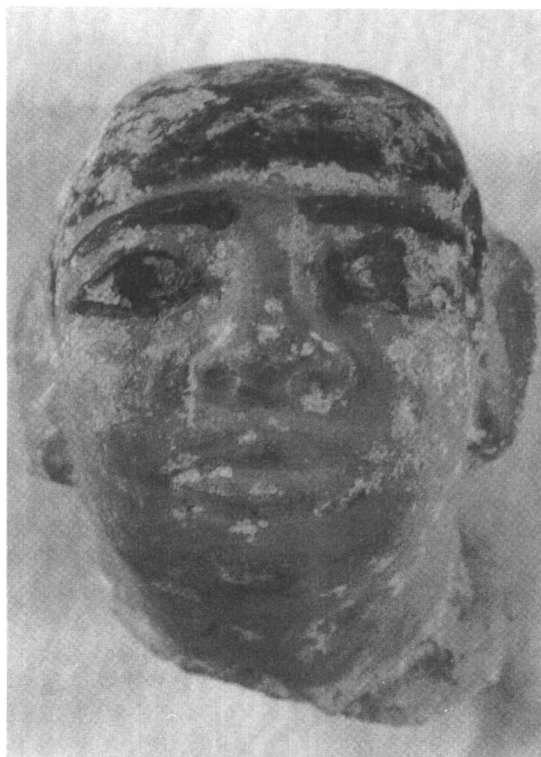


Figure 5. Chromatograms showing amino acid analysis results of collagenous materials. (A) Animal-glue paint binder from an Egyptian wooden figure sculpture, same group as Figure 4 (MFA excavation no. II-B-3; supernatant fraction); and (B) collagen (no extraction; supplied by Sigma Chemical Company). (Normalized percent composition listed in Table I, columns 1 and 2.)



**Figure 6.** Limestone head fragment, from Giza, Old Kingdom; approximately 2.5 in. high (6.3 cm); excavation no. 38-4-10; in the collection of the Museum of Fine Arts, Boston. This object is from a group of limestone fragments found to have been painted with the Group A binder (analytical results from a similar object are given in Figure 7A and Table I, column 8.) Reproduced with permission from the Museum of Fine Arts, Boston.

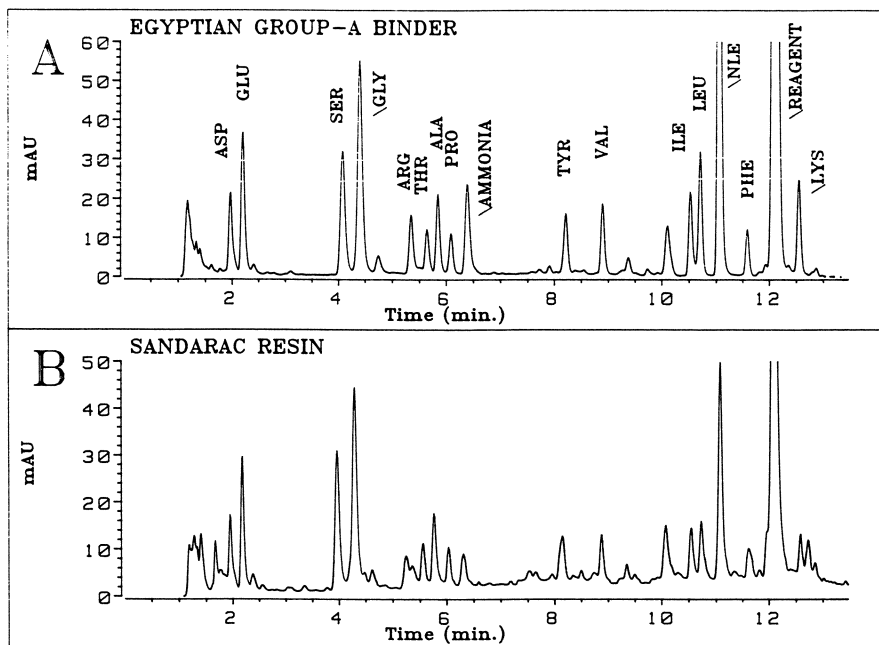


Figure 7. Chromatograms showing amino acid analysis results of Group A binder and sandarac resin. (A) Fragment of Egyptian limestone sculpture painted using the Group A binder (excavation no. 35-11-24; combined supernatant and precipitate fractions); and (B) sandarac resin, unaged (*Tetraclinis articulata*; after liquid-liquid extraction; supplied by Kremer Pigmente). (Normalized percent composition listed in Table I, columns 7 and 8).



**Moroccan Sandarac.** Of all the materials analyzed, Moroccan sandarac resin most resembled the composition of the unidentified binder in the Group A samples (Figure 7 and Table I, columns 7 and 8). Moroccan sandarac, from the conifer *Tetraclinis articulata*, grows on the North African coast and therefore may have been available to artisans in ancient Egypt. It is soluble in alcohol but insoluble in turpentine (soluble in hot turpentine only if it is first fused) (3, 4). Sandarac was once widely used; in fact, the word "varnish" comes from "berenice," a former name for sandarac, as this resin was exported in considerable quantities from Bengazi, then known as Berenice, on the Libyan coast (4).

**Ancient Egyptian Use of Resin.** In his book, *Ancient Egyptian Materials and Techniques*, Lucas makes several references to the Egyptian use of resins as paint binders and varnishes, as adhesives, and as materials used in the mummification process (55). Pine pitch was used as a sealant and gum resins such as frankincense and myrrh were burned as incense (56). Lucas found that a number of Egyptian resin samples were soluble in alcohol but not turpentine, although aware that, used as an identifying characteristic, the solubility of the resins might be expected to change over time (55). Despite sandarac fitting the solubility profile of these samples, Lucas nevertheless dismissed sandarac resin as a possible Egyptian resin because of the lack of evidence of its importation from northwest Africa (55).

Ancient Egyptian trade routes may have been more extensive than originally thought, however, since recent discoveries of Baltic amber tomb artifacts indicate contact with northern European cultures in addition to the more accepted transactions with Far Eastern cultures (Boon, J. J., FOM Instituut voor Atoom- en Molecuulfysica, personal communication, 1995, 55 and 57). Furthermore, sandarac was found on Carthaginian mummies dating from the first millennium BC (4, 58), a time period roughly corresponding to the New Kingdom in Egypt.

Lucas' claim that distillation was unknown to the ancient Egyptians (55) has led some to speculate that the aforementioned "Chios turpentine" (*Pistacia atlantica* [19] or *P. terebinthus*, var. *atlantica* [20]), a semi fluid oleoresin (55), was their primary source of resin (19). A higher concentration of alcohol than that provided by fermentation of wine and beer, at which the Egyptians were proficient, is necessary to dissolve most resins (55), including sandarac. Other references do indicate, however, that the Egyptians may have used a simplified form of distillation (59-62). For example, an early method for collecting a turpentine-like extract involved heating oleoresins in pots and condensing the vapors on woolen fleece (59, 63). Similar methods may have been used to collect a crude alcohol distillate. Distillation methods used today evolved from eighth-century Arabic distillation techniques (59), and these in turn may have developed from less sophisticated separation methods previously used in the area. How the Egyptians dissolved the resins for their various applications has still not been adequately determined and is beyond the scope of this discussion. A full understanding of the types of resins used during their long and enterprising history awaits further research into the materials and working methods used by the ancient Egyptians.

## Future Research Goals

Further study may lead to the characterization of resinous artists' materials and archeological samples based on their trace amino acid compositions. This line of investigation may also be of interest in botanical studies. To strengthen the chemosystematic value of this technique, nonprotein amino acids, such as n-methyl-4-hydroxyproline found in the leaves of resinous *Copaifera* tree (34), should also be investigated for trunk resins. The effects of seasonal and geographic variations must be evaluated for the constituent amino acids as they have been for the terpenoid components of resins. Furthermore, the possibility that certain commercially prepared resins and samples from art objects may consist of mixtures of resins should also be taken into account.

Amino acids are often more permanent than some of the more abundant organic constituents of resins. Amino acid analysis is sure to complement other analytical methods such as oil/resin analysis by GC-MS in the study of aged resinous materials. Certainly, when applying highly sensitive amino acid analysis techniques to the investigation of artists' materials, consideration should be given to the possible interference of trace amino acids derived from nonproteinaceous sources.

## Acknowledgments

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## Chapter 14

# Amino Acids in the Amber Matrix and in Entombed Insects

Xueyun S. Wang<sup>1</sup>, Hendrik N. Poinar<sup>2</sup>, George O. Poinar, Jr.<sup>3</sup>,  
and Jeffrey L. Bada<sup>1,4</sup>

<sup>1</sup>Scripps Institution of Oceanography, University of California  
at San Diego, La Jolla, CA 92093-0212

<sup>2</sup>Institute of Zoology, Department of General Biology, University  
of Munich, D-88021 Munich, Germany

<sup>3</sup>Department of Entomological Sciences, University of California,  
Berkeley, CA 94720

We have investigated the amino acids in both the bulk matrix and in insect inclusions in tree resins ranging in age from <100 years to 130 million years. The amino acid content of the resin matrix averages about 5 ppm and does not systematically vary with the age of the resin. The amino acids in the matrix are likely derived from either plant cells, or microorganisms, encapsulated when the resin solidified. The amino acid content of the insect tissues entombed in amber is less than that in modern insect specimens; this loss may be the result of oxidation reactions. The amino acid compositions of a fly and bee entombed in 30-40 million year old amber are somewhat different from the amino acid profiles of modern insects; this finding suggests that the preserved amino acid pattern under anhydrous conditions may not be the same as in aqueous environments. The amino acid racemization rate in amber insect inclusions is retarded by a factor of  $>10^4$  compared to other geochemical environments on the surface of the Earth. This is also apparently due to the anhydrous properties of the amber matrix. The excellent preservation of amino acids in amber insect inclusions suggests that other biomolecules would also be preserved much better than in other geochemical environments. This conclusion is consistent with the reported successful retrieval of DNA sequences from amber-entombed organisms.

We have recently shown that the amino acids present in a fly entombed in 40 million year old Baltic amber are remarkably well preserved [1]. Essentially no racemization, or decomposition of the unstable amino acid, serine, has taken place since the fly was encapsulated in the amber 40 million years ago. In contrast, in other geochemical systems on the surface of the Earth the L-amino acids initially present in the tissues of organisms are totally racemized ( $D/L = 1.0$ ) on time scales of  $10^5$  to  $10^6$  years [2]. The more ancient the amino acids, the closer the  $D/L$  ratios should be to 1.0, provided there is no modern L-amino acid contaminates present [3].

