POPPY

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POPPY

The Genus *Papaver*

Edited by

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FOREWORD

Extracts from the opium poppy *(Papaver somniferum)* have been used by man to relieve pain for at least 3500 years. The use of soporific tea of poppy capsules has also long been known in Europe, perhaps ever since the beginning of poppy cultivation. Opium is the air-dried sap of the incised unripe capsule of the poppy. The traditional production of opium still employed today, consists of incising the poppy seed capsule, and collecting and drying the extruded juice. Manufacture of morphine and other important alkaloids has been performed from opium for many years. Opium on the other hand, is a dangerous narcotic drug; it has high morphine content and opium can produce addiction by itself in the absence of any chemical conversion. Nowadays India is the only country in which the cultivation of opium poppies for opium is still legal.

The commercial manufacture of morphine alkaloids direct from the poppy plant was achieved in the 1920s when a young Hungarian pharmacist János Kabay devised a method for the production of morphine from poppy straw. In 1927, he founded a small chemical company with the name of 'Alkaloida'. The extraction of morphine became a 'by-product industry', as the poppy was already cultivated in Hungary for its edible and oil-producing seed. The advantage of utilization of poppy capsules is that the capsules are virtually useless as a raw material for illegal manufacturers, and they should therefore be considered quite differently from opium for control purposes. The introduction of straw processing has given a great impulse to the manufacture of morphine alkaloids during the past decades, not only in Europe but also throughout the world; because this process is economical, and because poppy straw cannot give rise to any abuse. At present, more poppy straw than opium is used for opiate manufacture in the world. There are large manufacturers in Europe (Macfarlan Smith, Boots, Francopia, Salars, etc.), in America (Mallincrodt, Penick, etc.), and in Australia (Glaxo, Extal, etc.) using straw or its concentrate as a raw material. ICN Alkaloida Hungary Company Limited—based on Kabay's traditional process—even now plays an important role in the morphine producers of the world.

This book presents the majority of the most important scientific results concerning the poppy plant. The information proves that the processing of poppy became a large international business, which is well supported by outstanding biological, chemical, technological novelty coming from many regions of the world. It is a great honor for me that since the time of Kabay's invention, Hungarian scientists have played a major role in both the scientific and industrial development of this branch of the pharmaceutical industry contributing to its world-wide advancement.

Lászlo Dupcsák
Executive VP and General Manager
ICN Alkaloida Hungary Co. Ltd

PREFACE TO THE SERIES

There is increasing interest in industry, academia and the health sciences in medicinal and aromatic plants. In passing from plant production to the eventual product used by the public, many sciences are involved. This series brings together information which is currently scattered through an ever increasing number of journals. Each volume gives an in-depth look at one plant genus, about which an area specialist has assembled information ranging from the production of the plant to market trends and quality control.

Many industries are involved such as forestry, agriculture, chemical, food, flavour, beverage, pharmaceutical, cosmetic and fragrance. The plant raw materials are roots, rhizomes, bulbs, leaves, stems, barks, wood, flowers, fruits and seeds. These yield gums, resins, essential (volatile) oils, fixed oils, waxes, juices, extracts and spices for medicinal and aromatic purposes. All these commodities are traded world-wide. A dealer's market report for an item may say "Drought in the country of origin has forced up prices."

Natural products do not mean safe products and account of this has to be taken by the above industries, which are subject to regulation. For example, a number of plants which are approved for use in medicine must not be used in cosmetic products.

The assessment of safe to use starts with the harvested plant material which has to comply with an official monograph. This may require absence of, or prescribed limits of, radioactive material, heavy metals, aflatoxin, pesticide residue, as well as the required level of active principle. This analytical control is costly and tends to exclude small batches of plant material. Large scale contracted mechanised cultivation with designated seed or plantlets is now preferable.

Today, plant selection is not only for the yield of active principle, but for the plant's ability to overcome disease, climatic stress and the hazards caused by mankind. Such methods as *in vitro* fertilisation, meristem cultures and somatic embryogenesis are used. The transfer of sections of DNA is giving rise to controversy in the case of some enduses of the plant material.

Some suppliers of plant raw material are now able to certify that they are supplying organically-farmed medicinal plants, herbs and spices. The Economic Union directive (CVO/EU No 2092/91) details the specifications for the obligatory quality controls to be carried out at all stages of production and processing of organic products.

Fascinating plant folklore and ethnopharmacology leads to medicinal potential. Examples are the muscle relaxants based on the arrow poison, curare, from species of *Chondrodendron,* and the antimalarials derived from species of *Cinchona* and *Artemisia.* The methods of detection of pharmacological activity have become increasingly reliable and specific, frequently involving enzymes in bioassays and avoiding the use of laboratory animals. By using bioassay linked fractionation of crude plant juices or extracts, compounds can be specifically targeted which, for example, inhibit blood platelet aggregation, or have antitumour, or antiviral, or any other required activity. With the assistance of robotic devices, all the members of a genus may be readily screened. However, the plant material must be fully authenticated by a specialist.

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The medicinal traditions of ancient civilisations such as those of China and India have a large armamentaria of plants in their pharmacopoeias which are used throughout South East Asia. A similar situation exists in Africa and South America. Thus, a very high percentage of the World's population relies on medicinal and aromatic plants for their medicine. Western medicine is also responding. Already in Germany all medical practitioners have to pass an examination in phytotherapy before being allowed to practise. It is noticeable that throughout Europe and the USA, medical, pharmacy and health related schools are increasingly offering training in phytotherapy.

Multinational pharmaceutical companies have become less enamoured of the single compound magic bullet cure. The high costs of such ventures and the endless competition from me too compounds from rival companies often discourage the attempt. Independent phytomedicine companies have been very strong in Germany. However, by the end of 1995, eleven (almost all) had been acquired by the multinational pharmaceutical firms, acknowledging the lay public's growing demand for phytomedicines in the Western World.

The business of dietary supplements in the Western World has expanded from the Health Store to the pharmacy. Alternative medicine includes plant based products. Appropriate measures to ensure the quality, safety and efficacy of these either already exist or are being answered by greater legislative control by such bodies as the Food and Drug Administration of the USA and the recently created European Agency for the Evaluation of Medicinal Products, based in London.

In the USA, the Dietary Supplement and Health Education Act of 1994 recognised the class of phytotherapeutic agents derived from medicinal and aromatic plants. Furthermore, under public pressure, the US Congress set up an Office of Alternative Medicine and this office in 1994 assisted the filing of several Investigational New Drug (IND) applications, required for clinical trials of some Chinese herbal preparations. The significance of these applications was that each Chinese preparation involved several plants and yet was handled as a **single** IND. A demonstration of the contribution to efficacy, of **each** ingredient of **each** plant, was not required. This was a major step forward towards more sensible regulations in regard to phytomedicines.

My thanks are due to the staff of Harwood Academic Publishers who have made this series possible and especially to the volume editors and their chapter contributors for the authoritative information.

<div align="right">Roland Hardman</div>

PREFACE

Looking back into the history of mankind, the development of therapy was promoted by the activity of some outstanding physicians and scientists throughout the ages who collated and expanded previous traditional knowledge. Egyptian medicine may be considered to be one of the first basic sources which affected European therapy for centuries afterwards.

The basics of pharmaceutical botany—which later determined Roman therapy—were laid down by the Greek physicians Hippocrates 'Father of Medicine' (460–357 BC) and Theophrastus 'Father of Botany' (370–285 BC). Dioscourides, in his work known as *De Materia Medica* (78 BC), compiled a complex system of medicines. Galenus (131 BC), incorporating previous works, described a system consisting of 473 species of plants, different minerals (lead, copper, iron, sea salt, sulphur, etc.) and preparations made of animal products (milk, whey, butter, fats, testicle, egg, blister-beetle, etc.). The number and form of medicines have changed from those times—some have disappeared for ever, some exist even now. The opium poppy *(Papaver sommferum)* is an example of a medicine used in all the therapeutic systems mentioned above, appearing in the first records, applied afterwards in the Middle Ages, and still used today.

As a result of the development of chemistry and biological sciences in the eighteenth century, the introduction of new medicines and therapeutic methods began. The isolation of active compounds of widely used plants, especially that of morphine from opium, started a new age in the development and application of medicines. Since that time hundreds and hundreds of plants used traditionally have given us valuable chemical compounds for use in medicine. The poppy has never lost its therapeutic and economical importance.

I have an emotional personal bond to the plant. Beyond the fact that Hungarian people use poppy seeds as a delicious food supply in large amounts, it was the Hungarian pharmacist János Kabay whose activity had a great effect on the development of modern opiate production. My first scientific work was concerned with poppy. I have researched this plant for 30 years, and two of my cultivars are produced on a large scale in Hungary.

This book, *Poppy—The Genus Papaver* is the third volume in the Medicinal and Aromatic Plants book series and our idea was to gather together the majority of the up-to-date information on poppy and related species. There is much information available on poppy in the published literature, however, the taxonomy and botanical descriptions of the genus given in this volume contain some new aspects, e.g. special morphology and structural differentiation of the genus. A thorough evaluation concerning the physiology of poppy and regional differences in poppy raw production is given here. In addition to a detailed analysis of ecological factors affecting the accumulation of opiates, the basic methods of both illicit and licit cultivation are also discussed. It is thought that this is the first occasion of the presentation of a complex evaluation of poppy production in Australia. Information on the pharmacological effectiveness and application of opiates and the international control of their processing and trade are discussed. Biotechnological and chemical data of poppy are presented in detail.

The aim of this book series and this volume on the poppy is to provide a wide range of information to arouse the further interest of different kinds of experts and professionals and any other people interested in the plants covered.

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I.   INTRODUCTION

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1 APPEARANCE OF POPPY IN ANCIENT CULTURES

Poppy seems to be one of the few species which was utilized, even cultivated, in prehistoric times. However its origin is not yet known conclusively and there are different statements in the literature concerning its first appearance. Until the 1930s *Papaver setigerum* D.C. was accepted as the first ancestor. Based on the native distribution of this species the Mediterranean area was thought to be its place of origin and this idea was supported by discoveries of fossilized remains. Analysis of the remains from the Neolithic age verified that poppy was known to cavemen living in the territories of Spain, France, Germany and Hungary 4–5 thousand years BC. This infers that some form of poppy was widely known and utilized in ancient times (Tétényi, 1997).

On the basis of genetic investigations (Hrishi, 1959) large differences between *P. setigerum* and the cultivated poppy have been proven. The gene centres of the two species were distinguished by the results of botanical expeditions. It seems to be accepted that the gene centre of the cultivated poppy is outside Europe, located in Middle Asia, especially in the territories of India, Iran and Afghanistan.

From written historical records, the gene centre of poppy was thought to be located in Western Asia (Simmonds, 1976). This theory is also supported by early historical data which show ritual and therapeutic-like applications of the plant in this area even in ancient times. The name of the plant appears in classic literature, for example, in Homers *Odyssey* and *Iliad*. Kritikos and Papadaki (1967a) summarized the historical evidence and concluded that the Greeks portrayed their divinities Hypnos, Nyx and Thanatos with poppies. The first written record has been dated as early as the eighth century BC. In the Corinth region there was a city named Mekone or Poppy-town. Some scientists believe the name of the town may reflect the fact that extensive poppy cultivation took place, while others think that the town was thus named as it was the place of the first discovery of the plant. In early records the plant was mentioned as a tool for an easy and painless death. Hippocrates (460–377 BC) was one of the first who emphasized the medical advantages of the poppy and its preparations. He mentioned that the plant was frequently used in medicinal preparations in unripe, ripe and baked forms. He also described poppy juice as a hypnotic, narcotic, styptic and cathartic agent. The nutritive property of the seeds was also recognized by him. Herakleides (340 BC) reported that the plant was used as a way to carry out euthanasia on some Greek islands—women in particular took poppy to shorten the time left until natural death.

At the time of Dioskorides (first century AD) a lot of information had already been accumulated on the applications and 'taxonomy' of poppy. Several kinds of poppy were distinguished by him. The 'cultivated' or 'garden' poppies were used in baking bread. Two types were known in this category: plants with elongated capsules and white seeds; and plants forming involuted and elongated capsules with black seeds. From present botanical knowledge this category can be stated to be the species *Papaver somniferum*. The next group named 'flowering' poppy showed strong hypnotic properties and may refer to species of *Papaver hybridum*. The 'wild' poppy group may be equivalent to species known today as *Papaver orientale*. Later, Pliny mentioned an 'intermediate' type between the 'wild' and 'cultivated' poppy—*Papaver rhoeas*. In medical applications the juice of the plants (leaves and capsules boiled in water), tablets (leaves and capsules pressed, rubbed) and opium (dried dew drops) were utilized. These types of medicines were taken to induce sleep, aid digestion and relieve coughs and stomach troubles. As an adverse reaction of overdose lethargic sleep and mortal effects were mentioned.

The curing system of Galen (second century AD) which had a great influence on the development of subsequent European therapy, included many different application forms of poppy. He stated:

"Opium is the strongest of the drugs which numb the senses and induce a deadening sleep; its effects are produced when it is soaked in boiling water, taken up on a flock of wool and used as a suppository; at the same time some can be spread over the forehead and in the nostrils. If it is mixed with a drug that mitigates its power, its effects are greatly reduced."

The opinion that poppy was unknown to the early Egyptians must be reconsidered. However, there are contradictory statements about the exact time of its actual appearance. Some scientists state that the plant was introduced from abroad, particularly from Greece and Babylon. The time of the introduction coincides with the period shortly before Roman times. From the data of Gabra (1956) an oleaginous ointment was found in a tomb from the XVIIIth Dynasty. It was also noted by Gabra that flowers and pieces of *Papaver rhoeas* were frequently found in tombs and on monuments from the time of the above mentioned Dynasty. There is a further evidence of the existence of poppy in Egyptian times in the Ebers papyrus (1500 BC). In the opinion of many scientists, poppy is mentioned in this document under the name of seter-seref. However the first data on large-scale cultivation and preparation of opium were registered only in the Egyptian Thebas.

There is no doubt about the evidence that the poppy was also cultivated by Sumerians, Babylonians and Assyrians about 3–6 thousand years BC. On the clay tables of the Sumerians the production method of poppy juice was described. It was collected very early in the morning, was called 'gil' and it was used for curing.

The name poppy and its preparations appear in the Bible and in the Talmud. Probably the plant head 'rosch' refers to the capsule of *Papaver setigerum*.

The exact time of the introduction of poppy into India is under discussion. It had probably been introduced at the time of the invasion of Alexander the Great (fourth century BC). The Persians took it with them for the needs of their army. There is no evidence afterwards of applications of poppy in India until the seventh century AD. It is hard to believe, however, that opium was unknown by Indian physicians until that time.

2 TRADITIONAL APPLICATIONS OF OPIUM AND ITS ALKALOIDS

The word opium as the definition of poppy latex was used in the first century AD. It seems to be derived from the Greek word opos, meaning juice, and transferred by the Romans. The term opium spread all over the world and is still used today.

Opium has been utilized throughout the centuries for many purposes. The most important ancient application forms were summarized by Kritikos and Papadaki (1967b) as follows.

- Inhalation of opium vapours by pipe smoking. This is considered to be a common application form in some countries nowadays.
- The taking of opium internally as a hypnotic and narcotic agent in a variety of preparations, including opium capsules, juice, tablets, etc.
- Application in the form of suppositories.
- External treatment with opium preparations for diseases of the eye, ear ache, etc.
- Opium was used traditionally for euthanasia mixed with hemlock and it was also used traditionally to commit suicide.

Ancient knowledge of the applications of opium was collated by the Roman physician Galenus (127–199 BC) who described the tranquillizing, cough releasing and febrifuge effects of opium. However, the adverse and toxic effects of the drug were not emphasized by him.

The application of opium in East Asian countries, especially around the 'Golden Triangle', still shows many traditional elements and several forms (Suwanwela *et al.*, 1978). Opium is used by the inhabitants as a therapeutic drug known by the villagers to be effective in the treatment of sickness, pain, headache, backache, diarrhoea and coughs. The hill tribes live under primitive conditions and suffer from many diseases. Sometimes the poor living conditions result in a general breakdown of health which is then often treated with opium. If the illness becomes chronic and the application of opium continues, it can lead to addiction. The tranquillizing and euphoric effects of opium are known by ethnic groups as well. Opium is taken to help at times of severe sorrow, insomnia or if socio-economic conditions result in serious concern and anxiety. There is also another aspect of opium use in these areas. Smoking the opium pipe is a tradition at local social events and occurs occasionally at funeral ceremonies and social gatherings.

3 MODERN APPLICATIONS OF OPIATES

The application of opium is known and practised by pharmacists nowadays. Its powder *(Pulvis opii),* tincture *(Opii siccum),* etc. are legal in many pharmacopoeias and they used to be added as constituents of Gallenic products. However, modern applications require more processed forms and products which adhere to strict industrial standards. The application of opiate alkaloids (mainly in hydrochloride, sulphate and phosphate forms) are restricted in some well defined therapeutic fields. Analgesics of morphine origin are used mainly to control severe pain and for their anti-diarrhoea and sedative effects. Codeine and to a lesser extent pholcodine and ethyl morphine and narcotine are utilized to promote anti-tussive activity. In some countries the application of

hydrocodeine and oxycodeine is also official in this therapeutic field (Bryant, 1988). Apomorphine hydrocloride can be used as an emetic in small quantities and its anti-Parkinson efficacy has been recognized and tested. The poppy alkaloid papaverin, which is made synthetically, is used as a smooth muscle relaxant. Having knowledge of the undesirable side effects of opiates, the importance of narcotic antagonists is considerable. Naloxone and naltrexon are used in this respect.

4 RECENT HISTORY OF POPPY

The utilization of poppy was spread by the growth of the Roman Empire. The cultivation of the plant for food and medicines probably started at that time in all the Provinces. After the Roman period the cultivation of the plant continued both in European and Asian countries. However, significant differences arose in the purposes of production: opium became the main product in the East, while seed and seed oil were sought after in Europe.

Opium production in Southeast Asia (Golden Triangle), West Asia (Golden Crescent) and some other territories even nowadays uses the traditional methods which have been applied for thousands of years. Production is regulated by local consumption, market possibilities and political considerations. Such political aspects became obvious during the 'opium war' between Great Britain and China in 1838–1842. At present the politics of international organizations (WHO, FAO, UNIDO) are oriented to gain control of production in order to reduce illicit trade and consumption.

In Europe large amounts of poppy seed oil were produced for food purposes at the end of the 18th century and during the first half of the 19th century. Producers known throughout the world were located in Provence, Alsace and some German states. The importance of poppy oil became diminished after this time and industrial applications became the main form of utilization of poppy.

The manufacture of morphine in Europe began in the 19th century in small pharmaceutical companies. Opium for processing was imported from Turkey and Persia at that time. Macfarlan and Smith was one of the first companies to become specialized in opium processing in 1837 in the UK. At the start of their business, Macfarlan and Smith processed 25 tonnes of opium annually. The processing of opium was developed by many other companies afterwards as a result of the economical and medical importance of the products, e.g. Francopia in France (1847) and Mallinckrodt in the USA (1898). About 45% of the worlds morphine production is even nowadays based on traditional methods (Bryant, 1988). About 800–1000 tonnes of Indian opium are legally processed annually. This is only about 5% of the estimated total world opium production; the majority of the opium is commercialized illicitly.

In the first half of the 20th century a remarkable advance was made in obtaining morphine and related compounds from the straw. The invention of the Hungarian pharmacist Kabay in 1928 opened a new perspective for the plant and for its industrial utilization. Based on his method the alkaloids, especially morphine, were extracted from the capsule of the poppy. Previously, this straw was waste, which had to be separated from the seed in the final step of the commercial poppy cultivation processes.

Using this method a high seed quality and a valuable raw material for the pharmaceutical industry became available at the same time. This invention resulted a continuous increase of the poppy cultivation area in Europe and most recently in Australia. Some of the European poppy-producing countries in order of their poppy cultivation area are: Hungary (6000–10000 ha) Czech Republic and Slovakia (7000–8000 ha), Romania (3000–5000 ha) and the Netherlands (1000–1500 ha).

Kabay's invention made the extraction of the dried poppy capsule economically viable. The first factory specializing in dry capsule processing was established by him in Hungary in 1928. The factory ALKALOIDA has processed poppy straw from that time with a high efficiency. Straw processing has many advantages over that of opium. In Europe poppy has traditionally been cultivated as a food crop. The cultivation technology of the poppy straw makes it possible to harvest the seed and the capsule in parallel. The seed, being the main product for the farmers, reduces the price of the industrial raw. On the other hand, the production of the straw can be highly mechanized, reducing labour, and from the official point of view the control of cultivation and handling is much easier than that of opium. Realizing these advantages, and simultaneously with chemical—technological developments, the ratio of straw to opium industrial processing is continuously increasing. According to Bryant (1988) about 1000 tonnes of poppy straw in morphine equivalent are processed in the world every year. The leading countries in the straw processing business are the USA, the UK, France, Australia and Hungary.

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II. BIOLOGY OF POPPY

1. TAXONOMY

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1 INTRODUCTION

The taxonomy of poppies has changed in recent decades of plant systematic studies as further characteristics of taxa have become known. In some works, *Papaveraceae,* the family which contains among others poppies, has been included into a larger order, *Rhoeadales,* together with the families of today's *Capparales* (Fedde, 1936; Engler, 1964; Tutin and Heywood, 1964; Hutchinson, 1969; Markgraf, 1986). The association of these groups was based only two morphologic characters—the existence of commissural stigma and the replum in the fruits.

Later on, many difference in traits of *Papaveraceae* and *Capparales* became evident: the presence of benzylisoquinoline alkaloids and laticifers (or alkaloid-containing cells) in *Papaveraceae;* glucosinolates accumulation in *Capparales;* the centripetal development of stamens in the former and centrifugal in the latter group. There are also differences in some palynological and embryological characteristics. Considering these data, it is not surprising that *Papaveraceae* was dissociated from *Capparales* (Hutchinson, 1959; Takhtajan, 1959).

Based on further evidence and evolutionary considerations, the former *Papaveraceae* family was elevated to order *Papaverales* divided into two families—*Papaveraceae* and *Fumariaceae*. The chemical difference between these families is the presence of meconic acid in *Papaveraceae* and the lack of it in *Fumariaceae* (Fairbairn and Williamson, 1978). Later on, *Papaverales* order was associated with *Ranunculales*. This association was verified mainly by serological studies and by the accumulation of benzylisoquinoline and aporphine alkaloids in both orders (Frohne and Jensen, 1979; Takhtajan, 1980; Dahlgren *et al.,* 1981; Cronquist, 1988).

The latest chemosystematic overview of the lower taxa of angiosperms by Gottlieb *et al.* (1993) and the revision of the phylogenetic relationships between them (Kubitzky, 1993) reveal a high level of affinities between *Papaverales* and *Ranunculales* which has lead to the incorporation of *Papaverales* into *Ranunculales* at family level *(Papaveraceae and Fumariaceae)* based on the characteristics summarized in Table 1.

Papaveraceae flavonoids are characteristic in their high number of substitution patterns and benzylisoquinoline alkaloids are significantly diversified in this family. Having these traits *Papaveraceae* (and *Fumariaceae*) represents the highest evolutionary level in *Ranunculales* order.

Table 1 Characteristics of *Ranunculales*, including *Papaverales*

Habit		herbaceous (with secondarily woody elements)
Pollen		tricolpate, pantoporate
Oil cell		absent
Endosperm		nuclear
Lignans		wanted
Flavonoid pattern:	flavones	dioxy-chrysin, apigenin, luteolin-glycosyde,
	flavonols	kaempferol, quercetin and their derivatives
	flavanones	eryodictyol
Benzylisoquinoline alkaloid pattern:		
aporphines and derivatives		benzyltetrahydroisoquinolines, aporphines, pavines, benzylisoquinolines, oxoaporphines, isopavines
berberines		tetrahydroprotoberberines, protoberberines, protopines, phthalidoisoquinolines
morphinanes and derivatives		morphines

2   FAMILY *PAPAVERACEAE* JUSS., GENERAL DESCRIPTION (AFTER KADEREIT (1993) WITH SOME MODIFICATION)

2.1 Habit and Morphology of Vegetative Organs

Usually annual, biennial, perennial herbs, sometimes evergreen *(Argemone, Romneya)* rarely small trees *(Bocconia)*. Buds for overwintering occur in the axes of leaves in basal rosettes or on the basal portion of branches (hemicryptophytes). Geophyte representatives of the family have buds covered by bracts on the stem base under the ground. Rosette leaves, if any, are usually petiolated, the upper leaves more or less sessile, sometimes amplexicaulous.

The primary root can develop into a fleshy taproot. Adventitious root formation occurs, for example, in *Chelidonium*. Rhizomes are characteristic in *Eomecon,* with runners.

Leaves are alternate, rarely opposite *(Canbya)* or whorled *(Meconella)*. Leaf venation is mostly pinnate, rarely palmate. The lamina is entire or variously incised or divided. The branching is monopodial or, in some cases, the axes are unbranched.

2.2 Anatomy of Vegetative Organs

The vascular strands of the stem are mostly arranged widely spaced in a ring or rings. In woody species (Carlquist and Zona, 1988) vessels have simple perforation plates. Libriform fibres exist in all woody species. The axial parenchyma are usually paratracheal. Sieve tube plastids are S-type (Behnke and Sjolund, 1990). The occurrence of laticifers is a predominant anatomical feature of the family. Laticifers can be articulated and anastomosing as in *Papaver* species (Esau, 1965; Fairbairn and Kapoor, 1960), non-anastomosing *(Chelidonium)* or non-articulated *(Stylophorum)* and occur in all parts of the plant (except the seeds), usually in association with phloem areas (Thureson-Klein, 1969). Craig and Mahlberg (1976) have even identified laticifers in stamens of *Papaver somniferum* as well.

Leaves in *Papaveraceae* are anomocytic and amphystomatic (Kidwai, 1972), or hypostomatic. Vernation of leaves can be involuted or revoluted (Weberling, 1992). The nodes are unilacunar or multilacunar with up to eight gaps (Ezelarab and Dorner, 1966).

Plants can be glabrous or an indumentum may be present. The indumentum can consist of unicellular or multicellular hairs which are terminally uniseriate *(Chelidonium)* or multiseriate throughout *(Papaveroideae)*. Glandular multicellular hairs are known in *Glaucium*. The hairs can be smooth or rough, from soft and silky to prickly.

2.3 Flower Structure and Anatomy

The calyx is uniseriate, 2–3-merous, usually free (but fused in *Eschscholzia*). The upper margin of the sepal can form a flap-like lobe. The situation of this lobe, if any, is variable. The vasculature of sepals is usually branched. The flower buds are often nodding before flowering. Petals are in two 2–3-merous series with the exception of *Bocconia,* where the petals absent, and *Sanguinaria* which is polypetalous. The vernation of the petals is strongly crumpled. The vasculature of the petals can consist of one or more branched trace.

Numerous stamens are characteristic of the family, with the exception of some *Canbya* and *Meconella* species, where only 4–12 stamens are present in the flowers. The filaments can be filiform, clavate or laterally expanded and the anthers dithecal, open with longitudinal slits.

The flowers are perigynous, with the exception of *Eschscholzia,* where they are hypogonous. The gynoecium can consist of two to sometimes over 20 carpels. Except in *Romneya*, the gynoecium is unilocular, with parietal placentae. The placentae of neighbouring carpels are fused into one in most cases. In *Romneya* the gynoecium is multilocular and here the placentae extend radially and are fused in a central column, which is sterile. In *Glaucium*, the entire locule is filled by a spongy proliferation of the placentae.

The style can be absent, indistinct or distinct. The stigma of each carpel is found along the apical margin (Gonnermann, 1980). The surface of the stigma is dry and its form varies in different taxa. In *Platystemonoideae* the apex of the capsule, and therefore the stigmas of neighbouring carpels, is free. In the bicarpellate *Eschscholzia* two additional stigmas are present. Apart from the former two taxa, the stigmas, being alternate with the placentae, are the so-called commissural stigmas (Weberling, 1992).

In *Papaver* genus the stigma is more or less discoid or conical; the lobes of stigmatic rays are missing in some cases. The carpels are vasculated usually with many traces. These are concentrated in the placentae or in the median of the carpel (dorsal trace). Dorsal traces can be present *(Meconopsis)* or absent *(Stylomecon)*. In the latter case the lateral branches of the placental bundle differentiate towards the carpel median forming a pseudodorsal trace. As laticifers are associated with the phloem, they are distributed diffusely in the ovary wall.

2.4 Inflorescence Structure, Embryology and Pollen Morphology

The flowers of *Papaveraceae* are either single or are arranged in determinate inflorescence: raceme, corymb, panicle or umbel. The terminate flower usually opens first, and the order of opening is then basipetal.

The ovules are anatropous or subcampylotropous, bitegmic and crassinucellate. Megaspore development follows the *Polygonum-type*. Synergids are hooked and have filiform apparatus. The polar nuclei fuse before fertilization. In *Papaver* sometimes five antipodes occur. The endosperm is nuclear and the embryo can be rudimentary. The anthers contain secretory tapetum. Cell wall formation is simultaneous and the pollens can be bi- or tricellular. Branched pollen tubes can occur in *Papaveraceae*.

The form of spheroid pollen grains is usually reticulate, rarely spinuliferous. The aperture is tricolpate, polycolpate *(Eschscholzia)* or polyporate *(Argemonidium)*, sometimes inaperturate *(Meconopsis)*.

2.5 Karyology, Pollination and Reproductive System

Chromosome numbers in the family are mainly multiples of x=6, 7, 8, 9, 10, 11. The highest number in the family exceeds $2n$=84.

The *Papaveraceae* species are mostly insect-pollinated, with the exception of the wind-pollinated *Bocconia* and *Macleaya*. The insect pollinators are mainly *Hymenoptera* and *Diptera*. As the flowers lack nectaries, they offer only pollen to pollinating insects which are mostly attracted by visual stimuli, e.g. the colour of the petals and sometimes their basal marks. The numerous colourful anthers and filaments also increase the attractiveness of the flowers. The flowers of one species, *Papaver alpinum* smell of cloves; *Romneya* also has fragrant flowers.

Protogynous species are *Bocconia* and *Sanguinaria;* in the other taxa the maturation of anthers and stigmas occurs at the same time. Self-compatibility often exists and self-pollination can even occur in the bud, causing cleistogamy.