<sup>4</sup>Corresponding author

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Abstraction of the amino acid  $\alpha$ -hydrogen by water or  $\text{OH}^-$ , producing a planar carbanion intermediate, is the rate limiting step in the racemization reaction [4]. This is also the first step involved in serine dehydration [5]. Thus, environments where amino acid stereochemistry is preserved would also favor the preservation of serine. Sufficient water for  $\alpha$ -hydrogen abstraction, and thus for racemization and serine dehydration to take place, is usually present in most natural geochemical environments. However, in desiccated samples [6], and in those from hydrophobic deposits such as tar pits [7], racemization is retarded. In the absence of water, racemization proceeds slowly, or is even quenched. Thus, the unusual preservation of amino acid stereochemistry, and the lack of extensive serine decomposition, in the fly entombed in amber is apparently the result of the anhydrous nature of the amber matrix. The importance of anhydrous media in molecular preservation is demonstrated by the observation that in anhydrous non-aqueous organic solvents, the stability of fragile biomolecules such as enzymes is greatly enhanced compared to aqueous solutions [8].

The anhydrous environment of amber has also been suggested to be important in the preservation of DNA in organisms encased in amber [9]. DNA has been successfully amplified from plant and insect tissues preserved in amber as old as 130 million years [10-14]. In most geochemical environments, DNA fragments containing several hundred base pairs should not be preserved for more than  $10^4$  years [15-17]. Depurination (hydrolysis of the deoxyribose/adenine or guanine bond), followed by rapid chain breakage, is thought to be the main reaction important in the fragmentation of DNA in the geologic environment [15,16]. We have recently shown that the rates of depurination and aspartic acid racemization at neutral pH are nearly identical over the temperature range of 45° to 120°C [1]. Also, the *in vivo* rate of aspartic acid racemization measured in human enamel, dentine and the eye lens nucleus [18] is similar to that estimated for *in vivo* depurination [19]. The retrieval of DNA sequences and the lack of any detectable racemization in amber-entombed insects suggests that DNA depurination and amino acid racemization are coincident with each other not only in aqueous solution, but apparently under anhydrous conditions as well.

In order to further investigate the preservation of amino acids in both the bulk amber matrix and in amber-entombed insects we have performed amino acid analyses on various copal and amber samples [20] which span an age range from <100 years to 130 million years.

## Experimental Methods

The various fossilized tree resins with insect inclusions which were analyzed in this study are listed in Table I. All samples were obtained from authenticated collections.

The procedures used in processing the various samples are essentially the same as that reported previously [1]. Only a generalized scheme will be given here. All the resin and amber pieces were first extensively washed with a variety of solutions in order to remove surface contaminants. The pieces with insect inclusions were placed in sterile petri dishes and cracked by the addition of liquid nitrogen. Fossil resins with inclusions usually crack along the plane of the inclusion. For pieces that did not readily crack, sterile needles were used to pry them open. Once the insect tissue was exposed, moistened needles were used to remove and transfer the tissue to a clean glass vial. The amount of insect tissue obtained from the various fossilized resins was too small to be weighed with the analytical balances available in our laboratories; we estimate that the sample weights were in the range of a few tenths of milligrams. These intact tissue samples were hydrolyzed in twice-distilled 6 M HCl

**Table I. The tree resins and insect inclusions analyzed in this study**

Location	Resin	Insect	Age (years)
East Africa	copal-- <i>Hymenaea</i>	bee	<100
Colombia	copal-- <i>Hymenaea</i>	none	<300
New Zealand	copal-- <i>Agathis</i>	none	20,000 to 30,000
Dominican Republic	amber-- <i>Hymenaea</i>	bee	25-40 million
Baltic	amber-- <i>Agathis</i>	fly	40 million
Lebanon	amber-- <i>Agathis</i>	none	130 million

for 24 hours at 100°C. For comparison, a modern fly and bee were collected from a laboratory window sill and a garden. These modern insects were washed with water, dried under vacuum and then hydrolyzed in 6 M HCl for 24 hours. Clear amber and copal chunks which had no inclusions were directly hydrolyzed in 6 M HCl for 24 hours. A reagent blank was processed by simply heating the same volume of 6 M HCl for 24 hours.

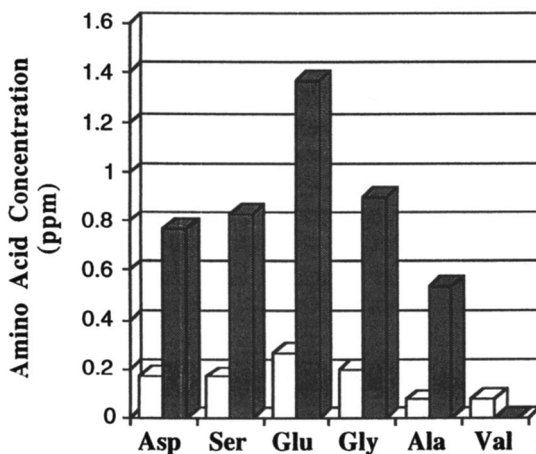
The 6 M HCl hydrolysates were evaporated to dryness, and the residues reconstituted in a small volume of twice distilled water. A portion of these amino acid extracts were derivatized with *O*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC), and analyzed by high performance liquid chromatography (HPLC) using fluorescence detection [21].

## Results And Discussion

The amino acid compositions of bulk Dominican Republic (*Hymenaea* resin) and Baltic (*Agathis* resin) amber are given in **Figure 1**. The amino acids shown are the major ones detected and are representative of the variety of functional groups present in the protein amino acids; other amino acids such as isoleucine and leucine were present in the matrix samples, but only in trace quantities. The amount of amino acids detected in each of these samples was several times greater than that in the procedural blanks.

The total amino acid contents (expressed as Asp + Ser + Glu + Gly + Ala + Val) of the various bulk copal and amber samples are shown in **Figure 2**. The amino acid contents ranged between about 1-18 ppm, and there is no systematic difference between the various copal and amber samples. The amino acids in the copal and amber matrix are probably derived from two possible sources: plant cells and microorganisms encapsulated when the resin solidified [22]. As is discussed in the chapter by Halpine in this volume, the degradation of resin cells is part of the process involved in resin formation, and amino acids would certainly be components of these cells. In addition, a variety of microorganisms have been identified in the amber matrix [22]. Assuming that the amino acids in the various resins are entirely derived from bacterial cells, then the amino acid contents we have measured can be used to make a rough estimate of the number of bacterial cells present in the resin matrix. Using an average amino acid content for the resins of about 5 ppm and a value of  $1 \times 10^{-13}$  g as the amino acid content of a typical bacterial cell [23], then we estimate





**Figure 1.** The concentrations (in parts per million, ppm) of aspartic acid, serine, glutamic acid, glycine, alanine and valine in the bulk matrix of Dominican Republic and Baltic amber:

□ Dominican Republic      ■ Baltic

The compositions of the Dominican Republic and Baltic amber are in general representative of the other specimens. Although other amino acids were present in the amber matrix, we have listed only the major ones detected.



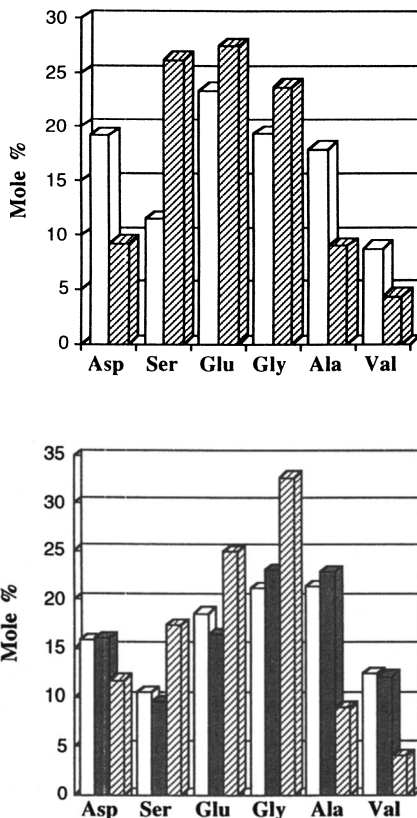
**Figure 2.** The total amino acid concentration (given as the sum of the amino acids shown in **Figure 1**) in the various copal and amber samples. Only the Colombian copal had an amino acid content significantly higher than the others.

that the various copal and amber pieces contain around  $5 \times 10^7$  bacterial cells per gram of resin. This value should be viewed as a maximum for the number of bacterial cells, however, because as we have mentioned previously, there are other possible sources of the amino acids.

The quantity of total amino acids isolated from the intact insect tissues retrieved from the various amber pieces was in the range 0.1–2  $\mu\text{g}$ . The amount of total amino acids obtained from the insects trapped in the copal samples was consistently greater than for the amber insect tissues and was in the range 10–20  $\mu\text{g}$ . Because none of the intact insect tissue samples were large enough to be weighed, it was not possible to determine the actual amino acid contents of these copal and amber entombed insect tissues. However, if we assume that the tissue weights were in the range of 0.1–0.5 mg, then the insect tissues preserved in amber contain roughly 0.1–1 % amino acids while the copal entombed insects contain about 2–10 % amino acids. We found that the modern fly and bee contained 15–20 % amino acids (dry weight). There does thus appear to be a net loss of amino acids from the insect tissues entombed in fossilized resins with increasing geologic age. Amino acids are prone to oxidation reactions [24]. Trapped gases, including oxygen, are present in the amber matrix, although the gas composition has been modified from air by microbial respiration, oxidative reactions and diffusion [25,26]. Thus the loss of amino acids we have observed in the insect tissues with increasing resin age may be the result of their slow oxidation.