2.6 Fruit and Seed

The papaveraceous fruits are composed of a single pistil. The superior ovary is usually unilocular or multilocular by the union of intruding placentae and bilocular in *Glaucium,* In this latter plant a septum divides the fruit into two halves.

The fruits (capsules) are mostly monomorphic, but dimorphic fruits occur in *Hypecoum*. The shapes of mature fruits are diverse: they can be clavate, ellipsoidal, globose, ovate, obovate, oblong, fusiform, etc. They may be dehiscent or indehiscent. Dehiscence, being a diagnostic characteristic in *Papaveroideae* subfamily, can occur in pores or valves, in most cases basipetally. *Platystemon* is an exception where the carpels break after maturity into one seeded mericarps. The capsules of *P. somniferum* cultivars remain closed as a result of selection for the retention of seeds.

The surface of the fruits can be smooth, wrinkled or nerved. If fruits are not glabrous, they are covered with bristles, prickles spines or smooth appressed hairs. Such hairs may have a diagnostic value.

The colour of the fruits is wide ranging: they are usually brownish or black, but can be greyish or green (Gunn, 1980).

Seeds are usually numerous per fruit. However, in some cases their number is much less and in the extreme, only one seed is present. Seeds arise from anatropous or semicampylotropous ovules oriented horizontally on the placentas. Brückner (1983) gives a summary of seed coat anatomy in 22 genera of *Papaveraceae*. The seeds develop from the bitegmic ovule. The outer integument bears the outer epidermis,

the parenchymatic layer (if it exists) and the crystal layer. The latter contains calcium oxalate crystals of different sizes and arrangements (Figure 1). The fibrous layer, middle layer and the inner epidermis develop from the inner integument. The ornamentation of the outer epidermis is a reliable form of identification of species (Figures 2 and 3). In some cases arils are present which originate from the funiculus or raphe. These arils usually contain oils and are known to aid seed dispersal by ants who transport the seeds (myrmecochory) and use arils for food. The seed coat can be glossy or dull and ranges in colour from white to black.

2.7 Phytochemistry

At subfamiliar and generic level it is not easy to delimit groups on a phytochemical basis. Berberines, tetrahydroberberines, protopines and dehydrogenated benzophenanthridines seem to be ubiquitous in the family. Aporphines, morphinanes, pavines, isopavines, narceines and rhoeadines have in some cases a systematically scattered distribution. Only *Chelidonioideae* can be characterized by the accumulation of reduced benzophenanthridines.

Meconic acid can be found in *Papaver*. Cyanogene glycosides, dhurrin and triglochinin occur in *Papaver* and *Eschscholzia,* but these compounds can also be found in some allied taxa.
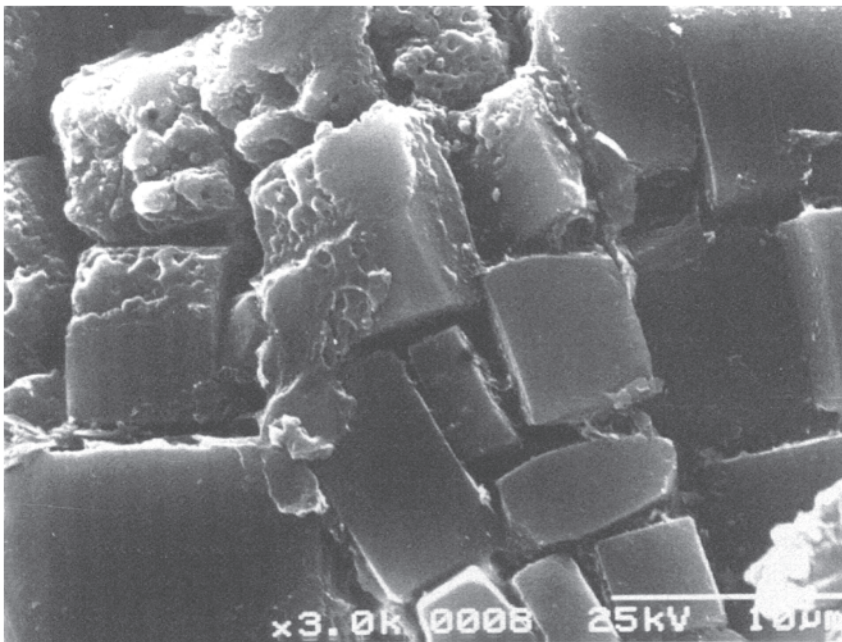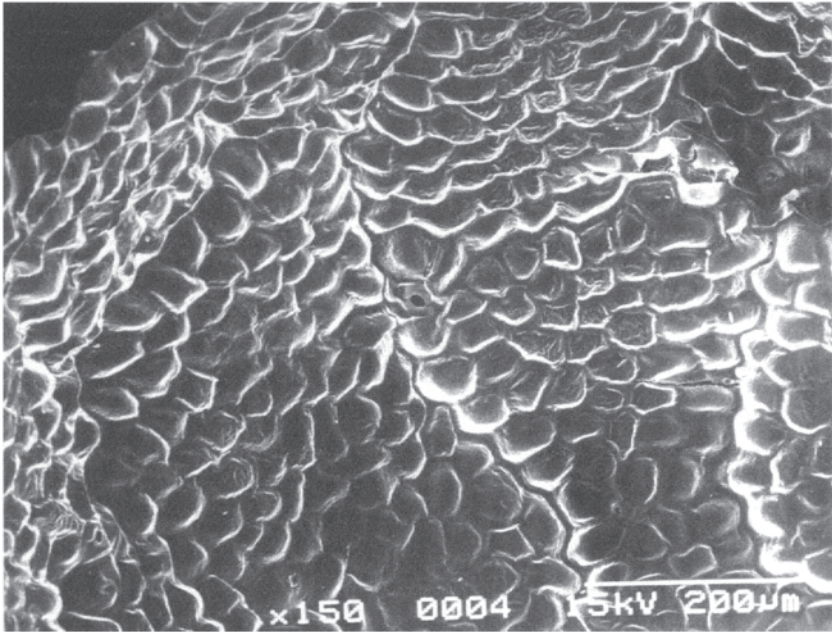

Figure 1 Crystals in *Chelidonium majus* seed coat

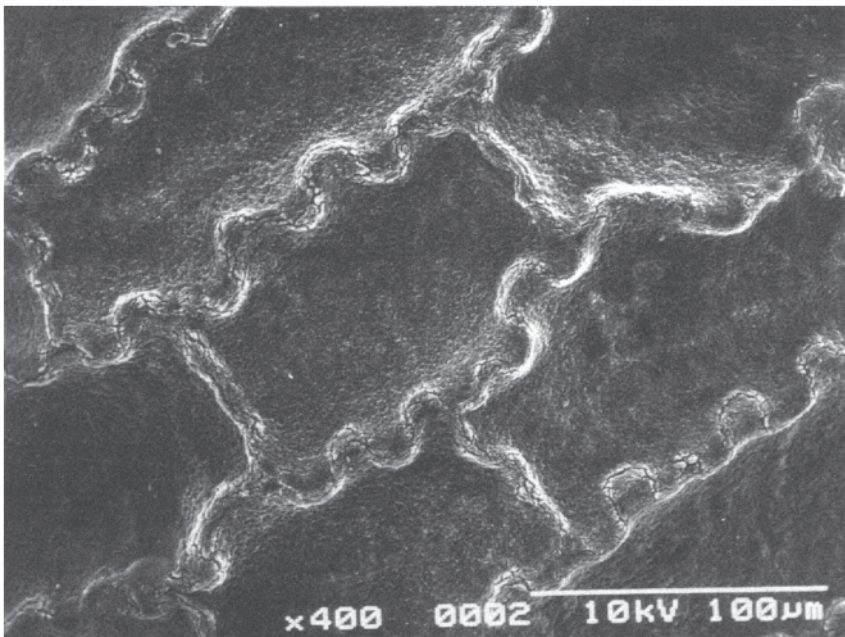**Figure 2** Outer epidermis of the seed coat of *Eschscholzia* (face view)

Figure 3 Outer epidermis of the seed coat of *Papaver atlanticum* (face view)

2.8 Distribution and Habitats

In its natural distribution *Papaveraceae* is a family of the northern hemisphere. Only one species has been found in South Africa *(Papaver aculeatum)* and one other is native to Central and South America *(Bocconid)*. American genera are, among others, *Arctomecon, Canbya, Stylomecon,* and a single *Papaver* species, *P. californicum.* The majority of Eurasian taxa are found in Central Asia.

Representatives of the family can occur in arid and warm climates, in alpine vegetation, in arctic areas (mostly in open vegetation) and also in deciduous and tropical mountain forests. There are also widespread ruderals in the family.

3 SUBDIVISION OF THE FAMILY

Different authors divided the family into 3–7 subfamilies. Fedde (1936) described the *Hypecoideae, Papaveroideae* and *Fumaroideae* subfamilies in his system. Takhtajan (1980) divided *Platystemonoideae, Papaveroideae, Chelidonioideae, Eschscholzoideae, Pteridophylloideae* and *Hypecoideae.* According to Kadereit (1993) the ≈260 species of *Papaveraceae,* are divided into four subfamilies involving 23 genera (Table 2).

3.1 Characterization of the Subfamilies

3.1.1 Chelidonioideae (Ernst)

This subfamily is heterogenous. Unifying characteristics are the bicarpellate gynoecium (with the exception of *Stylophorum diphyllum*) and the peculiarity to this group, the multicellular, terminally unicellular hairs. There are three groups of genera and one separate genus in this subfamily.

The first group, *Chelidonium* and *Hylomecon* have pinnately divided leaves and umbellate inflorescences with small simple bracts. The basic number of chromosomes is $x=6$.

The second group has four genera—*Eomecon, Sanguinaria, Macleaya* and *Bocconia* are characterized by more or less palmately veined leaves. Their chromosome number is $x=9$ or 10. The chromosome number is $x=20$ in *Bocconia,* separating this genus from the other three to a certain extent, although many characteristics are unifying. The seeds of both the above groups are arillate.

The third group contains *Glaucium* and *Dicranostigma* with $x=6$ basic chromosomes, pinnate divided leaves and seeds without arils. The flowers present in this group are united in a racemose inflorescence with large leafy bracts.

Stylpohorum occupies an uncertain position with its complex characteristics: it has chromosome number $x=10$, as does the *Eomecon* group, pinnately divided leaves and umbellate inflorescence in common with *Chelidonium* and its allies.

3.1.2 Eschscholzioideae (Ernst)

Eschscholzia, Hunnemannia and *Dendromecon* are well characterized by unicellular hairs (if an indumentum is present) and the bicarpellate, explosively acropetal dehiscent capsules with ten longitudinal ribs and linear cotyledons. Specialities of the genera

Table 2 Subdivision of the *Papaveraceae* family (Kadereit, 1993)

Family	*PAPAVERACEAE*			
Subfamily	*Chelidonioideae*	*Eschcholzioideae*	*Platystemonoideae*	*Papaveroideae*
Genus	*Chelidonium* (Tourn.)	*Eschscholzia* Cham.	*Platystemon* Benth.	*Meconopsis* Viguier
	Hylomecon			*Papaver* (Tourn.) L.
		Hunnemannia A. Juss	*Hesperomecon*	*Romeria* Medic.
	Stylophorum Nutt.			*Stylomecon* Benth.
		Dendromecon	*Meconella* Nutt.	*Arctomecon* Torr. et Frém.
	Eomecon Hance			
	Sanguinaria Dill			*Argemone* (Tourn.) L
	Macleaya			*Canbya* Parry
	Bocconia (Plum) L.			*Romneya* Harv.
	Glaucium (Tourn.) Adans			
	Dicranostigma Hook. et Thoms.			

include: fused sepals in *Eschscholzia;* entire leaves in *Dendromecon* (woody plants) while the other two genera have finely dissected leaves.

3.1.3 Platystemonoideae (Ernst)

The three genera are characteristic in their apically apocarpous gynoecia and free simple stigmas. The calyx and corolla are trimerous, x=6-8

Platystemon and *Hesperomecon* have a dense indumentum with multicellular, multiseriate hairs. In *Platystemon* special characteristics are the ovary with 6–25 carpels and dehiscence of the capsule along the placentae and between the seeds.

Meconella genus involves glabrous plants with very few hairs, three carpels (as in *Hesperomecon*) and short stigmas. Dehiscence occurs along the placentae. The stamens are 4–6 in one series or 21 in two series.

3.1.4 Papaveroideae

The indumentum is multicellular, multiseriate. The gynoecium is polycarpellate, syncarpous. Within this subfamily two groups of genera can be recognized.

The first group contains *Arctomecon, Argemone, Canbya* and *Romneya.* The calyx and corolla usually occur in 2–3-merous whorls, the ovary has 3–12 carpels, the stigmas are sessile or on short styles and the flowers opens by basipetal valves. This group is known to be not a natural one (Kadereit, 1993) there are many uncertainties in the affinities to each other and to other genera. For example, in *Argemone* there are woody species, like in *Dendromecon,* and the seeds of some *Arctomecon* species can be arillate, as in *Chelidonioideae.* At the same time the chromosome number is usually higher than that in the other groups.

The second group includes *Meconopsis, Papaver, Romeria* and *Stylomecon.* Their ovules are semicampylotropous. The presence of meconic acid is characteristic and the seed coat layer contains fine crystals. *Papaver, Roemeria* and *Stylomecon* have more or less undulating cell walls in the outer layer of the seed coat.

Among these genera the genus *Papaver* has the greatest importance, comprising about 100 species, involving the native ancestors and close allies of cultivated poppies.

3.2 Phytochemical Overview of the Subfamilies

It is evident that the occurrence and amount of special compounds are dependent on the geographical location, on the local climatic conditions (Bernáth *et al.,* 1978), on the ontogenetic state of plants and on the plant parts studied (Mika, 1955). Furthermore, taking into consideration the great number of taxa and the detected compounds and the existence of chemical races it is not easy to delimit taxa and describe their relationships on a phytochemical basis. The following provides a short summary of alkaloid types of the subfamilies using results reported by Hegnauer (1990), Kalav and Sariyar (1989), Preininger (1986) and Tétényi (1993).

3.2.1 Chelidonioideae

Benzophenanthridines and protoberberines are characteristic to *Chelidonium* genus. The most frequently examined species is *Chelidonium majus.*

Bocconia and *Macleaya* contain allocryptopine and protopine as major alkaloids. In one species (*Bocconia frutescens* L.) rhoeadine and papaverrubine have been detected. These compounds are widespread in *Papaveroideae* subfamily.

The genus *Hylomecon* contains benzophenanthridines, protopines and protoberberidines.

In *Sanguinaria,* benzophenanthridines—especially sanguinarine and chelerythrine—have been detected.

The alkaloid spectrum of *Stylophorum* genus is closely related to that of genus *Chelidonium,* as the same quaternary alkaloids have been detected in both. A particular alkaloid which has been isolated from the aerial plant parts is stylopine and the presence of this characterizes this genus.

A characteristic of the genus *Dicranostigma* is the predominance of aporphines, which indicates a close relationship with the genus *Glaucium.*

In *Glaucium* genus the presence of an enantiomer of chelidonine indicates a close relationship with *Chelidonium*. Aporphine, protopine, protoberberines and benzophenanthridines are the characteristic alkaloids of this genus. Chemical differences between plants of different geographical origin have been noted, i.e. chemical races exist.