The amino acid compositions (expressed as mole %) of the intact tissues obtained from the various copal and amber specimens compared to the modern insects are shown in **Figure 3**. The bee entombed in <100 year old East African copal has an amino acid composition identical to a modern bumble bee collected in a La Jolla garden. Thus, there appear to be no change in the amino acid composition of the tissue during the initial period of encapsulation of insects in tree resins. However, there does appear to be an enrichment of serine, glutamic acid and glycine in the fly entombed in 40 million old Baltic amber in comparison to that found in the modern fly (see **Figure 3**, top). In addition, the relative amounts of aspartic acid, alanine and valine are lower in the Baltic amber entombed fly in comparison to the modern fly tissue. These changes are also found in the comparison of the amino acid profiles of bee tissue obtained from 25–40 million year old Dominican Republic amber with that of a modern bee (**Figure 3**, bottom). Even though serine is one of the most unstable amino acids [2] it is still preserved and apparently even enriched in 25–40 million year old amber entombed insect tissue.

The reason(s) for the enrichment of some amino acids and the depletion of others in the insect tissues preserved in amber is not known at this point. It is unlikely that the amino acid compositional differences are due to evolutionary changes in the proteins in the insect tissues over the 25–40 million year time period [27]. In fossil calcified tissues such as bone and shell, a series of complex reactions generate changes in the original amino acid composition. Most of these changes are due to peptide bond hydrolysis and decomposition reactions of the amino acids [2, 5, 28]. In aqueous conditions, peptide bonds containing aspartic acid and serine residues are generally the most rapidly cleaved, whereas those containing hydrophobic amino acids such as valine are much more resistant to hydrolysis [for example, see discussion in 2]. However, virtually nothing is known about the relative hydrolysis rates under anhydrous conditions such as those characteristic of the amber matrix. Also, as was discussed previously [1], in the anhydrous amber matrix the rates of reactions such as racemization and serine dehydration are greatly retarded. The data in **Figure 3** suggest that in comparison to aqueous solutions, in anhydrous environments peptide bonds which are generally the most labile in water may be much more stable. In addition, as is demonstrated by the presence of serine



**Figure 3.** Amino acid compositions (expressed as mole % of the listed amino acids) of modern bee and fly tissue compared to the insect tissue entombed in Baltic and Dominican Republic amber and in East African copal.

**Top:** □ Modern laboratory fly    ▨ Baltic fly  
**Bottom:** □ Modern bumble bee    ■ East African bee  
           ▨ Dominican Republic bee

in the amber entombed insect tissues, decomposition reactions which readily take place in the presence of water are quenched in anhydrous media. Thus, the peptide fragments remaining in insect tissues encased in amber apparently contain a different ensemble of amino acids than would be expected under aqueous geochemical conditions.

The D/L ratios of aspartic acid and alanine in the various resin entombed insects are given in **Table II**. These two amino acids were selected because they represent

**Table II. D/L aspartic acid (Asp) and alanine (Ala) ratios in resin entombed insects and a fossil shell**

Age (years)	Organism / Source	D/L Asp	D/L Ala
Modern	Fly / laboratory	0.02	<0.01
Modern	Bee / garden	0.02	<0.01
<100	Bee / East African copal	0.08	<0.01
30-40 million	Bee / Dominican Republic amber	<0.01	<0.01
40 million	Fly / Baltic amber	0.04	<0.01
1 million	Mollusk shell / North Carolina*	0.91	0.95

\* Taken from [29]

one of the fastest racemizing amino acids (Asp) and one with an intermediate racemization rate (Ala). In the 30-40 million year old amber entombed insects no significant amino acid racemization could be detected in comparison to the modern specimens (the small amount of racemization detected in these samples is likely entirely caused by racemization during the 6 M HCl hydrolysis procedure). In contrast, the amino acids in a fossil mollusk shell from North Carolina which is only around 1 million years old have been found to be completely racemized [29]. The half life for aspartic acid racemization for insects entombed in amber is estimated to be about  $1 \times 10^9$  years based on the difference of 0.02 in the D/L aspartic acid ratios determined for the Baltic amber fly inclusion and the modern fly. This half life is  $10^4$  to  $10^5$  times longer than the aspartic acid racemization half lives measured in geochemical samples from temperate environments [2].

The retardation of amino acid racemization in insect tissues encased in amber suggests that the degradation of other biomolecules might also be inhibited. If DNA depurination is retarded by more than a factor of  $10^4$  to  $10^5$  as is aspartic acid racemization in the amber insect inclusions, then DNA fragments containing many hundreds of base-pairs could be preserved over time scales of  $10^7$  to  $10^8$  years. This conclusion is consistent with the reports that DNA fragments have been successfully amplified from a number of organisms entombed in amber [10-14]. Considering the reported enhanced stability of enzymes in non-aqueous anhydrous solvents [8], it is intriguing to speculate that even some enzyme activity may still be preserved in amber entombed insect tissues. The preservation of enzymatic activity would help support recent claims that revivable bacteria are present in the abdomens of insects entombed in 25-40 million year old Dominican Republic amber [30].

## Conclusions

The anhydrous nature of amber apparently retards geochemical reactions such as amino acid racemization and DNA depurination. Both unaltered amino acids and retrievable DNA fragments are preserved in amber entombed insects. Amino acid racemization and DNA depurination have now been found to occur in concert with each other in both aqueous and anhydrous environments. We suggest that amino acid racemization in ancient remains provides an excellent probe for accessing the preservation of important biomolecules such as DNA.

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## Chapter 15

# Dammar Resin: A Chemical Model for Reactions of Utah Resinite

Richard Dutta and Harold H. Schobert

Fuel Science Program, Pennsylvania State University,  
University Park, PA 16802-2303

The hydrogenation, dehydrogenation and cracking reactions of resinite found in Utah coal are of importance if a coal liquefaction process is to be fully optimized. Dammar resin is very similar in structural composition to Utah resinite, and was used in this study. A series of reactions of various temperatures and times was undertaken and product distributions were analyzed. The polymeric fraction of dammar was successfully depolymerized and dealkylated to naphthalenes and benzenes. The triterpenoids were defunctionalized and cracked to produce bicyclic components and phenols. The results from the dammar compared well with DECS-6 resinite liquefaction. The naphthalenes were hydrogenated to tetralins using palladium-on-carbon catalyst. Analysis of the data shows that a high temperature, short reaction time process step, followed by a low temperature catalytic hydrogenation step, is a favorable route to produce cycloalkanes from resinite models.

For the past several years we and our colleagues have been investigating the hydrogenation and dehydrogenation chemistry of a variety of polycyclic compounds. This work has aimed at investigating some of the fundamental chemical processes involved in various aspects of fuel utilization, including direct coal liquefaction, stability of aviation fuels in the pyrolytic decomposition regime, *cis-trans*- and ring shift isomerization, petroleum residua upgrading, and carbonization or graphitization reactions leading to carbon materials. The compounds investigated have included decalin (1), tetralin (1), anthracene (2,3), phenanthrene (3,4), pyrene (3,5) and chrysene (3).

Recently we have sought to extend this work to other polycyclic cycloparaffins, particularly terpenoids that would have structures relevant to various aspects of fuel chemistry. Dutta (6) has shown that rosin (a recent pine resin predominantly made up of compounds with an abietane structure) could be converted

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to bicyclic compounds in high concentration. A practical motivation for this effort has been the recognition that decalin and tetralin confer significant stability on jet fuels thermally stressed in the pyrolytic decomposition regime (i.e.,  $\geq 350^{\circ}\text{C}$ ) (7). As will be discussed in more detail below, feedstocks that could readily be converted to decalin could be desirable starting materials for production of jet fuels having superior high-temperature thermal stability.

### Jet Fuel Stability

The major factor that controls the stability of fuels is its chemical composition (1). The major reason for development of jet fuels with improved thermal stability is two-fold. First, the fuel can be used for thermal management (i.e., the absorption of heat to protect electrical and mechanical components) on the next generation of aircraft. These fuels will be subjected to temperatures exceeding  $400^{\circ}\text{C}$ . At high temperatures, pyrolysis reactions of certain components in the fuel become significant and result in the formation of gums and insoluble solids. These materials will reduce heat transfer efficiency, degrade valve performance and deposit solids in the fuel line and in fuel combustor nozzles. Second, a fuel that can withstand thermal degradation above its critical temperature could be used, possibly even in today's aircraft, for "supercritical combustion". This could significantly reduce the production of thermal  $\text{NO}_x$  from jet engines.

### Selection of Feedstocks

With the increasing interest to develop advanced jet fuel comes an increasing need to find new feedstocks that can be converted to thermally stable compounds. Jet fuels are traditionally produced from petroleum and thus they contain a large proportion of alkanes. Song et al. have shown alkanes to be thermally unstable at elevated temperatures (8). In the same study decalin, tetralin and cyclohexanes were shown to resist the formation of solids at temperatures above  $400^{\circ}\text{C}$ . Therefore an ideal chemical composition of advanced jet fuels would be one that is predominantly cycloparaffinic. With a desired product in mind, it would be advantageous to select a feedstock that can be converted to the product in as few steps as possible. Compounds that are, or could be easily converted to cycloalkanes, would therefore make ideal feedstocks.

Certain coals can be considered potential feedstocks if their chemical structures are suitable. For the purpose of advanced jet fuel production, coal liquefaction can be considered a simple synthesis reaction, whereby we start with a complex, macromolecular, crosslinked structure, and we want to produce bicyclic cycloalkanes. Therefore, in selection of a coal, ideal properties would be a low crosslink density, few aromatic rings per cluster and a relatively high H/C ratio. The petrographic make-up of the coal would be ideally one high in hydrogen-rich macerals, e.g. resinite. Coal from the Blind Canyon seam in Utah fulfills all the above criteria, including an unusually high concentration of resinite (6%).

Studies on liquefaction and hydrogenation of resins are not a new concept. Francis established the relative ease of hydrogenation of coal resins in comparison to other components of coals (9). Storch and his co-workers corroborated this observation (10). Although the structure of the coal resins was not established by this work, it was inferred, from their ease of liquefaction, that the resins likely contain fewer condensed polycyclic structures than other constituents of the coal (11,12). The products of hydrogenation of resins were shown to be oils of relatively low density and rich in saturated hydrocarbons (13). During World War II, the use of modern pine resins as a source of aviation fuel was investigated in Japan (14).

Detailed studies of the liquefaction of resinite or resinite models will help evaluate the prospects for liquefaction of resinite-rich coals, and of resinite macerals concentrated from such coals.

### Background Discussion on Resin Research

**Early Research.** Because our work focuses mainly on the chemical reactions of dammar resin and related materials, rather than on the geochemistry of resins and resinites, we do not attempt to provide a comprehensive summary of the geochemical literature. It is worth noting that investigations of the chemical properties of resins have been conducted for nearly two centuries, at least since the work of Klaproth (15) and, shortly thereafter, Johnston (16,17) on characterization of these materials. Schrötter was apparently the first to investigate resins associated with brown coals (18). Some of the details of these very early studies are summarized in the treatise by Redwood (19). In the United States, White provided some of the first reports on resins in paleoflora and in coals (20). Many of the early studies of coal resins were based on solvent extraction; none provided exact structural information about the resins, but an extraordinary array of names for these substances appeared in the literature in the late nineteenth and early twentieth centuries. A useful summary is provided by Hinrichsen and Taczak (21). Teichmüller suggested the separation of resinites into three genetic groups: terpene, lipid, and secondary resinites (22).

**Recent Studies.** With the emergence of advanced analytical techniques, the overall understanding of the structure, chemistry and maturation trends of resinite has increased. Through these studies, a large variety of chemically different resinites have been identified (23-29). Anderson et al. (30) have proposed a classification system of resinites based on their structural characteristics and composition. They have separated resinites into four classes (with some sub-classes). For this study, the class II resinites are the ones under investigation. These include Utah resinites and some Indonesian resinites.

**Structural Information on Utah Resinite and Dammar.** The chemical composition of resinite from Cretaceous coal seams in Utah has been shown to contain a regular polymer consisting of sesquiterpenoid repeat units (31). In addition to this polymer, resinite also contains other occluded cyclic triterpenoids. The botanical origin of this resinite is still under discussion. Based on chemical analysis, an affinity with Dipterocarpaceae has been suggested (31,32). Dammar is a recent resin obtained from Dipterocarp species from South East Asia. It consists of a methanol soluble fraction and a methanol insoluble fraction (33). The soluble fraction has been shown to be made up of triterpenoids (34). Attempts to elucidate the structure of the polymeric component have been made by several authors. Shigemoto et al. (35) suggested a macromolecule made up of  $C_{15}H_{26}$  sesquiterpene units with a 1,6-dimethyl-4-isopropyl-1,2,3,4,5,6,7,8,8a-octahydronaphthalene structure. Brackman et al. (26) analyzed fossilized dammar and suggested the insoluble fraction was made up of a polymerization between triterpenoids and sesquiterpenoid moieties. Van Aarssen et al. (27) have provided the most complete structural identification of the dammar polymer using various pyrolysis techniques. Their results show fossilized resin and recent dammar resin polymer are almost identical, and they proposed a polycadinene structure that fit their pyrolysis data. Spectroscopic data also supported their proposed structure (36). It therefore seems as though a possible 'model' for Utah resinite could be recent dammar resin. (The chemical composition of dammar may vary from sample to sample. In this investigation, the polymeric fraction of dammar is what we are comparing to



fossilized Utah resinite. Although the proportions of the various fractions in dammar may vary, the structure of the polymer should be the same for any dammar sample).

Here we report some aspects of the hydrogenation, dehydrogenation, and cracking chemistry of dammar resin, with particular focus on the reactions of the insoluble, polymeric fraction which could be a model for the reactions of resinite found in Utah coals.

## Experimental

**Samples and Sample Preparation.** Dammar resin was obtained from Sigma. It was used without further treatment for reactions of unseparated dammar. (In the remainder of this Chapter we will use the term "whole dammar" to refer to the material used without an initial separation of the polymeric and low molecular weight components). To precipitate the polymeric component, methanol was added to the whole resin and the white insoluble solid was separated from the soluble fraction by vacuum filtration. The methanol soluble fraction was recovered by rotary evaporation of the methanol. A sample of Utah resinite was obtained by Soxhlet extraction of DECS-6 coal (petrographic data shown in Table I) with hexane for 24 hours.

**Table I. Data for DECS-6**

<b>Seam</b>	Blind Canyon	<b>Petrographic Composition</b>	
<b>State</b>	Utah	<b>Vitrinite</b>	69.1%
<b>ASTM rank</b>	hvA b	<b>Liptinite</b>	17.0%
<b>Mineral Matter</b>	6.7wt%	<b>Inertinite</b>	13.6%

**Reaction Procedure.** All reactions were carried out in 25ml microautoclave reactors. Approximately 4g of resin was loaded into the reactor (catalysts and solvents were not used in this investigation because the reaction chemistry did not want to be complicated further). The reactor was purged with nitrogen twice, and then pressurized to 7MPa with hydrogen. Heating was accomplished by lowering the reactor into a fluidized sand bath preheated to the desired temperature. After a measured reaction time, the reactors were quenched to room temperature by immersing in a cold water bath. The products from the reaction were removed from the reactor with tetrahydrofuran (THF). It was found that in all cases, all products were soluble in THF. Therefore, the THF was removed by rotary evaporation and the reaction products were dissolved in hexane. The insoluble fraction was separated by filtration and the soluble liquid fraction was obtained by rotary evaporation of the hexane. The liquid fraction and the solid fraction were weighed and conversion data were obtained using the original weight of resin in the reaction.

**Analysis of Products.** The liquids produced in the reaction were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 5890II GC coupled with a HP 5791A mass selective detector. Quantitative analysis of the products was performed using a Perkin Elmer 8500 GC. The column used was a 30m x 0.25mm i.d. fused silica capillary column (DB-17) coated with 50% phenyl-50% methylpolysiloxane with a coating film thickness of 0.25 $\mu$ m. The column temperature was programmed from 40 to 280°C at a heating rate of 4°C/min after a 5-min isothermal period. Heptadecane was chosen as the internal standard, with concentration of products being calculated as below.

Concentration of component = (Area component / Area IS) \* concentration IS \* RF.

The solids produced in the reaction were analyzed by solid-state  $^{13}\text{C}$  CPMAS NMR using a Chemagnetics M-100 spectrometer.

## Results

Figure 1 shows the chromatograms of dammar liquefaction products at three different temperatures (see Table II for peak identification). The figure is split into three sections.

1 - C<sub>1</sub>-C<sub>5</sub> benzenes (RT 4-20 minutes).

2 - C<sub>1</sub>-C<sub>5</sub> naphthalenes and indenes (RT 21-45 minutes).

3 - Dimers/trimers of cadinane and high molecular weight triterpenoids (RT 46-90 minutes).

**Table II. Peak Identification for Figures 1, 3, 6**

I toluene	VIII dimethyltetralin	XV C <sub>15</sub> -phenol
II dimethylbenzene	IX dimethylnaphthalene	XVI cadalene
III trimethylbenzene	X dimethylnaphthalene	XVII C <sub>15</sub> -phenol
IV p-cymene	XI C <sub>4</sub> -tetralin	XVIII 5-ring compounds
V C <sub>4</sub> -benzene	XII calamenene	XIX Bidadinanes
VI C <sub>2</sub> -dihydroindene	XIII trimethylnaphthalene	XX ursenal
VII methylnaphthalene	XIV trimethylnaphthalene	XXI ursenal

At 350°C, cracking of the high molecular weight compounds is minimal, with only calamenene (XII) and dimethylnaphthalene (X) being produced, as shown in Figure 1, section 2. It has been reported previously that removal of the isopropyl group along with hydrogen atoms is possible under these conditions (37). This can be seen more clearly at 400°C. Calamenene decreases in concentration, as the concentration of dimethylnaphthalene increases. This reaction proceeds via dehydrogenation of calamenene to cadalene (XVI) which then dealkylated to dimethylnaphthalene. Section 3 of the chromatogram shows cadinane dimers (XIX) and ursenal (XX, XXI). It can be seen that as the temperature increases, the bidadinanes dealkylate to dibenzhydroanthracenes (XVIII). These compounds then crack to produce naphthalenes. Ursenal and other triterpenoids are the likely sources of the C<sub>15</sub>-phenolic compound (XVII) seen at 400°C. At 450°C, the high molecular weight compounds are almost totally cracked to benzenes and naphthalenes. Isomerization of the tetrahydronaphthalenes to C<sub>2</sub>-dihydroindenes (VI) occurs at this temperature, along with ring opening to produce C<sub>1</sub> to C<sub>4</sub> benzenes (I-V). This has been also been observed in previous work by Penninger (38). He demonstrated that tetralin will undergo isomerization and an  $\alpha$ -ring opening mechanism at elevated temperatures and pressures.

Figure 2 shows quantitative data of the product distribution from Figure 1. It can be seen that if a large conversion to bicyclic compounds is to be accomplished, a temperature of 450°C is necessary. With this foundation to work on, the next part of this investigation was to determine how the product distribution varied with reaction time at a fixed temperature.

Figure 3 shows the products of dammar liquefaction at various reaction times. Figure 4 shows quantitative data on the three sections of the chromatograms.

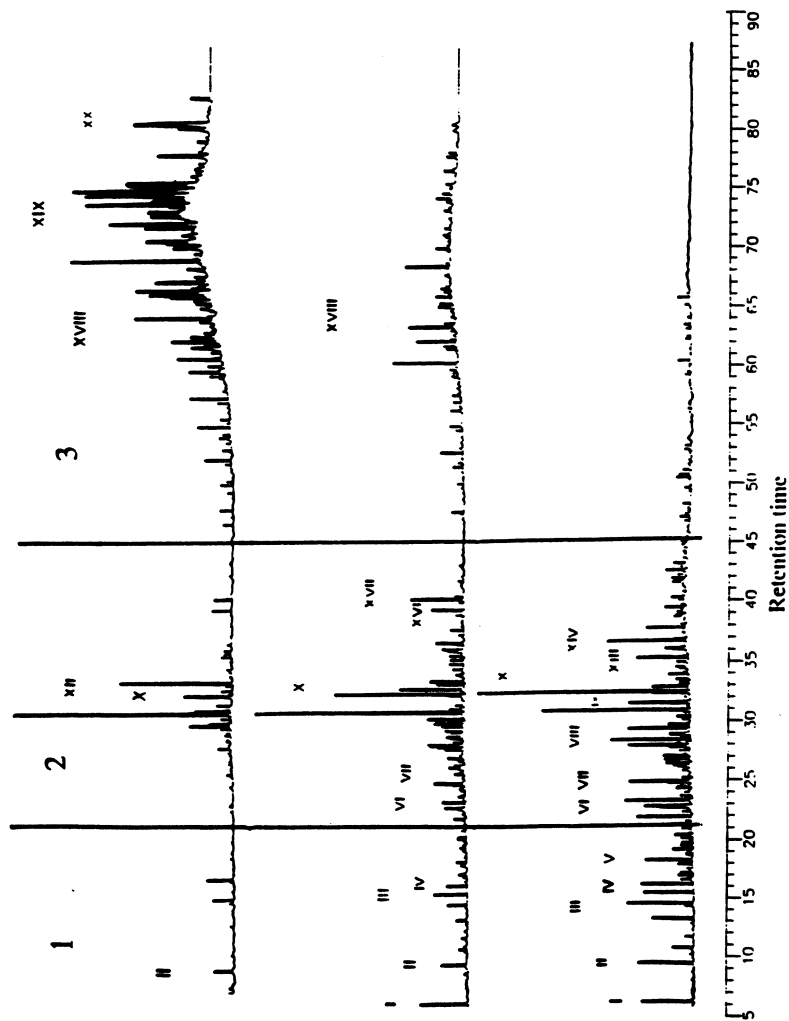


Figure 1. Products from dammar reactions at 350°C (top), 400°C (middle), and 450°C (bottom). Reaction time 60 minutes. H<sub>2</sub> pressure 7MPa.