3.2.2 Escbscholzioideae

The three genera *(Eschscholzia, Hunnemannia, Dendromecon)* accumulate protopine, hunnemannine and allocryptopine. A species of the *Eschscholzia* genus is characterized by the presence of pavine alkaloids.

3.2.3 Platystemonoideae

Platystemon and *Meconella* accumulate protopine, protoberberines and benzophenanthridines.

3.2.4 Papaveroideae

The *Argemone* genus is chemically a very heterogenous group. Some species have a close chemical relationship with *Eschscholzia* on the basis of pavine accumulation, while armepavine (detected in *Argemone turnerae* Powel) is general in the *Papaver* genus.

Stylomecon genus is characterized by its small quantities of alkaloids. The main alkaloids are cryptopine, allocryptopine and protopine.

Genus *Papaver* has the highest level of botanical and phytochemical variability, embracing many species with numerous subspecies and varieties yielding approximately 170 alkaloids from 13 alkaloid groups. In this genus the papaverrubines, benzylisoquinolines, phthalideisoquinolines and the protoberberine alkaloids dominate.

In genus *Romeria,* besides the aporphine alkaloids, roemeridine and rhoehybrine are characteristic.

The major alkaloids in genus *Meconopsis* are protopine and amurensinine in Asian species and aporphine and magnoflorine in the only UK species (*Meconopsis cambrica* L.) Vig., indicating the existence of chemical races in this genus. Rhoeadine and amurensinine accumulation indicates its relationship with *Papaver genus.*

Table 3 Groups of sections in genus *Papaver* (Kadereit, 1988)

Meconella	*Argemonidium*	*Pilosa*	*Carinatae*
Meconidium		*Pseudopilosa*	*Rhoeadium*
Californicum		*Horrida*	*Macrantha*
			Papaver

In genus *Romneya* benzylisoquinolines, protopines and benzophenanthridines have been detected.

4 SUBDIVISION OF GENUS *PAPAVER*

Papaver genus has been divided by different authors into five to eleven sections (Elkan, 1839; Fedde, 1936; Günther, 1975). The latest revision was made by Kadereit in 1988 who divided the eleven sections of the genus into four groups based on morphological traits, primarily on the characteristics of the capsules (Table 3).

In the first group valvate dehiscence of the capsule and the pyramidal stigmatic disc is characteristic, with the exception of *Meconella* where the stigmatic disc is flat.

Argemodinium is separated from the other sections by the special shape of its stigmatic disc which appears to be a solid continuation of the bristly poricide capsule, in the form of a 'plug'.

The poricide capsules in the third group are long and narrow with flat stigmatic discs; the leaves are narrow, incised or pinnatifid.

The last group is united by more or less obovoid glabrous capsules with pores which open or remain closed, and with a flat deciduous stigmatic disc.

Some capsule types of *Papaver* genus are shown in Figure 4.

5 GENERAL PHYTOCHEMICAL CHARACTERS OF GENUS *PAPAVER*

In this genus twelve alkaloid groups (tetrahydroisoquinolines, benzylisoquinolines, aporphines, promorphinanes, morphinanes, protoberberines, phthalideisoquinolines, secophthalideisoquinolines, rhoeadines, benzophenanthridines, rhoeadines, papaverrubines) have been detected containing almost 200 compounds. The number of detected compounds is continually increasing with the ongoing development of analytical methods. The occurrence of different alkaloids is usually not in close connection with morphological traits, but in some taxa Tétényi (1997) has observed a close relationship between morphology and chemistry.

6 CHARACTERISTICS OF SECTIONS OF *PAPAVER* GENUS

6.1 Sectio *Meconella* (Spach.)

6.1.1 Morphology and Karyology

All species of this section are perennial and are characterized by their scapose growth habit (Figure 5.1). The leaves, which are simple or often dissected, form rosettes. The basal part of the stem may be horizontal. The epidermis is hairy or glabrous.
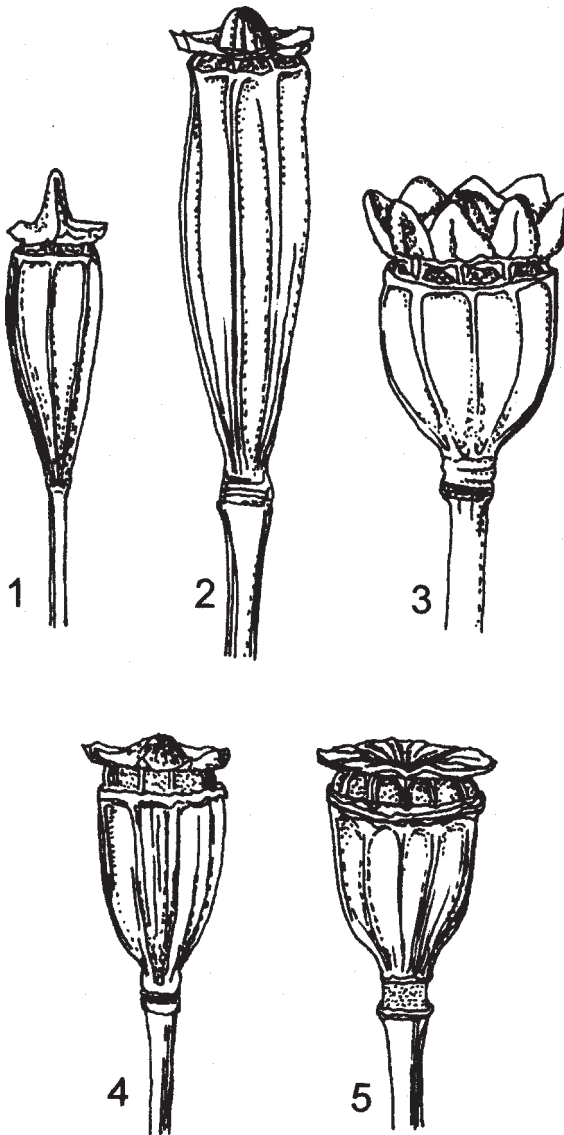
Figure 4 Capsules of some *Papaver* species: 1, *P. stylatum*; 2, *P. umbonatum*; 3, *P. carmeli*; 4, *P. humile*; 5, *P. rhoeas*

The floral axis bears single flowers and has no lateral branches or leaves. The floral axis is glabrous or covered by appressed or erect white or brownish-black hairs. The flower buds are oval or round, glabrous, or covered with light or dark hairs. The two oval sepals usually fall off after flower opening, with the exception of some arctic species where they remain for a longer period after flowering.
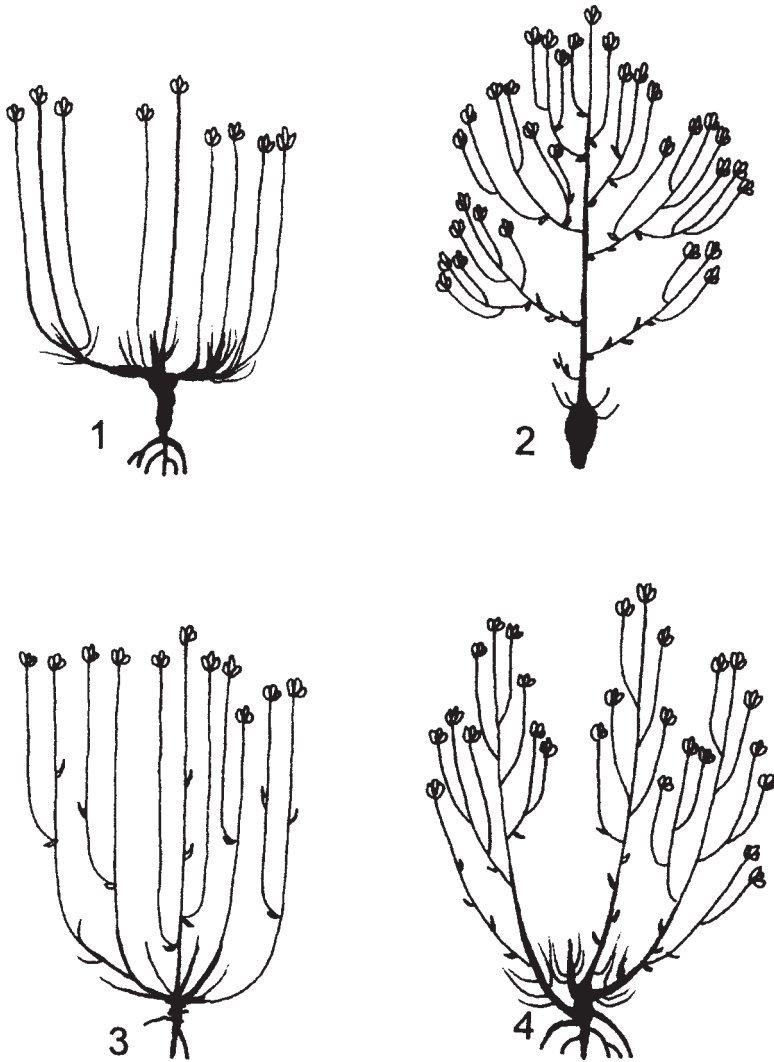
Figure 5 Growth habit of:1; sectio *Meconella (Papaver alpinum)*; 2, sectio *Meconidium (Papaver fugax)*; 3, sectio *Argemonidium (Papaver argemone)*; 4, sectio *Pilosa (Papaver pilosum)*

The petals are wedge-shaped, growing laterally and more or less overlapping. They usually fall after flowering, but in some cases can remain attached to the surface of the ovary. Flowers are highly variable in colour; white, yellow, orange, reddish, brownish flowers occur, sometimes with basal marks of different colours. Even in the same population different flower colours can appear.

The species in this section have numerous stamens (20–100 per flower) and the anthers are light green or greenish-orange with a yellow filament.

The stigmatic discs, which are usually flat, have very deep incisions between the stigmatic rays. The capsules are bristly and open valvately along the placentae, resulting in distinct valves. The capsule shape is also variable: they can be cylindrical, barrelshaped, clavate, or almost entirely spherical. The seeds may be elongated (Figure 6) and the radial walls of the outer epidermis are wavy along the longitudinal axis of the seed and straight perpendicular to it (Figure 7).

In Asian and usually in Central European species, the ploidy level is low: $2x$, $4x$, $6x$, and the level of ploidy increases with geographical latitude (Rändel, 1974), with the exception of *P. alboroseum* ($2n=28$) and *P. microcarpum* ($2n=14$) which are found in North East Eurasia and Alaska (Rändel, 1975). In Scandinavia, North America and Greenland the ploidy level is up to $12x$ (Rändel, 1977a,b). The maximal number of chromosomes is 84, the basic chromosome number n is 7.

6.1.2 Photochemical Characteristics

The characteristic alkaloids in this groups are: amurensin, amurensinin, amurin, nudaurin, alpinigenin, alpinin, papaverrubin and muramin (Boit and Flentje, 1960; Maturevá *et al.,* 1966; Rändel, 1974). Tétényi (1993) also describes the accumulation of isopavine, retroprotoberberine and protomorphinane alkaloids. It seems that the lack of aporphine alkaloids is the main chemical characterisitc of this section (Preininger, 1986).
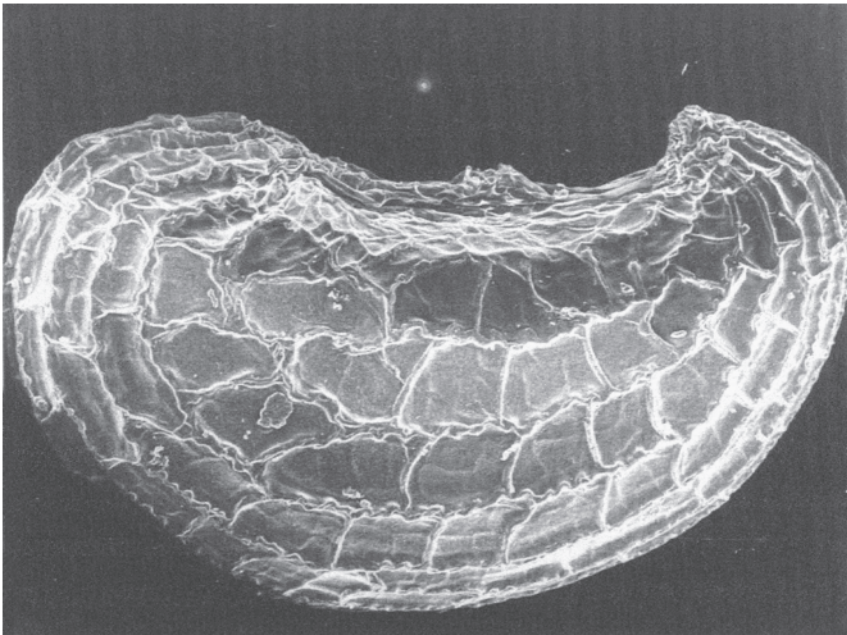
Figure 6 Seed of *Papaver nudicaule*
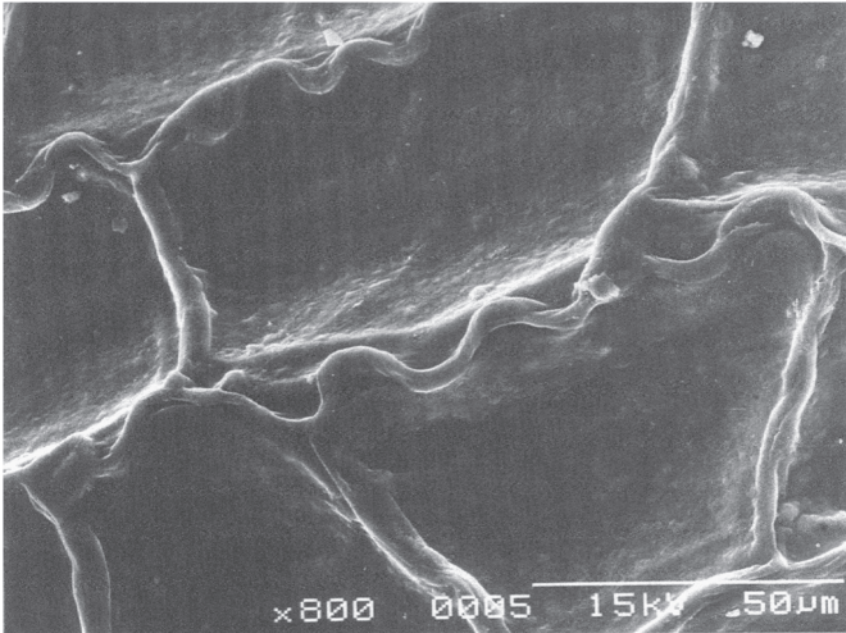
Figure 7 Outer seed coat epidermis of *Papaver nudicaule*

6.1.3 Species and Geographical Distribution

This section contains about 30 species. In the high mountains of Central Europe *P. alpinum* L. is the most typical. Six of its subspecies are spread over the Alps, one subspecies is found in East and South Karpatian regions and one in Macedonia. Species of *Meconella* having many subspecies and synonyms are listed in Table 4.