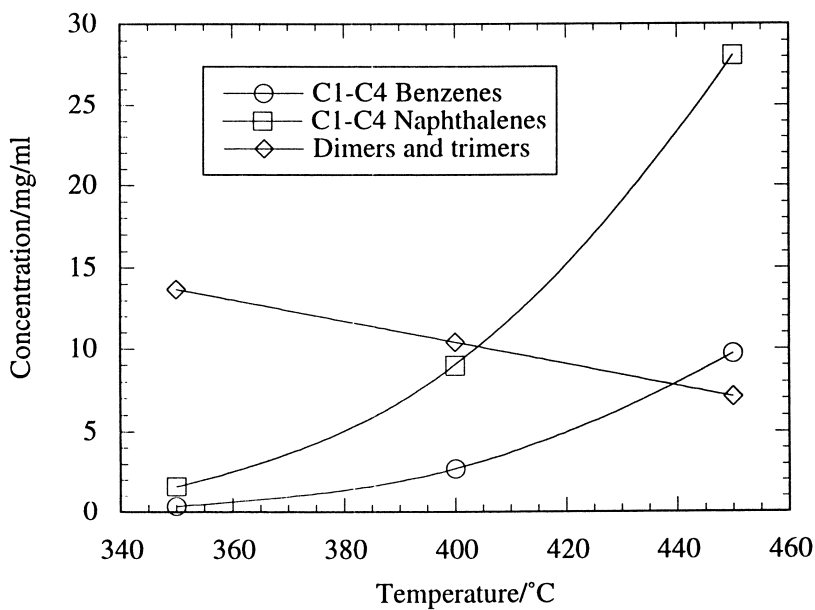


Figure 2. Quantitative data from dammar reactions at various temperatures. Reaction time 60 minutes. H<sub>2</sub> pressure 7MPa.

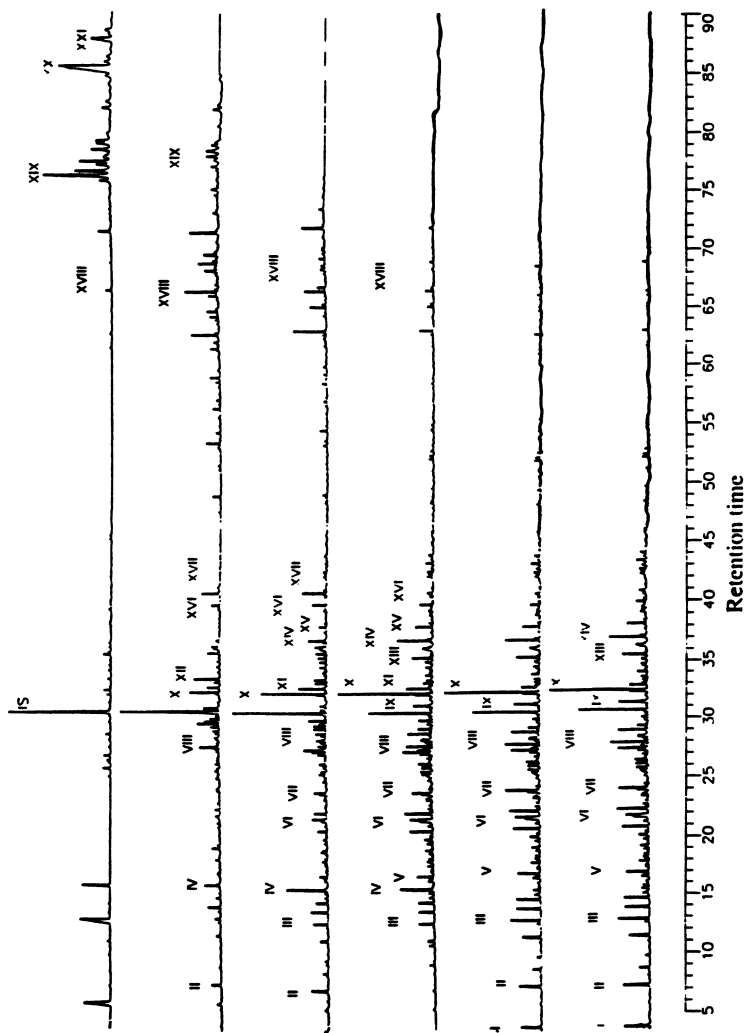


Figure 3. Products from dammar reactions at 450°C for 1, 5, 10, 30, 45, and 60 minutes (descending order). H<sub>2</sub> pressure 7MPa.

After 1 minute, very little reaction has taken place. After 5 minutes, ursenal (XX, XXI) has been converted to lower molecular weight compounds. Exact identification of these compounds is difficult, but the appearance of C<sub>15</sub>-phenols (XV, XVII) at longer reaction times suggests that the triterpenoids are being cracked to sesquiterpenoid compounds. (A C<sub>15</sub>-phenol, 2,6-di-*tert*-butyl-4-methylphenol is added to THF as a stabilizer. The C<sub>15</sub>-phenol observed in the products could not come from THF for two reasons. Firstly, it is not seen in the products from all the reactions and secondly, when the polymeric fraction is separated and reacted, no phenolic compounds are seen. Therefore, they must be coming from the methanol soluble (triterpenoid) fraction). The high molecular weight bicadinanes are being dealkylated to 5-ring compounds (XVIII) after 5 minutes. These are cracked to almost zero concentration after 60 minutes. As the reaction proceeds, calamenene is dealkylated and dehydrogenated to dimethylnaphthalene. Isomerization of tetralins starts to occur after 10 minutes. The data from Figure 2 suggest that most of the cracking reactions are complete after 30 minutes reaction time. Conversion data (Figure 5) show that after this time, solids are starting to be formed in the reactor, with no increase in the conversion to liquids. Given et al. (39) showed resinite could be converted to 59% oils at 350°C, and 73% oils at 400°C. The fact that only a small increase in conversion was seen may be due to solids being produced by retrogressive reactions, e.g. repolymerization and condensation reactions. NMR data on the solids produced show that they are very aromatic, and therefore undesirable products.

Because dammar consists of two discernible fractions, it is difficult to ascertain exact mechanisms by reacting the whole dammar. In order to determine which products are derived from which fraction, the polymeric component of dammar was separated and reactions were run on this component at 450°C for 10 and 60 minutes. Figure 6 shows the products from these reactions. It can be seen that section 3 of the chromatogram of the reaction products from the polymer at 10 minutes is different than the chromatogram for products of the whole dammar reaction. Bicadinanes (XVIIIa) are seen (identification based on mass fragmentation pattern and compared to mass spectra of C<sub>15</sub>-compounds), whereas the 5-ring compounds seen after 10 minutes in Figure 3 are not. These compounds (XVIII, Figure 3) can therefore only come from the triterpenoid fraction. This is backed up by the appearance of C<sub>15</sub>-phenol (XV) after 10 minutes (Figure 3). This compound is from the triterpenoid fraction because it is seen in the methanol-soluble fraction but not in the reacted polymeric fraction. The exact source of the benzenes in the products is difficult to find, because they are tertiary cracking products (i.e., produced from hydrogenated naphthalenes, which themselves are cracking products). Qualitatively, it appears that the majority of benzenes are coming from triterpenoid-derived dibenzohydroanthracenes and naphthalenes. The substituted naphthalenes originate mostly from the polymeric fraction, but it has been reported that pentacyclic triterpenoids can be a source of naphthalene derivatives (40,41).

### Processing Implications and Conclusions

Figure 7 compares the products from dammar liquefaction at 450°C for 60 minutes with Utah resinite at 450° for 60 minutes. It can be seen that they are very similar. If they are to be compared however, the difference in composition of the original materials has to be taken into account. Dammar contains approximately 16% polymeric material, whereas resinite is much more polymerized, with an approximate content of 75-90% polymer. Therefore, the breakdown of the triterpenoids will not be a major source of benzenes and naphthalenes in resinite liquefaction. What will be important is the breakdown of the polymer. From the dammar results, it can be seen that a high temperature is required (450°C), along with a short residence time (30

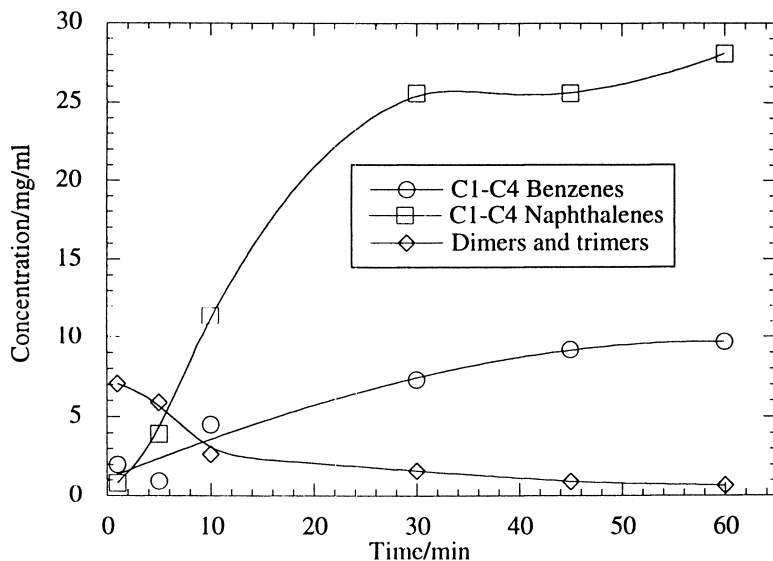


Figure 4. Quantitative data from dammar reactions at various reaction times. Temperature 450°C. H<sub>2</sub> pressure 7MPa.