 Meconella is mainly distributed in Central, Inner and East Asia, and the South East part of the former Soviet Union (North Siberia, Kamchatka). It is supposed (Rändel, 1974) that the area of Asian diploid taxa is the centre of origin, but it could be hypothesized, based on the change of ploidy level, that the spread of these taxa follows two directions: North-East and West-West-North. The former direction results in the species of the Behringian region and Western North America. The great morphological similarities between North European and North East American species could be a consequence of transatlantic transport of this group, also covering Iceland and Greenland. The distribution of species in this section occurs in the arctic-alpine, steppe habit and also in mesic circumstances.

 All representatives of section *Meconella* exhibit large variability. A strong relationship exists between morphological traits, e.g. leaf form and habitat. Some traits, for example, chromosome number and flower colour, may be the same in more than one species. In addition, genetic recombination between the different ploidy types increases the level of diversity. As a consequence of all these factors it is extremely difficult to delimit subspecies and even species.

Table 4 Species of sectio *Meconella*

Species	Subspecies	Synonym
Papaver alpinum L.	rhaeticum (Ler. Mgf),	P. pyrenaicum ssp. rhaeticum Fedde
		P. pyrenaicum Willd,
		P. rhaeticum Ler.,
		Argemone pyrenaica L.
	ernesti-mayeri Mgf.	
	Kerneri (Hay.) Fedde	P. kerneri Hay.
	Sendtneri (Kern.) Schinz and K	P. pyrenaicum ssp. sendtneri Fedde
	tataricum Nyár.,	
	alpinum L.	P. burseri
	corona-sancti-stephani Zap	P. pyrenaicum
	degenii Urum et Jáv	
P. nudicaule L.	nudicaule	
	baicalense Tolm.	
	Amurense Busch	
	radicatum Rottb.	
	Rubro-aurantiacum (DC) Fedde	P. rubro-aurantiacum Lundstr.
	Xanthopetalum (Trautv.) Fedde	
	leiocarpum Turz.	
P. croceum Ledeb.	Croceum	
	chinense (Rgl.) Rändel	
	rubro-aurantiacum	
	longiscapum Rändel	
P. radicatum Rottb.	polare Tolm.	
	dahlianum (Nordh) Rändel	
	ovatilobum Tolm.	
P. canescens Tolm.		
P. tianshanicum Popov.		
P. angrenicum Pazij.		
P. lapponicum Tolm.		
P. indigirkense Jurc.		
P. minutifolium Tolm.		
P. leucotrichum Tolm.		
P. laestadianum Nordh.		
P. pulvinatum Tolm.		
P. nivale Tolm.		
P. microcarpum DC.		
P. alboroseum Hult.		
P. walpolei Pors.		
P. relictum (Lundstr.) Nordh.		
P. macounii Greene.		
P. miyabeanum Tatew.		
P. pygmaeum Rydb.		
P. czekanowskii Tolm.		

Figure 8 Radial walls of seed coat epidermis in *Papaver fugax*

6.2 Sectio *Meconidium*

6.2.1 Morphology and Karyology

Section *Meconidium* is the least well known section in the genus and contains both biennial and perennial species.

Species in this section have a well developed more or less evergreen leaf rosette and a thick main root. Its growth form is shown in Figure 5.2. The axis emerges in the second year and has a horizontal base with numerous lateral branches. Leaves on the axis are usually small, sometimes even reduced to scales or absent.

The capsules can be bristly or glabrous with a pyramidal stigmatic disc. The radial walls of the epidermis of the seed coat are almost straight (Figure 8). The species of this section are diploid.

6.2.2 Phytochemical Characters

The major specific chemical constituents in this section are rhoeadanes and armepavine as 1-benzyltetrahydroisoquinoline type alkaloids (Phillipson *et al.,* 1981a,b; Phillipson, 1983). In *P. curviscapum* 1-methoxiallocryptopine was also detected (Sariyar *et al.,* 1989). This section is heterogenous not only in the morphology of its species, but also in chemical characteristics. At least three chemical races exist: benzylisoquinoline-proaporphine type, morphinane type and rhoeadane type (Preininger, 1986).

Table 5 Species of sectio *Meconidium*

Species	Synonym
P. acrochaetum Born.,	
P. armeniacum (L.) DC	
P. curviscapum Nab.	
P. cylindricum Cullen	
P. persicum Lindl	*P. tauricolum* Boiss., *P. hyoscyamifolium* Boiss. and Hausskn.
P. triniifolium Boiss	
P. fugax Poir	*P. caucasicum* Marsch.-Bieb., *P. floribundum* Desf.
P. libanoticum Boiss	
P. polychaetum Schott et Kotschy	
P. arcochaetum Born.	*P. fugax* Poir. var. *microcarpum*, Boiss.
	P. tauricolum Boiss. var. *microcarpum* (Brodz.) Boiss.
P. armeniacum (L.) DC	*P. caucasicum* M.B var. *stenocarpum* Boiss.
	P. roopianum (Brodz.)Sosn.

6.2.3 Species and Geographical Distribution

Meconidium section comprises about ten species (Table 5). The section is distributed in North West Iran, Caucasus, and the Middle East. One species exists in Lebanon.

6.3 Sectio *Californicum* Kadereit

The only native annual species of North America, *P. californicum* A.Gray, is a member of this section. It has slender, ribbed capsules with pyramidal stigmatic discs. The nervature of the leaves is pinnate; the leaves are mostly glabrous; the filaments are yellow. Chemically it is characterized by the presence of rhoeadine and protopine (šantavý *et al.,* 1960), muramine and benzylisoquinoline latericine.

6.4 Sectio *Argemonidium* Spach.=Argemonorhoeades

6.4.1 Morphology and Karyology

This section contains annual species. They are the so-called half-rosette plants. The peculiarity of their branch system is the few and very long internodes (Figure 5.3). The branches are straight and hairy. The upper leaves are sessile, more or less hairy. The leaves may amphistomatic, e.g. *P. fugax* (Figures 9.1 and 9.2).

The filaments are violet—black. The pollen colour can differ from species to species and may be pink, purple or blue (Landolt, 1967).

The capsules are long, narrow, bristly and valvate, without an 'ordinary' stigmatic disc. In species of this section the stigmatic disc, as an 'apical septa' is a continuation of the capsule, forming a solid 'plug'. There are four to eight stigmatic rays. The seeds may differ in shape from that of the 'ordinary' *Papaver* seeds (Figure 10).

The chromosome number *n* is six *(P. hybridum)* or seven *(P. argemone);* the level of ploidy is maximally *6x* in *P. argemone* ssp. *argemone,* where 2*n*=40 or 42 (Kadereit, 1990). Although *P. apulum* lies morphologically between *P. hybridum* and *P. argemone* cytologically *P. apulum* and *P. hybridum* are diploids while *P. argemom* is hexaploid.
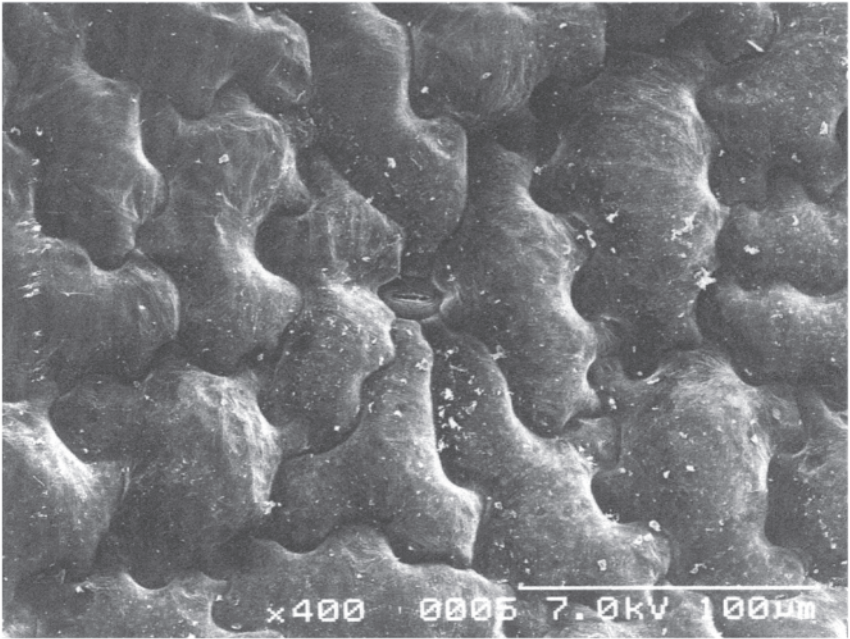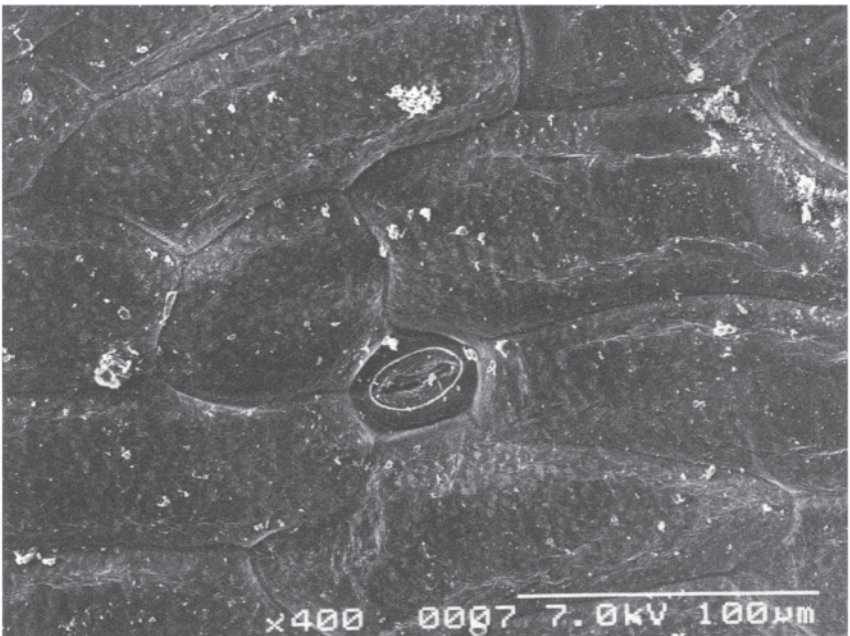
Figure 9 1, Lower epidermis of *Papaver fugax* leaf; 2, upper epidermis of *Papaver fugax* leaf
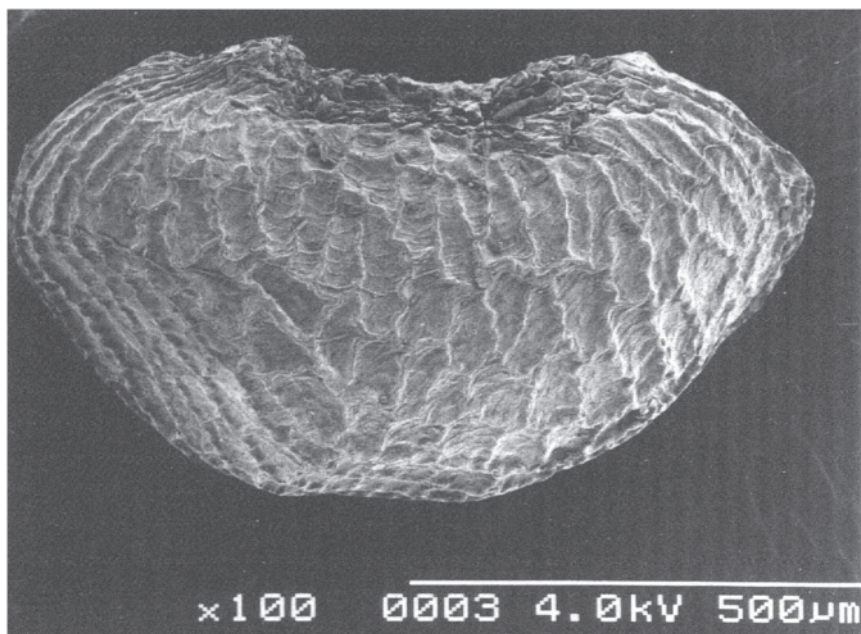
Figure 10 Seed of *Papaver pavonium*

6.4.2 Photochemical Characters

Species of *Argemonidium* section contain only very small amounts of meconic acid. The alkaloid composition of this section resembles that of the *Rhoeadium* section, but the alkaloid content is much more lower and more specific to the different species. For example, in *P. argemone* rhoeadine and isorhoeadine are present; *P. hybridum* accumulates glaudine and pahybrine; *P. pavonium* contains roemeridine and its carbonyl derivative (Tétényi, 1993). Characteristic alkaloid groups found in this section are protoberberine, benzophenanthridine, protopine, rhoeadine and papaverrubine (Preininger, 1986).

6.4.3 Species and Geographical Distribution

The section contains four species as listed in Table 6. These species occur in the southern Alps and Adriatic Sea to the western Himalayas; *P. hybridum* can also be found in some islands in the Pacific.

6.5 Sectio *Pilosa* (**Prantl**)

6.5.1 Morphology and Karyology

This section contains perennial half-rosette subscapose plants with racemose inflorescence (Figure 5.4). The basal leaves are petiolated, long-lanceolate, dentate, and in some cases dissected.

Table 6 Species of sectio *Argemonidium*

Species	Subspecies	Synonym
P. apulum Ten.		
P. argemone L.	*argemone* Kadereit	
	nigrotinctum (Fedde) Kadereit	
	davisii Kadereit	
	meiklei Kadereit	
	belangeri Taht.	*minus* (Boiv.) Kadereit
P. pavonium Fisch et Mey	*ocellatum* (Woron) Kadereit	
	pavonium Kadereit	
P. hybridum L.		*P. hispidum* Lam.

Table 7 Species of sectio *Pilosa*

Species	Synonym
P. pilosum Sibth. et Sm.	
P. apokrinomenon Fedde	
P. strictum Boiss et Bal.	
P. feddei Schwz.	
P. spicatum Boiss et Bal.	*P. heldreichii* Boiss., *P. pannosum* Schw.

The axes bear sessile leaves with convolute vernation. The flowers are large—4–6 cm in diameter—with orange petals. The pedicels are long and the filaments filiform. The capsules are narrow and glabrous and the stigmatic disc is more or less flat with few (4–8) rays. Capsules open poricidally.

The level of ploidy in this group is maximally $4x$; chromosome numbers $2n=14$ or 28.

6.5.2 Phytochemical Characters

The species of this group accumulate morphinane, amurine and aporphine, glaucine and roemerine alkaloids (Öztekin *et al,* 1985). Rhoeadine, papaverrubine and protopine alkaloids are found. The presence or absence of amurine (promorphinane alkaloid) is the chemical difference between the sections *Pilosa* and *Pseudopilosa* (Preininger, 1986).

6.5.3 Species and Geographical Distribution

The species of this section are listed in Table 7. *P. pannosum* Schw. and *P. heldreichii* Boiss. are regarded as the synonyms (or allies) of *P. spicatum* based on both morphological (Cullen, 1965) and chemical (Öztekin *et al.*, 1985) data.

The occurrence of the section is limited to Western Anadolu (Anatolia) in Turkey.