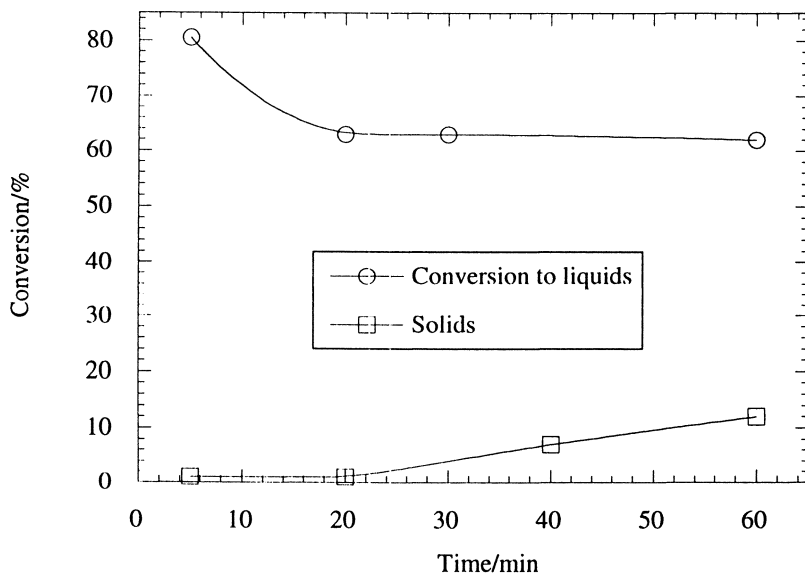


Figure 5. Conversion of dammar to liquids and solids. Temperature 450°C. H<sub>2</sub> pressure 7MPa.

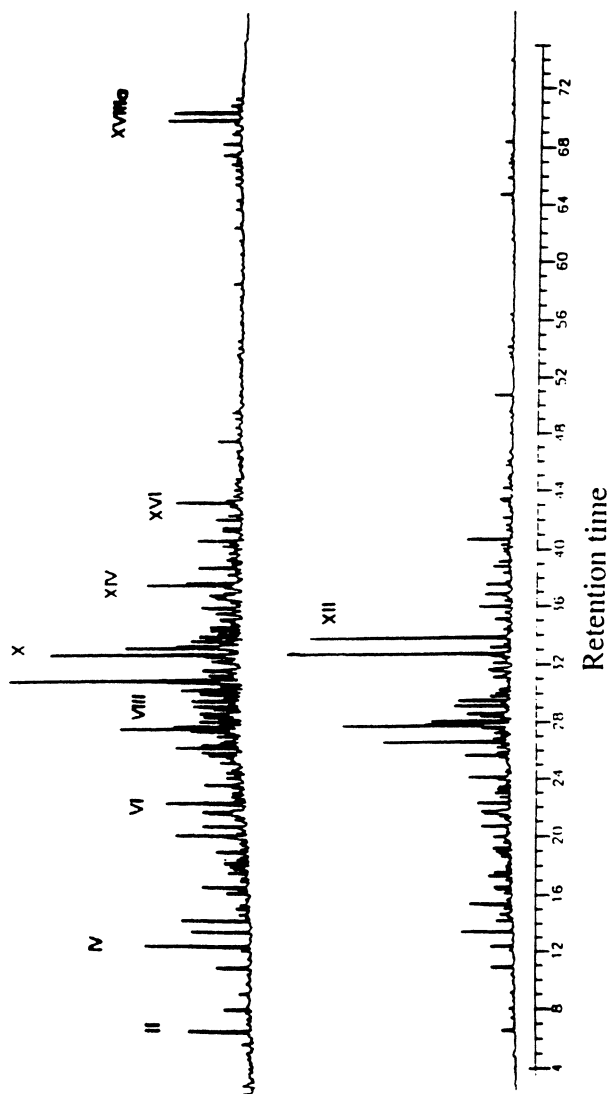


Figure 6. Products from dammar polymeric fraction at 450°C for 10 minutes (top) and 60 minutes (bottom). H<sub>2</sub> pressure 7MPa.



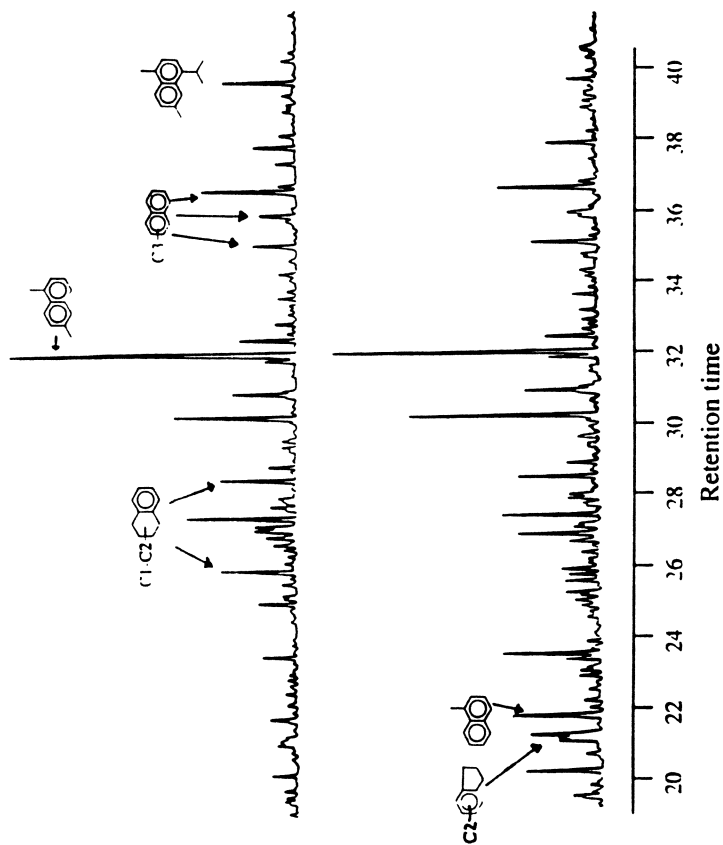


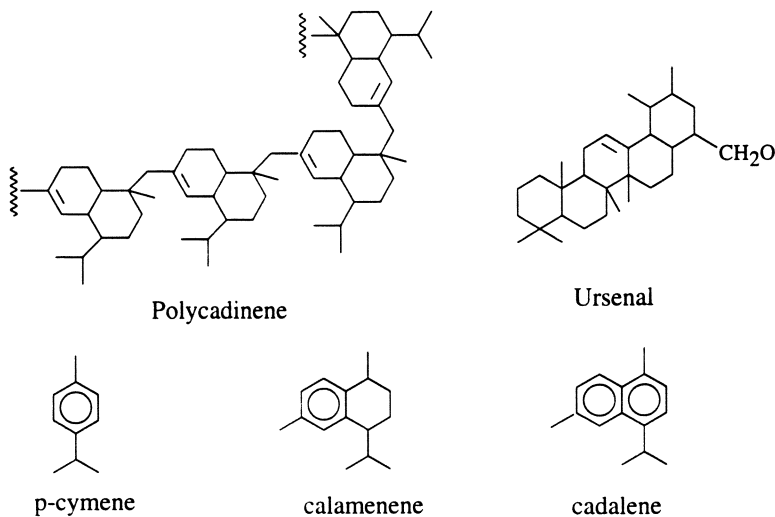
Figure 7. Comparison of DECS-6 resinite (top) and whole dammar (bottom) after reactions at 450°C and 60 minutes. H<sub>2</sub> pressure 7MPa.

minutes). After 30 minutes, most of the cracking reactions are complete. If the reaction is allowed to continue, the quality of the distillate may be affected by aromatization and isomerization. After this high-temperature stage, another reaction stage would be necessary to hydrogenate the naphthalene fraction to tetralins and decalins. Figure 8 shows the products from upgrading dammar liquefaction products after 3 hours hydrogenation with Pd/C catalyst at 300°C. It can be seen that the C<sub>1</sub>-C<sub>3</sub> naphthalenes have been converted to tetralins. The monocyclic fraction did not hydrogenate under these conditions. This temperature strategy is termed reverse-temperature stage programming, and been shown to be effective in other resin hydrogenation experiments (6). This strategy allows the resin to crack/depolymerize at a high temperature, and then, because of the thermodynamic shift to dehydrogenation at this temperature, the temperature is lowered for the hydrogenation (catalytic) stage.

Therefore, if a resin is to be used in the manufacture of advanced jet fuel, several conclusions can be reached from this work. Utah resinite is an excellent source of the types of compounds that are desirable in advanced jet fuels. But careful selection of temperature and reaction time is necessary to produce these desirable compounds in high concentration.

It is not likely that the entire demand for jet fuel, in both commercial and military sectors, could be supplied by liquefaction of resinite. However, our work suggests a possible process strategy in which the resinite can be used advantageously. 'Skimming' coal to concentrate the hydrogen-rich macerals, e.g. by flotation, solvent extraction or selective grinding (42-44), could give a distillate which is of very high quality with respect to thermal stability. The rest of the coal could then be used to produce a transportation fuel where the high-temperature stability is not critical. The process of liquefying resin-rich coals may not have to be as severe as trying to liquefy whole coal with low resinite content, because hydrogen costs could be kept down. Resinite is itself cycloparaffinic in nature, and therefore could serve as a hydrogen-donor solvent for the low H/C ratio vitrinite maceral.