6.6 Sectio *Pseudopilosa* (Pop. et Günther)

6.6.1 Morphology

The growth habit of species in this section differs from that of *Pilosa* species in its subscapose form. There are some leaves on the lower part of the flower axis carrying a single flower (Figure 11.1). The leaves have revolute vernation in buds.
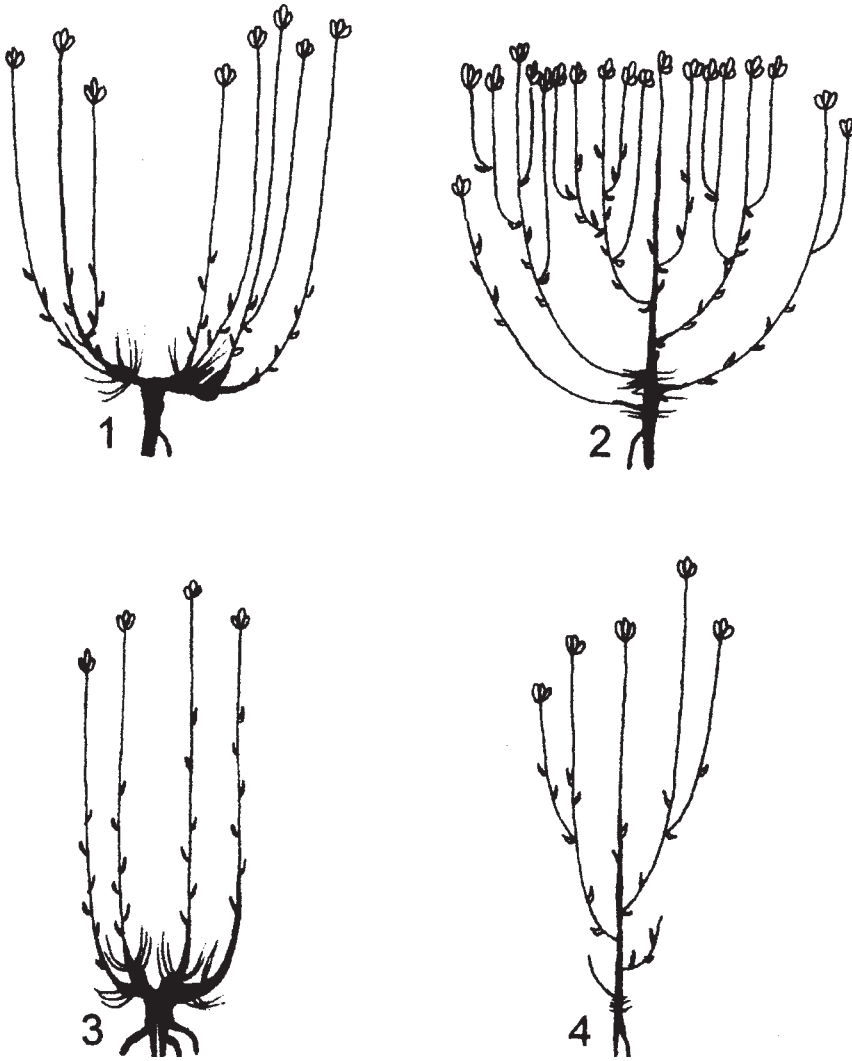
Figure 11 Growth habit of: 1, sectio *Pseudopilosa (Papaver atlanticum)*; 2, sectio *Rhoeadium (Papaver rhoeas)*; 3, sectio *Macrantha (Papaver bracteatum)*; 4, sectio *Papaver (Papaver somniferum)*

Table 8 Species of sectio *Pseudopilosa*

P. atlanticum Ball et Cross.
P. lateritium Koch.
P. oreophilum Rupr.
P. rupifragum Boiss et Reut.
P. montanum Trautv.

6.6.2 Photochemical Characters

The most characteristic special compounds are protopine and rhoeadine; the species of *Pseudopilosa* lack amurine and glaucine (Tétényi, 1993).

6.6.3 Species and Geographical Distribution

The species of this section are listed in Table 8. The species are separated into two areas of geographical location: *P. lateritium* is endemic in North East Turkey and also occurs in Caucasus; *P. rupifragum* and *P. atlanticum* are species of North West Africa and southern Spain.

6.7 Sectio *Horrida* (Elkan)

6.7.1 Morphology and Karyology

The single representative of this section is *Papaver aculeatum* Thunb.

The habit of this species is usually annual, but in some circumstances it may be biennial (Günther, 1975). It is a rosette plant with rarely branching axes, and oval to narrowly elliptical incised leaves. The vegetative parts are covered by setae—long prickly bristles occur on the leaf tips and major veins (Figure 12.2).

Flowers develop mostly in racemose clusters. The filaments are filiform and both filaments and anthers are yellow in colour. The capsule is glabrous, narrow, long and poricidal with a flat stigmatic disc carrying 5–11 stigmatic rays (**Figure 12.1**). Its dark brown seeds are small, 0.5–0.7 mm long, and reniform. The chromosome number $2n$=22.

6.7.2 Phytochemical Characters

The chemical composition of this species resembles that of *P. pilosum* species, both containing salutaridine and aculeatine (Maturová *et al.,* 1966).

6.7.3 Geographical Distribution

P. aculeatum as the only representative of *Papaver* genus in the Southern hemisphere, occurs in Easter in South Africa as a species of temperate mixed grasslands. It also appears in Australia, as a result of synanthropic spread (Kadereit, 1988a,b).
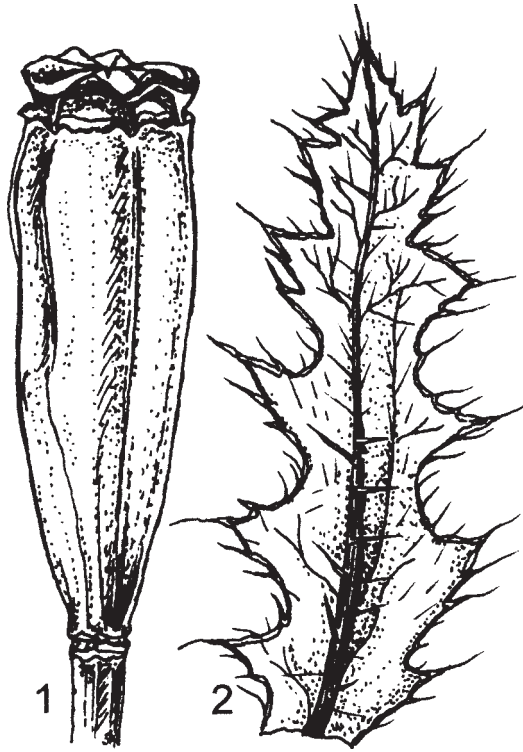
Figure 12 *Papaver aculeatum* (sectio *Horrida*): 1, capsule; 2, lower surface of cauline leaf

6.8 Sectio *Carinatae* Fedde

6.8.1 Morphology and Karyology

This section contains only one species—*Papaver macrostomum* Boiss et Huet. with three varieties: var. *bornmülleri* (Fedde) Kadereit; var. *macrostomum* Kadereit; and var. *dalechianum* (Fedde) Kadereit (Kadereit, 1987). It is characterized by its annual habit. The branches and form and arrangement of stem leaves resembles that of *P. rhoeas*. The leaves and sepals are covered by sparse or dense patent hairs. The lower leaves are petiolated and are larger than the sessile upper leaves. The lamina is 1–2 pinnatripartite.

The filaments are filiform and black. The anthers have small appendages on their apices. Capsules are mostly glabrous, ovoid or cylindrical with 6–8 stigmatic rays. The special characteristic of this section is that stigmatic discs become detached from the capsules at maturity (Figure 13). The chromosome number is diploid ($2n$=14).

6.8.2 Phytochemical Characters

The characteristic alkaloid of this species is macrostomine (Hegnauer, 1990). In its natural habitat (Central Asia) macrostomine and sevanine accumulation has been
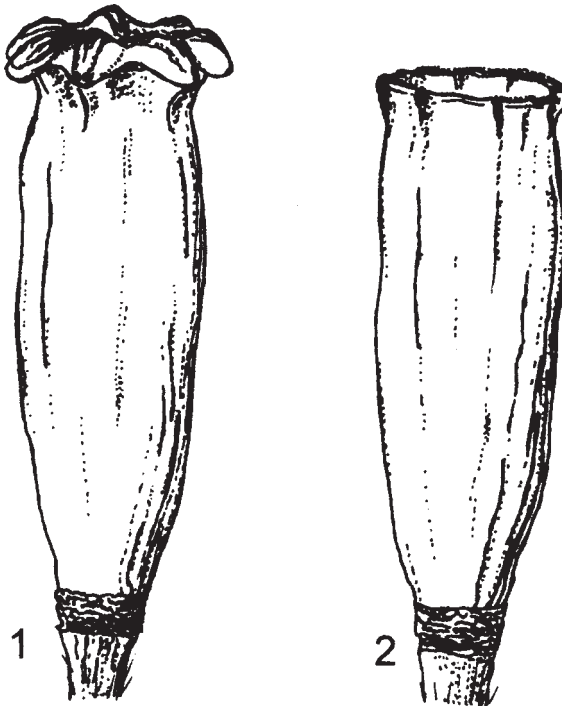
Figure 13 Capsule of *P. macrostomum* (sectio *Carinatae*): 1, closed capsule; 2, open capsule, without stigmatic disc

detected in *P. macrostomum*. In the same species cultivated in Europe, rhoeadine, papaverrubine and protopine alkaloids were found, showing the existence of chemical races (Preininger, 1986).

6.8.3 Geographical Distribution

The species occurs in Iraq, Iran, Turkey and in the South of the former Soviet Union.

6.9 Section *Rhoeadium* Spach

6.9.1 Morphology and Karyology

The characteristic habit of species in this section is annual, although some species may be biennial in special circumstances (Markgraf, 1986; Günther, 1975).

The stem may be simple or branched to different degrees (Figure 11.2). The leaves are sessile; in some species they are covered by spiny hairs.

The capsules are glabrous, ellipsoid, with poricidal dehiscence. The stigmatic disc is usually flat with 5–18 stigmatic rays (Figure 14). The shape of capsule is very similar to those of *P. rhoeas* and *P. humile*. The difference between these species is the presence or absence of a petiole of the upper stem leaves (Kadereit, 1990). The shape
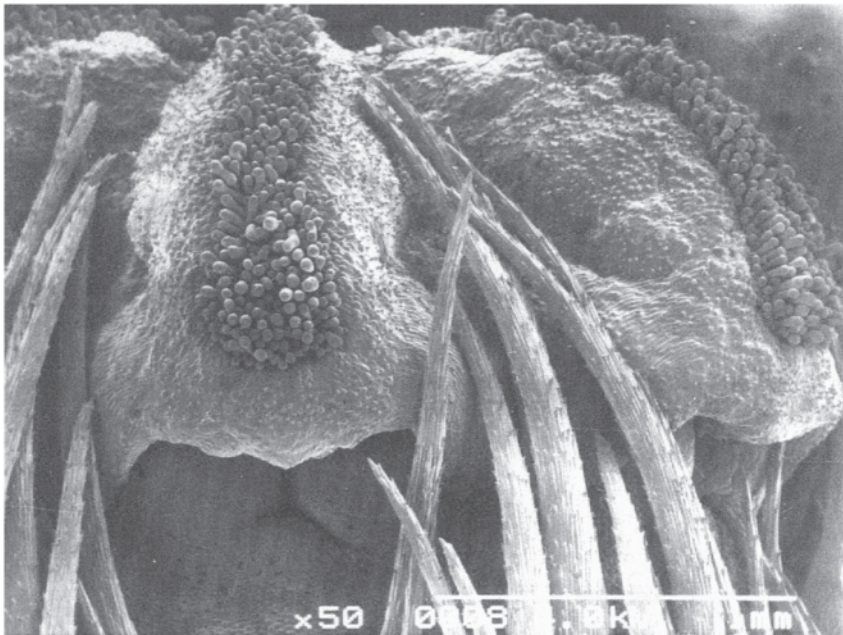
Figure 14 Stigmatic rays with stigmatic papillae of *Papaver rhoeas*

of the seeds resembles that of the seeds of *Papaver somniferum,* but the outer epidermis cells are smaller and their radial walls are wavy (Figure 15).

The species are mostly diploid, e.g. *P. rhoeas* (2*n*=14), tetraploid, e.g. *P. dubium* (2*n*=28), or hexaploid (2*n*=42) in *P. dubium* ssp. *dubium* (Kadereit, 1990). Hybridization often occurs among *P. rhoeas P. carmeli, P. humile* and *P. umbonatum.* Spontaneous hybrids occur quite often, for example *P. rhoeas*×*P. dubium*=*P. exspectatum* Fedde; *P. rhoeas* var. *rhoeas*×var. *strigosum* Boenn=*P. feddeanum* K. Wein recognized in Harz (Markgraf, 1986; Kadereit, 1990). The uncertain delimitation of taxa results in difficulties in nomenclature as well: new synonyms have recently been reported in such a common species as *P. rhoeas* (Wendt, 1996).

According to Fehér (1932), *P. rhoeas* and *P. dubium* may exhibit pseudocleistogamy. In several poppy species dehiscence of anthers occurs in the closed bud stage accompanied by ripening of the stigma. The lack of self-incompatibility makes fertilization possible even in closed bud. In certain meteorological circumstances (cold and rainy weather) the buds remain closed during the whole developmental period of the capsule. The carpels and petals remain attached to the top of the ripening capsule as a particular visual indicator of pseudocleistogamy.

6.9.2 Phytochemical Characters

This group is chemically quite unclear. The rhoeadine content (Kalav and Sariyar, 1989) and the various latex colours in *Papaver rhoeas* allies and the existence of
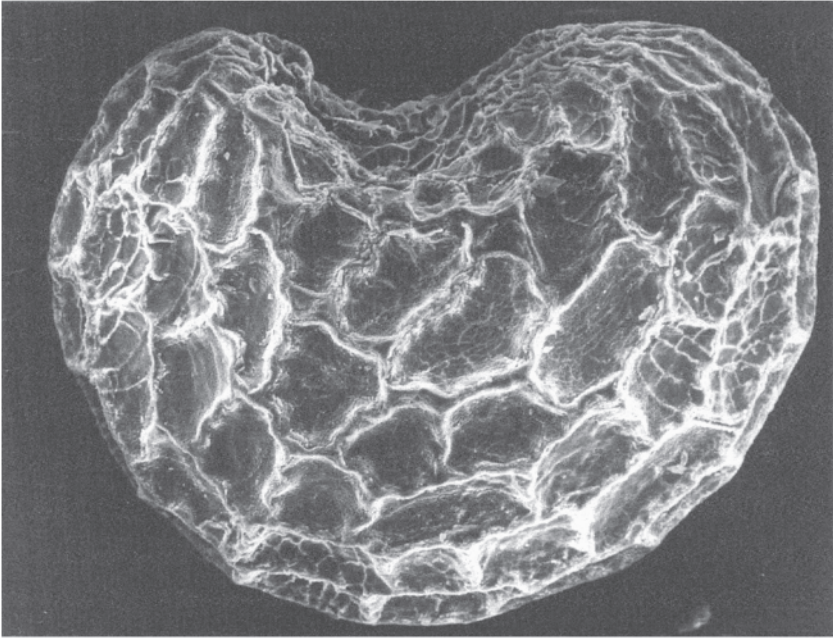
Figure 15 Seed of *Papaver rhoeas*

different phthalide, morphinoide and aporphine alkaloids in *P. arenarium* illustrate the great chemical diversity of these species. Thebaine has also been detected in a tetraploid cytotype of *P. dubium* (Espinasse, 1981).

Studies of this section have shown the occurrence of chemotypes (Preininger, 1986). One chemotype contains rhoeadines, the other aporphines and proaporphines. The occurrence of latericine in this section (in *P. californicum*) is interesting because this compound was formerly detected in the section *Pilosa*.

6.9.3 Species and Geographical Distribution

About 16 species and their allies have been reconized in this section (Table 9). *P. postii* and *P. rumelicum* may be biennial (Günther, 1975).

The native distribution area of this group is bicentral: species occur in South West Europe and North West Africa, and in areas from the East Mediterranean to Central Asia. According to Kadereit (1988a, 1990), *P. rhoeas* and *P. dubium* were introduced to Europe along with cereal crops.

6.10 Sectio *Macrantba* (Elk.)=Oxytona Bernh.

6.10.1 Morphology and Kaiyology

The species in this section are characterized by their scapose perennial habit. They have a rosette with long, thick and bristly pinnately dissected or incised leaves, a

Table 9 Species of sectio *Rhoeadium*

Species	Subspecies	Synonym
P. albiflorum Pacz.	*Albiflorum*	
	austromoravicum Kubát	
P. atlanticum Ball.		
P. arenarium Marsch. et Bich.		
P. commutatum Fisch et Mey.		
P. guerlekense Stapf		
P. stylatum Boiss et Bal. ex.Boiss		
P. carmeli Feinbrun		
P. clavatum Boiss and Hausskn. ex Boiss		
P. dubium L.		*P. confine* Jord.
P. dubium	*dubium* Kadereit	
	laevigatum (M. Bieb.) Kadereit	
	erosum (Litv.) Kadereit	
	albiflorum (Boiss) Dust	
	glabrum (Royale) Kadereit	
	lecoqii (Lamotte) Syme	
P. lacerum Pop.		
P. litwinowii Fedde		
P. rhoeas L.		*P. intermedium* Bedker O Ktze,
		P. strigosum Schur.
		P. ramosissimum Fedde
P. rechingeri Kadereit		
P. pinnatifidum Moris		
P. purpureomarginatum Kadereit		
P. umbonatum Boiss.		
P. postii Fedde		
P. rumelicum Velen		
P. syriacum		
P. humile Fedde(Kadereit 1988)		

thick main root with fleshy lateral branches and a long, almost always unbranched, flower axis (Troll, 1964). The growth habit is shown in Figure 11.3.

The morphological peculiarity of this section is that overwintering buds are situated axillary in the central rosette leaves while in other sections these buds develop distally on the thickened ends of branches (Günther, 1975).

According to Nyman and Bruhn (1979) the species also differ in the position of the upper leaves, the bracts, the flower buds and petals.

The uppermost leaf insertion is on the upper third of the stem in *P. bracteatum* and *P. pseudo-orientale,* and in the median area in *P. orientale*. The leaves are hypostomatic in *P. bracteatum,* with multicellular, multiseriate hairs on the adaxial epidermis. Bracts are found in *P. bracteatum,* several bracts may occur in some individuals of *P. pseudo-orientale,* while in *P. orientale* bracts are absent.

Table 10 Species of sectio *Macrantha*

Species	Synonym
Papaver bracteatum Lindl.	*P. lasiothrix* Fedde
P. orientale L.	*P. paucifoliatum* (Trautv.) Fedde,
	P. orientale var. *paucifoliatum* Trautv.
	P. orientale var. *parviflora* Busch.
P. pseudo-orientale (Fedde) Medv.	

P. bracteatum and *P. pseudo-orientale* display erect flower buds. The pendulous bud of *P. orientale* straightens only just before flower opening. The flowers of *P. bracteatum* have red petals with long, dark, stripe-like basal marks. The other two species have orange-red petals; basal marks may or may not be present in *P. pseudo-orientale* while they are absent in *P. orientale*. The stigmatic disc is conical in *P. bracteatum* but is more or less flat in the other two species.

The ploidy level provides clear delimitation of species in this section. According to a systematic study by Goldblatt (1974), the hexaploid *P. pseudo-orientale* ($2n=42$) is regarded to be a transient form between (or an allopolyploid hybrid of) the diploid *P. bracteatum* ($2n=14$) and the tetraploid *P. orientale* ($2n=28$).

6.10.2 Photochemical Characters

P. bracteatum accumulates thebaine as its dominant alkaloid. Alpinigenine, codeine, protopine and some other alkaloids exist to a much lesser degree (Nyman and Bruhn, 1979; Meshulam and Lavie, 1980). In *P. orientale* isothebaine is a characteristic alkaloid (Vágújfalvi, 1970) and mecambridine has also been reported (Phillipson *et al.,* 1981a). The main alkaloids in different populations of *P. pseudo-orientale* are isothebaine (Tétényi, 1986), macranthaline, orientalidine and salutaridine (Sariyar and Baytop, 1980). In species of this group morphinane-type alkaloids are detectable only in traces or are completely lacking (Böhm and Nixdorf, 1983). The most important and intensively studied species of this section is *P. bracteatum,* for its thebaine content and its lack of morphine. The great chemical diversity of this section is indicated by the existence of at least four chemical races (where isothebaine-salutaridine, thebaine-salutaridine, macranthaline and salutaridine are the major alkaloids), and some chemovarieties (Hubert *et al.,* 1994).

6.10.3 Species and Geographical Distribution

This group contains only three species (Table 10) which occur in different areas of Caucasus, Iran and Turkey. *P. bracteatum* tolerates dry habitats, *P. orientale* can be found in alpine conditions and *P. pseudo-orientale* prefers moist situations.

6.11 Sectio *Papaver* (Tourn.) L.

6.11.1 Morphology and Karyology

The members of this section are annuals with more or less branched flower axis. The habit is shown in Figure 11.4. The lower leaves are usually petiolated and the upper

Figure 16 Cauline leaves of sectio *Papaver*. 1, *P. somniferum* ssp. *somniferum;* 2, *P. somniferum* ssp. *setigerum;* 3, *P. glaucum,* 4, *P. gracile*

leaves take various forms from dissected to undivided (Figure 16), sessile, or strongly auriculate—amplexicaulous (Kadereit, 1986a,b). Plants may be glabrous or with different setae covering various organs. The petals are rotund or reniform, of various colours (from white to dark violet) and sizes, and basal marks may exist (Figure 17). The filaments are filiform or clavate. The capsules are globose or ovoid with flat stigmatic discs; they can be poricidal or indehiscent (Figure 18). The stigmatic rays, which can be overlapping or non-overlapping, occur in very diverse forms. The seeds are reniform and again are found in various colours.

The ploidy level in this section is between $2x$ and $8x$. Species are self-compatible with the exception of *P. glaucum.*

The latest revision on the section *Papaver* was reported by Kadereit (1986a). According to his findings, four species are found in this section; these are listed in Table 11 along with their subspecies and synonyms (Table 11). The morphological characteristics of the species of the section are summarized in Table 12.

Based on morphological traits, the delimitation of species is not convincing. According to Kadereit (1986a) this is the consequence of the close genetic correlation between taxa: *P. somniferum* is regarded to be an intermediate between taxa similar to *P. glaucum* and *P. gracile* and hypothetically it is a triploid hybrid of these species. The segregation of ancestral traits has resulted in the appearance of subspecies *somniferum* and *setigerum. P. decaisnei* is a hexaploid derivative of the triploid *P. somniferum.*
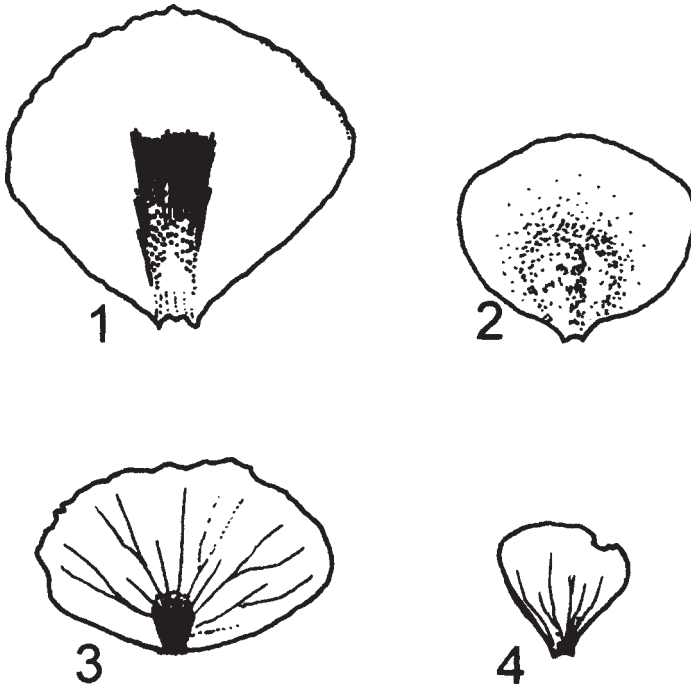
Figure 17 Petal shapes in sectio *Papaver*. 1, *P. somniferum* ssp. *somniferum*; 2, *P. somniferum* ssp. *setigerum; 3, P. glaucum*; 4, *P. gracile*

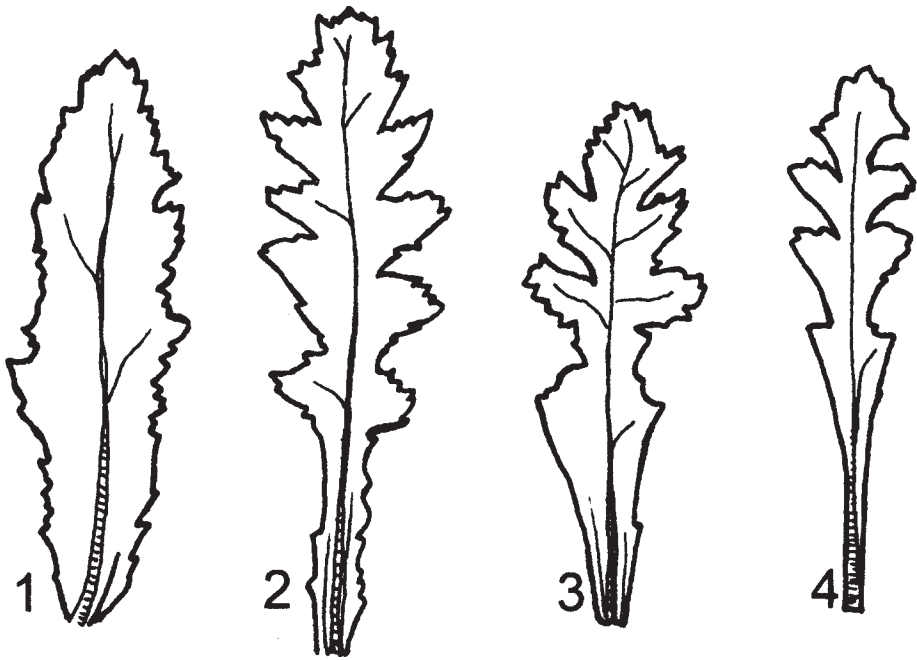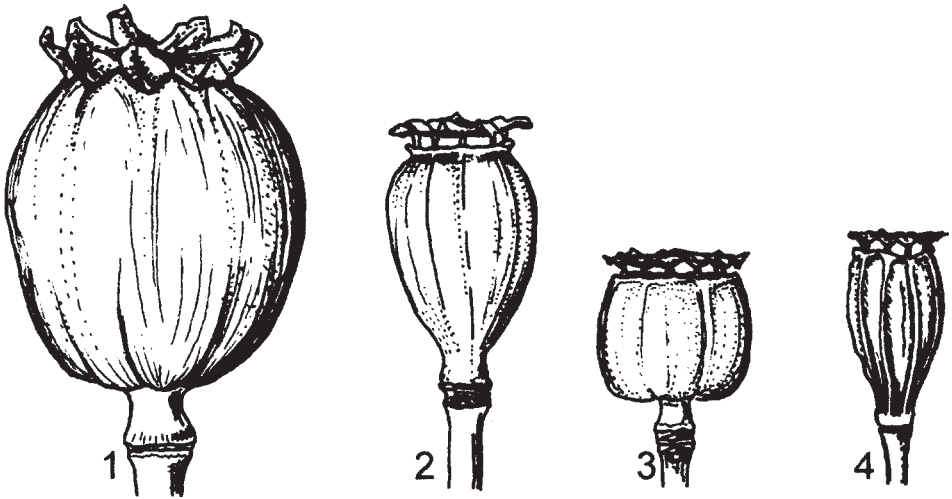**Figure 18** Shape of the capsules in sectio *Papaver*. 1, *P. somniferum* ssp. *somniferum*; 2, *P. somniferum* ssp. *setigerum; 3, P. glaucum* 4, *P. gracile*

Table 11 Species of sectio *Papaver*

Species	Subspecies	Synonyms
P. glaucum Boiss. and Hausskn.		*P. rogersii* Exell.
		P. gaube Cullen and Rech.
P. gracile Boiss.		
P. decaisnei Hochst.Steud. ex Elkan		*P. decaisnei* var. *dielsianum* Fedde
P. somniferum L.	ssp. *somniferum* Kadereit	*P. album-et-nigrum* Crantz
		P. amplexicaule Stokes
		P. somniferum var. *album* DC.
		P. indehiscens Dumort
		P. somniferum var. *hortense* Hussenot
		P. somniferum var. *stipitatum* Hussenot,
		P. somniferum var. *leptocaulotum* Fedde
		P. somniferum ssp. *songaricum* Basilevs.
		P. somniferum ssp. *indicum* (Vesselov.) Rothm.
		P. glaucum ssp. *indicum* (Vesselov) Rothm.
	ssp. *setigerum* (DC.) Corb.	*P. setigerum* DC.
		P. setigerum var. *cylindrocarpum* Fedde

6.11.2 Phytochemical Characters

This section is extremely heterogenous in its chemical composition. On the basis of the presence or absence of morphinanes it can be divided into two groups: *P. somniferum* and *P. decaisnei* are characterized by the presence of morphine alkaloids, thebaine, codeine and morphine, with narceine; *P. glaucum* and *P. gracile* lack morphinanes, but rhoeadine and papaverrubine occur to a greater extent (Preininger et al., 1981; Preininger, 1986). In *P. somniferum* ssp. *setigerum* both morphinane and rhoeadine have been detected (Fairbairn and Williamson, 1978). *P. somniferum* also accumulates oripavine (Nielsen et.al., 1983).

6.11.3 Geographical Distribution

The geographical distribution of this group is quite clear. *P. somniferum* ssp. *setigerum* occurs in the West Mediterranean region, *P. glaucum* is the native species of the Irano-Turanian region, *P. decaisnei* is spread from Sudan to Iran, Pakistan and Afganistan and *P. gracile* is found in South-East Mediterranean areas.