### Structure Key



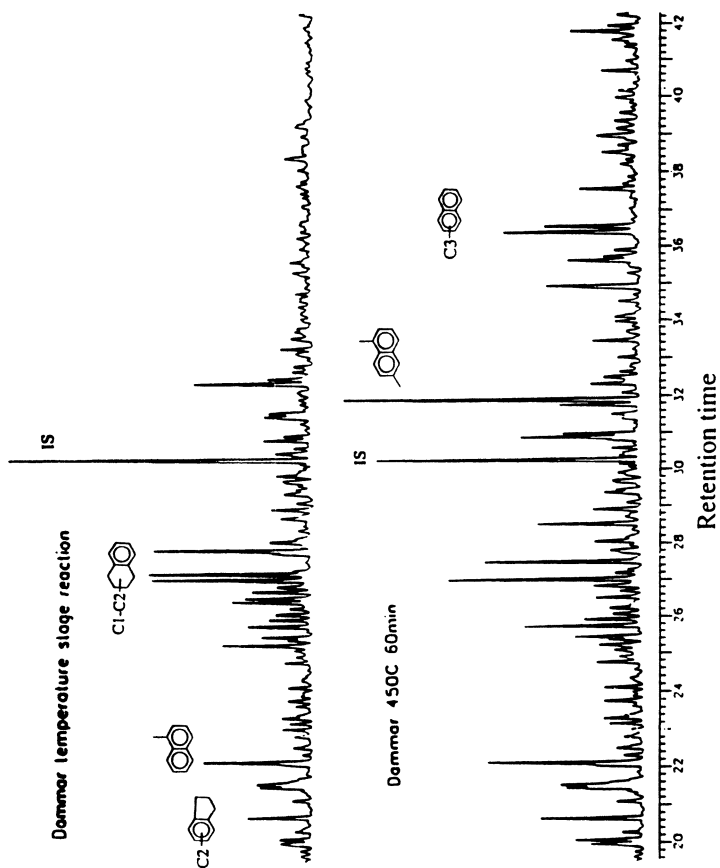


Figure 8. Comparison of products from single-stage dammar hydrocracking at 450° and 60 minutes (bottom) and reverse temperature stage hydrogenation (top). Catalyst Pd/C. Temperature 300°C. Reaction time 3 hours.

## Acknowledgments

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## Chapter 16

# Recovery and Characterization of Macroscopic Fossil Resins from Western Coals

Q. Yu<sup>1</sup>, L. Li, and J. D. Miller

Department of Metallurgical Engineering, University of Utah,  
Salt Lake City, UT 84112

Improved process technologies for the recovery of fossil resins from Utah coal have been developed and include selective flotation of fossil resin from coal and solvent refining of fossil resin concentrate. These technologies are reviewed based on the characteristics features of the resinite and other coal macerals. In this regard, four macroscopic fossil resin types can be distinguished by their color --- light-yellow, orange, light-brown, and dark-brown. The physical and chemical properties of each color type are evaluated and discussed in order to better appreciate the process technologies.

Macroscopic fossil resin or resinite (with grain sizes  $> 0.1$  mm) is a unique resource in the western United States (1,2). Such resinous coals are found in the states of Arizona, Colorado, New Mexico, Utah, Washington, and Wyoming (3,4). Among these, the Wasatch Plateau coal field in south central Utah is particularly significant because of its high fossil resin content. Many seams in this coal field have been reported to contain as much as 5% fossil resin by weight (4).

Fossil resin is friable and easily liberated from other coal macerals. Consequently the fossil resin particles tend to concentrate into the fine sizes during coal preparation and handling. Because of this property, it is not unusual to find that the fine coal streams in a coal preparation plant contain more than 10% fossil resin (hexane-soluble), even when the run-of-mine coal contains 3% fossil resin. Fossil resin obtained from the Wasatch Plateau coal field generally exhibits low density and good solubility in hexane, heptane, and toluene. It has been recovered intermittently from the Utah coal field since 1929 at a small production rate by gravity and/or flotation processes. The fossil resin concentrates thus produced are refined by solvent extraction and finally an acceptable fossil resin product is recovered by the evaporation of the solvent. This fossil resin product has a market value of \$0.50-0.70/lb (depending on its color and softening point) as a chemical additive used in the adhesives, rubber, varnish, paints, coatings, and thermoplastics industries, and particularly in the ink industry (5-7). A brief chronology of the Utah fossil resin industry is given in Table 1. Since 1992 there has been no production.

<sup>1</sup>Current address: USG Corporation, Research Center, 700 North Highway 45,  
Libertyville, IL 60048

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Table 1. Historical Review of the Utah Fossil Resin Industry

Year	Company	Concentration Process	Refining Process
1930s	Andriaan Nagelvoort	Sink/Float	Toluene Extraction
1940s	Combined Metals Corp. I.P.I.	Gravity Flotation	Hexane Extraction
1973	U.S. Resin Corp. Hercules Powder Co. (Blackhawk Resin)	Flotation Flotation	Hexane Extraction Hexane Extraction
1983	American Resins	Flotation	Hexane Extraction
1985	US Fuel, CPS	Flotation	Hexane Extraction

Due to the lack of technology for the efficient recovery of fossil resins from coal and competition from synthetic resins, this valuable resource has been wasted, being burned together with coal for electric power generation. It is estimated that the fossil resin from the Wasatch Plateau coal field which is burned each year for electric power generation has a chemical value of \$200 million - equivalent to the value of the coal itself. Continual development of other technologies in the 80's has gradually brought attention to the utilization of fossil resins as a chemical commodity because of their unique properties.

For example, solvent-refined fossil resins from the Utah coal field can be used as a feed stock in the ink industry (5). These fossil resins have thermosetting properties superior to most synthetic resins available from the petrochemical industry. This feature has become of particular significance today due to the development of high speed printing. As another example, fossil resins in coal have a higher BTU value than other coal macerals and are the easiest to be liquefied (8). It has been suggested that these fossil resins could have special value as a feed stock for high-density jet fuels by proper hydrogenation.

### CHARACTERIZATION OF MACROSCOPIC FOSSIL RESIN TYPES

Macroscopic fossil resin types obtained from the Wasatch Plateau coal of central Utah vary in color from light-yellow to dark-brown and they exhibit a natural hydrophobicity (9,10). It appears that these fossil resins are derived from terpenoid plant resins and are associated with other coal macerals in the coal seams (11-15). It has been found that these fossilized terpenoids are of predominantly aliphatic character when compared to normal bituminous coal (15,16). Four macroscopic fossil resin types were distinguished by their color --- light-yellow, orange, light-brown, and dark-brown --- and were physically isolated by hand-sorting. The differences among the four hand-sorted fossil resin types have already been recognized and quantitatively categorized on the basis of their major bulk and surface characteristics (9,10,15).

**Bulk Characteristics.** Some typical bulk characteristics of the four fossil resin types and the parent coal are listed in Table 2. As seen from Table 2, the ash contents of all fossil resins are extremely small compared to that of coal. The fossil resins are considered to contain hydrocarbon compounds which are highly volatile. The volatile matter contents decrease gradually from 100.0% for the light-yellow fossil resin to 96.9% for the dark-brown fossil resin. On the other hand, coal consists of about half (44.4%) volatile matter, much less than that for fossil resins. The only physical property that appears to remain

Table 2. Some bulk characteristics of four resin types and the parent coal (9,10)

Fossil Resin Type	Ash Content* (wt.%)	Volatile Matter* (wt.%)	Softening point (°C)	True Density (g/cm <sup>3</sup> )	Hexane Solubility (wt.%)
Light-Yellow	0.0	100.0	169-174	1.037	100.0
Orange	0.0	99.1	168-172	1.042	98.1
Light Brown	0.0	97.9	168-170	1.049	92.6
Dark Brown	0.2	96.9	168-172	1.054	88.2
Parent Coal	4.5	44.4	-----	1.293	0.2

\* Dry, ash free basis;

virtually constant among the four resin types is the softening point (168-174 °C). In this regard, softening points were not expected to yield any significant information to distinguish the resin types. However, the softening point of fossil resin plays a very important role in its utilization for printing inks, coatings, and varnishes. For a certain range of temperatures, the economic value of fossil resin increases with an increase in softening point (5,6).

Results from true density measurements reveal some small differences among the four fossil resin types and the density is found to range from 1.037 g/cm<sup>3</sup> for light-yellow fossil resin to 1.054 g/cm<sup>3</sup> for dark-brown fossil resin. These values increase monotonically from light-yellow through orange to light- and finally dark-brown fossil resins. Coal exhibits a significantly greater density (1.293 g/cm<sup>3</sup>) than fossil resins which might be explained by two factors 1) the difference in chemical structure (coal consists mostly of aromatic compounds while fossil resins are composed mostly of aliphatic compounds) and 2) the mineral matter content of the coal is greater than that of the fossil resins. Further, the hexane solubility of the fossil resins is found to gradually decrease from light-yellow to dark-brown fossil resin (Table 3). The light-yellow fossil resin is completely soluble in hexane, the dark-brown fossil resin has a solubility of only 88.2%. Coal is essentially insoluble in hexane (~0.2%).



With respect to elemental analyses of the fossil resin types and the parent coal some trends are also observed among these four fossil resin types (10,15). It appears that, the carbon content increases from light-yellow to dark-brown fossil resin, and that the hydrogen content decreases in the same sequence. The molecular weight and aromaticity of the four fossil resin types were shown to increase in the following order light-yellow < orange < light-brown < dark-brown fossil resin (10). The color difference observed among the four hand-sorted fossil resin types is explained in part by the presence of finely dispersed coal inclusions in the fossil resin matrix (10,15).

**Surface Characteristics.** Two typical hydrophobic properties of the four fossil resin types and the parent coal, contact angle and vacuum tube flotation response, have been determined (9,17) and the results are given in Table 3. It is found that all fossil resin types

Table 3. Surface hydrophobicity of the four fossil resin types and the parent coal

Fossil Resin Type	Advanced Contact Angle (degree)	Reagentless Vacuum Flotation Response (wt.% float)
Light-Yellow	100.8	97.6
Orange	99.2	84.5
Light Brown	92.8	60.8
Dark Brown	90.9	34.8
Parent Coal	57.1	11.5

exhibit significantly higher hydrophobic character than the parent coal as evidenced from their greater contact angle and higher vacuum tube flotation yield. The four fossil resin types show distinct difference in their hydrophobicities which are found to gradually increase as fossil resin color becomes lighter. Among the four fossil resin types, the light-yellow fossil resin exhibits the highest hydrophobicity with a contact angle of 100.8°. At the other extreme the dark-brown fossil resin has the lowest hydrophobicity with a contact angle of 90.9°. Of course, the coal also exhibits a certain degree of hydrophobicity with a contact angle of 57.1°. It should be noticed that the hydrophobic difference between fossil resin types and coal is found to gradually decrease as fossil resin color becomes darker.

Generally, in the conventional flotation process the greater the hydrophobic difference between fossil resin and coal the easier the separation will be. As revealed from the reagentless vacuum tube flotation response it is expected to be more difficult to separate dark-brown fossil resin from coal by conventional flotation. In addition, due to the small differences in their native hydrophobic character, heterocoagulation takes place between fossil resin and coal particles in suspension in order to minimize the system's

interfacial energy. Such an aggregation cause fine coal to float along with fossil resin particles into froth phase during flotation process, and consequently a fossil resin concentrate of a poor grade was obtained by conventional flotation (9,17,18).

The reagentless flotation rate or floatability from vacuum tube flotation tests was found to follow the order of light-yellow fossil resin > orange fossil resin > light-brown fossil resin > dark-brown fossil resin > coal as revealed from their flotation yield (Table 3). These results indicate that the natural floatability gradually increases as the fossil resin color becomes lighter. Coal, in comparison with the fossil resins, is shown to have a relatively low floatability under the same conditions. For example, within two minutes, almost all of the light-yellow fossil resin particles (97.6%) floated while a small amount of dark-brown fossil resin particles (34.8%) and some coal particles (11.5%) floated. It appears that in order to recover dark-brown fossil resin, either a greater flotation time or the addition of flotation reagents is required. However, with increasing flotation time or reagent addition, more coal particles are floated into the froth product, and again the separation efficiency decrease (9,17,19).

The four fossil resin types were also observed to exhibit a distinct difference in the floatability during a time release bench flotation of a composite fossil resin sample (9,17,19,20). The color of the froth products was found to gradually increase from yellow at the beginning of the flotation sequence to dark brown at final stage of flotation. This gives additional evidence that during the flotation process light-colored fossil resins (light-yellow or orange), which exhibit high degree of floatability, float first and faster than the dark-colored fossil resins (light-brown and dark-brown). In the later stages of flotation some coal particles were found to report to the froth product (9,17,19).

## SELECTIVE FLOTATION OF FOSSIL RESIN FROM COAL

Because both fossil resin and its parent coal display a distinct hydrophobic character and because the difference in their hydrophobicities is small, a heterocoagulation occurs between resin and coal particles in suspension due to van der Waals attraction and hydration forces (9). Consequently, most conventional flotation techniques for the recovery of fossil resins from coal have not been particularly successful. Selective separation of fossil resin from coal is difficult with conventional flotation reagents and a multi-stage flotation process is usually required to produce a fossil resin concentrate of modest quality (20).

During the past several years, significant research efforts have been made to develop process technology for the selective flotation of fossil resin from western coals. As a result of these efforts, several new flotation technologies have been developed and a number of papers and research reports have been published (9,17,20-22). Three U.S. patents which describe the new flotation technologies have been granted (18,19,23). Improved fossil resin flotation technologies which have been developed by researchers at the University of Utah are summarized in Table 4. Of these, selective fossil resin flotation by pH control is the most cost effective process (17,18,20). In this process, the pH of the resinous coal suspension is adjusted with lime to a high pH typically above pH 11, followed by addition of a suitable frother and the selective flotation of fossil resin carried

out for the necessary retention time. In one example, a concentrate containing 72% fossil resin at 90% recovery was obtained at pH 12 by single-stage flotation from a feed containing approximately 7% fossil resin. By contrast, flotation at neutral pH can only produce a product containing 20~30% fossil resin at about the same level of fossil resin recovery. This fossil resin separation technology is based on the findings that the heterocoagulation between fossil resin and coal particles, which contributes to the inefficiency of fossil resin separation from coal, can be controlled by pH adjustment. In this regard, the state of dispersion and coal hydrophobicity can be controlled for selective fossil resin flotation if the pH is adjusted to an appropriate level, between pH 8 and 12, depending on the resinous coal type and previous treatment (9,19,20).

The results from pilot-plant testing of two Utah resinous coal samples (CO-OP Mines and UP&L Mines) have demonstrated the success of this new flotation technology for the potential development of a fossil resin industry in the western US (17). Overall, the pilot-plant testing has shown that the new flotation technologies developed at the University of Utah provide a highly efficient means for the selective recovery of fossil resin from coal as demonstrated by a continuous flotation circuit (about 0.1 tph) which resulted in fossil resin recovery with the same separation efficiency as was obtained in laboratory

Table 4. Process strategies for the recovery of fossil resins from coal by froth flotation

---

**Conventional Flotation (~minus 28 mesh)**

Conditioning -- conventional reagent schedule

Flotation -- Fossil Resin Concentrate Product

Grade 50% and Recovery 50%

Product will have to be solvent refined prior to utilization.

**pH Controlled Flotation Technology (~minus 28 mesh)**

Conditioning -- new reagent schedule

Flotation -- Fossil Resin Concentrate Product

Grade 80% and Recovery 80%

It is expected that product will have to be solvent refined prior to utilization.

**Column Flotation with Wash Water Chemistry Control (~minus 28 mesh)**

Conditioning -- wash water chemistry control and new reagent schedule

Column Flotation -- Fossil Resin Concentrate Product

Grade 90% and Recovery 80%

Direct use of the product may be possible without further refining.

**Ozone Process (~minus 200 mesh)**

Conditioning -- Pregrinding and Ozonation

Flotation -- Fossil Resin Concentrate Product

Grade 95% and Recovery 80%

Direct use of the product may be possible without further refining.

---

bench-scale testing (> 80% recovery at ~ 80% concentrate grade). Another selective fossil resin flotation technology was developed with column flotation (19). Of particular interest in column flotation is the opportunity to operate the system by control of the wash water chemistry and under these conditions excellent separation efficiencies can be achieved.

## SOLVENT REFINING OF FOSSIL RESIN CONCENTRATES

Because light-colored fossil resin is preferable and is of greater commercial value than the dark-colored fossil resins, particularly in the ink industry, solvent refining is a necessary step to purify fossil resin concentrates and produce a light-colored fossil resin product as a chemical commodity (5,6,15,24). For some time, hexane and other solvents have been known to selectively dissolve fossil resins from other coal macerals and based on this property hexane extraction for refining of fossil resin concentrates has been practiced industrially on a small scale beginning in the 1940's (see Table 1). However, the quality of the solvent refined fossil resin product can vary considerably depending on the refining practice and quality control of the final fossil resin product is of concern.

It is evident that a major portion of the composite fossil resin from the Wasatch Plateau coal appears dark-brown in color and contains inclusions of what appear to be fine coal particles (10,15). Thus solvent refining is required to remove the fine coal inclusions and dark-color inducing compounds (hexane insoluble) from fossil resin concentrates in order to produce a premium fossil resin product.

**Solvent Extraction of Fossil Resin by Hexane/Toluene.** Of particular concern in this step is the natural color of the refined fossil resin product. Preliminary studies indicate that two major factors contribute to the natural color of fossil resins: 1) relative abundance of chromophores (mostly heteroatoms and conjugated double bond structures); and 2) finely dispersed inclusions of coal macerals (15). Since these compounds vary in their properties such as solubility, polarity, and molecular weight, they can be recovered by solvent extraction and evaporation of the solvent after filtration of the insoluble residue.

It is found that most of the fossil resin concentrate is hexane soluble and shows a yellow color, low density, high volatile matter, and low molecular weight, while the hexane insoluble but toluene soluble fossil resin appears dark in color and has a slightly lower volatile matter, higher density, and higher molecular weight (15). The toluene insoluble residue resembles coal and has a relatively high density and ash content and very low volatile matter.

The GC-MS and FTIR studies (15) also demonstrate that fossil resins consist generally of three major components: 1) sesqui- and tri-terpenoid (monomers, dimers and trimers); 2) some content of alcohols, ketones, and acids; and 3) a small amount of increasingly aromatized hydrocarbons.

**Kinetics of Fossil Resin Extraction by Heptane.** Both solvent type and extraction kinetics have a significant impact on the final fossil resin product (15,25). It is evident that the extraction rate decreases with an increase in extraction time and that there is a strong dependence of fossil resin extraction rate on particle size (25). The heptane extraction rate was found to decrease dramatically with an increase in particle size. For example, after 40 minutes of extraction, the percent dissolved was approximately 20% for the 8x10 mesh fossil resin particles while about 80% dissolved for the 100x150 mesh fossil resin particles.

After 2 hours of extraction, the 100x150 mesh sample had reached 92% of its ultimate extraction whereas only 40% had been extracted from the 8x10 mesh sample. The rate shows an inverse 1st-order dependence on particle size and the results suggest that the extraction rate, at dilute solids concentration, is limited by a surface reaction mechanism involving the solvation of resin molecules by the heptane solution (25).

The fossil resin extraction rate was found to increase with an increase in extraction temperature (25). The effect of temperature is very significant. A study of heptane extraction for the 48x60 mesh fossil resin sample indicated that almost complete extraction was observed in 20 minutes at 60 °C while only 25% extraction was observed at 0 °C. An activation energy of 15.5 kJ/mole was obtained based on the initial extraction rates (25). In view of these results, a moderate extraction temperature should be considered for continuous extraction circuits in order to establish reasonable retention times.

**Precipitation of Ultra Pure Fossil Resin Product.** As mentioned previously, colorless or nearly colorless fossil resins demand a premium price in the marketplace. The economic value of fossil resins can be dramatically enhanced if they are decolorized without affecting their other properties. The color of the resins can be attributed, in part, to the presence of various chromophores that are present even in refined resins. Among these chromophores are the conjugated olefinic linkages in association with such functional groups as carbonyl, amine, and thionyl groups (15,24,25). The oxygen and nitrogen content of fossil resin is small but significant, and the presence of carbonyl and amine groups has been verified by infrared characterization studies (15).

Because of the differences in molecular structure, molecular weight, and polarity of the chemical components which constitute fossil resin preferential precipitation and/or crystallization occurs among these components when the polarity of the solvent is adjusted. Under certain conditions colorless fossil resin compounds can be caused to precipitate and/or crystallize from the solvent. Depending on the solvents used and conditions of precipitation and/or crystallization (temperature and agitation intensity) the colorless and/or nonpolar resin compounds will precipitate and/or crystallize first and such a fashion of an ultra pure fossil resin product can be prepared.

## SUMMARY AND CONCLUSIONS

Improved process technologies has been developed for the efficient recovery of fossil resins from Utah coal and include selective flotation of fossil resin from coal and solvent refining of fossil resin concentrates. However, the development of a fossil resin industry still remains uncertain. Eventhough significant advances in process technology have been made, further economic analysis is required to establish the extent of the resource and the market potential.

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