Table 12 Morphological characteristics of species in sectio *Papaver*

Characteristic	*P. somniferum*	*P. gracile*
Plant height	15–150 cm	30–70 cm
Branching	unbranced or branching in the upper 2/3	unbranced or branching in the upper 2/3
Plant colour	green or glaucous	green or glaucous
Leaf size	2 × 1–33 × 18 cm	2.5 × 1.5–9 × 2.5 cm
Leaf outline	obovate, elliptical, ovate, oblong, cordiform	obovate, oblong, ovate
Leaf form	deeply divided or incised, serrate or dentate, obtuse or acute, apiculate	deeply divided, incised, serrate or crenate, obtuse or acute
Lobes	antrose, ovate, oblong, serrate, dentate, crenate, apiculate	antrose, ovate, triangular, incised, crenate or entire, obtuse or acute
Upper leaves	sessile, with amplexicaulous base	sessile with amplexicaulous base
Hairs on leaves	setose or glabrous	sparsely and densely setose
Pedicel	5–26 cm long, with patent or appressed setose	15–35 cm, glabrous, with patent or appressed hairs
Buds	ovoid to oblong, glabrous or setose, 1 × 0.7–3 × 2 cm	ovoid to elliptical, glabrous, few setae, 0.8 × 0.5–1.2 × 0.7 cm
Petals	broadly obovate to round of different colours and with markings	obovate, pink
Filaments	filiform or clavate, whitish to black	filiform, light red
Anthers	elliptical, ovoid, yellow to greenish brown	oblong, light blue
Capsule	obovoid, elliptical, cylindrical, ovoid, globose. stipitate or not, poricidal	narrowly obovoid, indistinctly stipitate 5 × 2.5–8 × 4 cm
Stigmatic disc	5–22 rays, free lobes	4–8 rays, without free lobes
Seeds	reniform, yellowish to blackish	reniform, brown, glaucous
Plant height	10–70 cm	10–35 cm
Branching	unbranched or branching, often from the base	branched from the base
Plant colour	strongly glaucous	glaucous
Leaf size	2 × 1–13 × 4 cm	1.2 × 0.5–8 × 3,5
Leaf outline	elliptical, oblong, ovate	obovate, elliptical, ovate
Leaf form	deeply divided to incised, coarsly serrate or crenate, obtuse or acute	divided, obtuse, acute
Lobes	antrorse, ovate, triangular, linear, serrate or entire	antrorse, ovate to oblong, serrate or entire, obtuse to acute
Upper leaves	sessile, with amplexicaulous base	sessile with rounded base
Hairs on leaves	glabrous	glabrous
Pedicel	17–25 cm, glabrous or with appressed setae	10–25 cm, glabrous, few patent

Table 12 (*continued*)

Characteristic	*P. somniferum*	*P. gracile*
Buds	ovoid to elliptical, 1.5×0.9–3×2 cm	ovoid to elliptical, 0.7×0.4–1.2×0.8 cm
Petals	broadly reniform, elliptical, dark red	obovate, red
Filaments	filiform, dark violet or blackish	filiform, dark violet or almost black
Anthers	greenish brown, with globose appendage	oblong, brown, with small apical appendage
Capsule	broadly obovoid, ovoid, elliptical, cylindrical, 8×6–13×7 cm	narrowly to broadly obovoid, 8×3.5–16×8.5 cm, stipitate
Stigmatic disc	5–14 stigmatic rays, free lobes	4–8 rays, short free lobes
Seeds	reniform, brown	reniform, glaucous

The geographical distribution was used for intraspecific classification of *P. somniferum* by Basilevskaja (1928). She separated eight subspecies. Seven of them—ssp. *songoricum,* ssp. *tarbagaticum* ssp. *turcicum,* ssp. *austro-asiaticum,* ssp. *chinense,* ssp. *tianchanicum,* ssp. *Eurasiaticum*—contain varieties of a distinct area and the last one—ssp. *Subspontaneum*—includes the cultivated taxa provar. *oleiferum* and provar. *opiiferum.*

7 AFFINITIES BETWEEN THE SECTIONS

Considering the great diversity of *Papaver* genus, it is not easy to find characteristics which can be used to organize the sections. Following the data of Kadereit (1988a,b) a possible arrangement of affinities between the sections based on their morphological differences and similarities is shown Figure 19.

8 CLASSIFICATION OF DOMESTICATED *PAPAVER SOMNIFERUM*

Danert (1958) divided cultivated *P. somniferum* at subspecific level into four convarieties on the basis of the colours of petals and seeds and the shape of stigmatic rays as follows: (I) convar. *somniferum* with 14 varieties; (II) convar. *nigrum* (Hayne) Alef.; (III) convar. *orientale* Danert; and (IV) convar. *rotundilobum* Danert each with 12 varieties.

The subspecies of *P. somniferum* show transient morphological characteristics among species of the sectio. The differences among them are mostly quantitative, e.g. the chromosome number in ssp. *Somniferum* is 20 (Hrishi, 1960) or 22 (Hammer and Fritsch, 1977).

Using the system described by Danert (1958), Hammer (1981), Maas (1986) and Hanelt and Hammer (1987) divided the species *P. somniferum into* three subspecies: ssp. *somniferum* and ssp. *songaricum* comprise the cultivated races (Table 13) and ssp. *setigerum* represents the ancestral wild one. These subspecies differ in their geographical distribution and form of their stigmatic lobes. Within a subspecies the

Table 13 Classification of cultivated *Papaver somniferum* L. (Hammer 1981, modified)

	Papaver somniferum			
	Ssp. somniferum		*Ssp. songaricum*	
Seed colour	*Indehiscent capsule*	*Dehiscent capsule*	*Indehiscent capsule*	*Dehiscent capsule*
	var. *somniferum*	var. *dinocarpum* Alef.	var. *albescens* Vess.	var. *orientale* Danert
White	var. *candidum* Vess.	var. *rubrospermum* Vess.	var. *rubicundum* Vess.	var. *apertum* Danert
Yellowish	var. *roseolum* Vess.	var. *sanguineum* Vess.	var. *rhodanthum* Vess.	var. *foratum* Danert
Pink	var. *paeonifolium* Alef.	var. *rutilum* Danert	var. *igneum* Danert	var. *fulgidum* Danert
	var. *macrocarpum* Coss.	var. *hussenotii* Alef.	var. *parmulatum* Danert	var. *maculosum* Danert
	var. *papyrinum* Danert	var. *pictiflorum* Danert	var. *apiatum* Vess.	var. *hapalanthum* Danert
	var. *clausum* Danert	var. *tenerum* Danert	var. *limboflorum* Danert	var. *palleolum* Danert
	var. *haageanum* Alef.	var. *contrasticum* Danert	var. *mundum* Danert	var. *leucomelum* Danert
	var. *coerulescens* Rothm.	var. *pallidum* Rothm.	var. *livens* Vess.	var. *gaucescens* (Rothm) Danert
Light grey	var. *oculatum* Danert	var. *madritense* Vess.	var. *holonatum* Danert	var. *praetextum* Danert
Light blue	var. *nigrum* Hayne	var. *quassandrum* Alef.	var. *rubidum* Vess.	var. *rotundilobum* Danert
Dark blue	var. *serenum* Danert	var. *spilanthum* Danert	var. *sigillatum* Danert	var. *ocellatum* Danert
	var. *subgriseum* vess.	var. *subviolaceum* Vess.	var. *nubeculosum* Danert	var. *poriferum* Danert
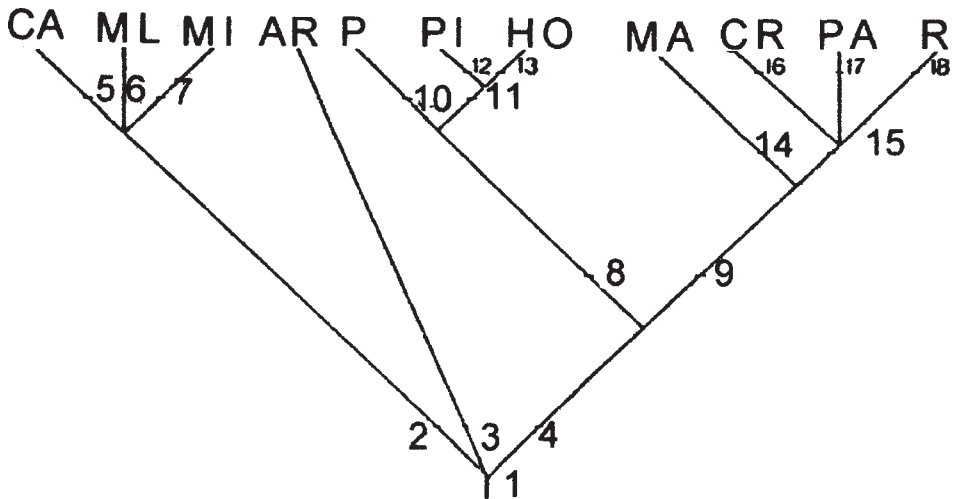
Figure 19 Hypothetical affinities between sections in genus *Papaver*. CA, *Californicum,* ML, *Meconella;* MI, *Meconidium;* AR, *Argemonidium;* P, *Pseudopilosa;* PI, *Pilosa;* HO, *Horrida;* MA, *Macrantha;* CR, *Carinatae;* PA, *Papaver,* R, *Rhoeadium*. 1, stigmatic disk; 2, valvate capsules, disc often pyramidal 3, capsules poricidal, disc with 'plug'; 4, capsules poricidal, disc without 'plug'; 5, annual; 6, perennial; 7, biennial; 8, capsules, narrow leaves yellow, filaments; 9, black filaments; 10, leaf vernation revolute, long pedicel; 11, leaf vernation convolute, short pedicel; 12, perennial; 13, annual; 14, perennial; 15, annual; 16, deciduous stigmatic disc; 17, upper leaves amplexicaulous; 18, upper leaves petiolated or sessile

varieties can also differ in dehiscence or indehiscence of capsules and in seed colour (in modern cultivars indehiscent capsules and light-coloured seed are typical).

Such a classification system is, however, not perfect as the characteristics used are influenced by domestication and high levels of combination—it is therefore difficult to give the exact taxonomic status.

ACKNOWLEDGEMENTS

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2. MORPHOLOGICAL-ANATOMICAL ASPECTS

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1 VEGETATIVE ORGANS

1.1 Root

From the radicle develops a taproot system with numerous lateral branches (Figure 1). In the primary state the stele is diarch, delimited by the pericycle. In the endodermis there is a casparian strip, the primary cortex contains parenchyma, with cells of thin cellulose walls and with many intercellular spaces. In the absorption zone the primary root is covered by a typical rhizodermis.

In transversal sections of the branching zone, where the root is in the state of secondary thickening, the main mass of the xylem is parenchyma. The xylem vessels
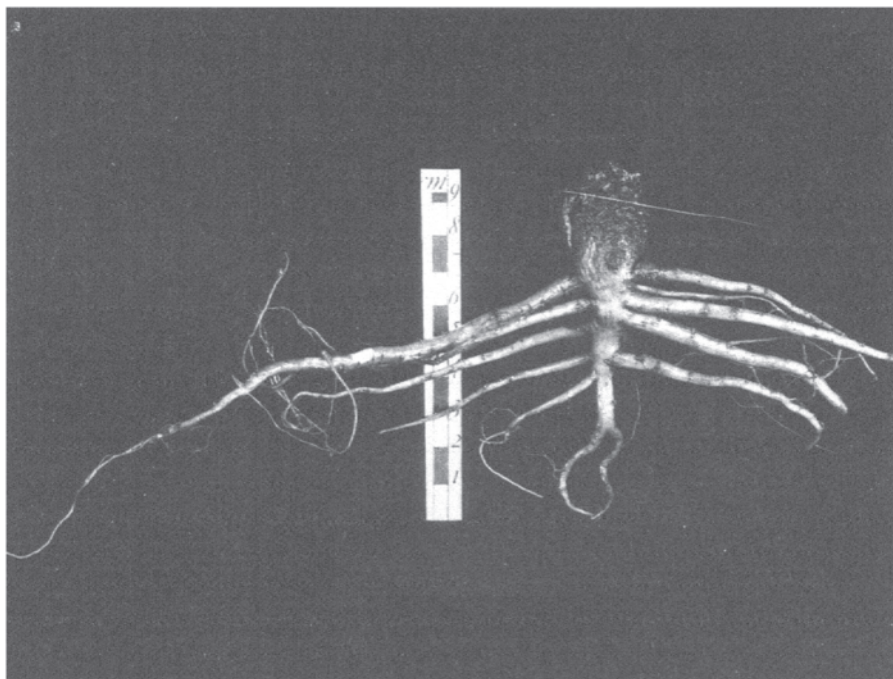
Figure 1 Taproot system of *P. somniferum*

47
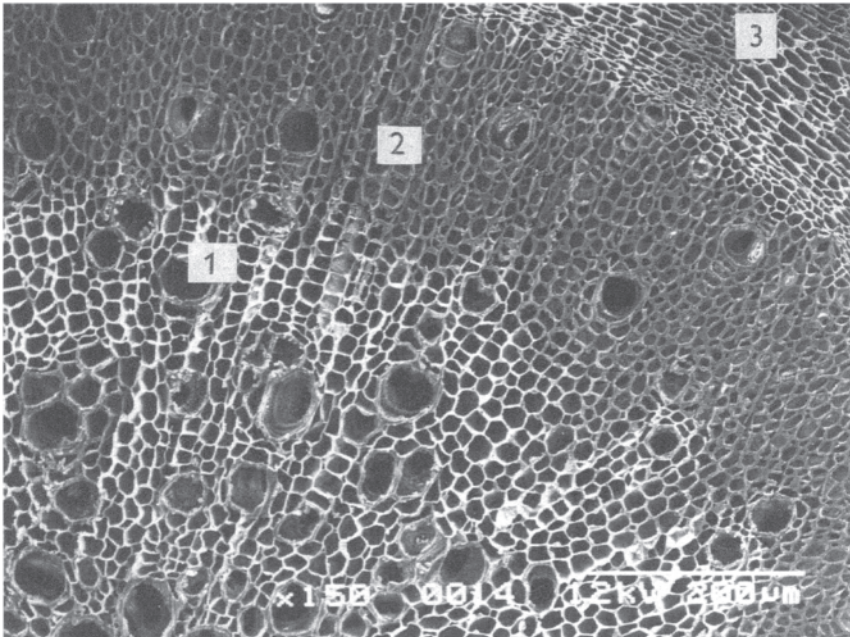
Figure 2 Transversal section of the secondary root of *P. somniferum:* 1, xylem vessel; 2, xylem parenchyma; 3, phloem bundle

are single or arranged in groups accompanied by fibres. The phloem merges almost imperceptibly with the parenchyma. Both phloem and parenchyma have tangentially elongated cells; laticifers are detectable as cells of larger diameter and lesser elongated form in the phloem bundles (Figure 2).

1.2 Shoot

1.2.1 Stem

After germination for a period of a few weeks, the nodes of the stem are short, i.e. leaves are in the rosette state (Figure 3). In parallel with the appearance of floral buds, the internodes start to elongate. This elongation is quicker after fruit setting and is continuous until capsule ripening. The degree of elongation decreases basipetally.

The outermost cell layer of the transversal section of the stem is the epidermis. Under the epidermis there is a collenchymatous hypodermis and a primary cortex comprising parenchymatic layers containing chloroplasts (chlorenchyma). Cells of chlorenchyma are small, with narrow intercellular spaces.

The collateral vascular bundles (Figure 4) are arranged in concentric rings separated from each other by interfascicular parenchyma. Between two large bundles there are two or three smaller ones. These smaller bundles give projections to traces of leaves. The vessels of the xylem can easily be recognized by their thick walls and spiral secondary thickening. Vessels are embedded in xylem parenchyma.
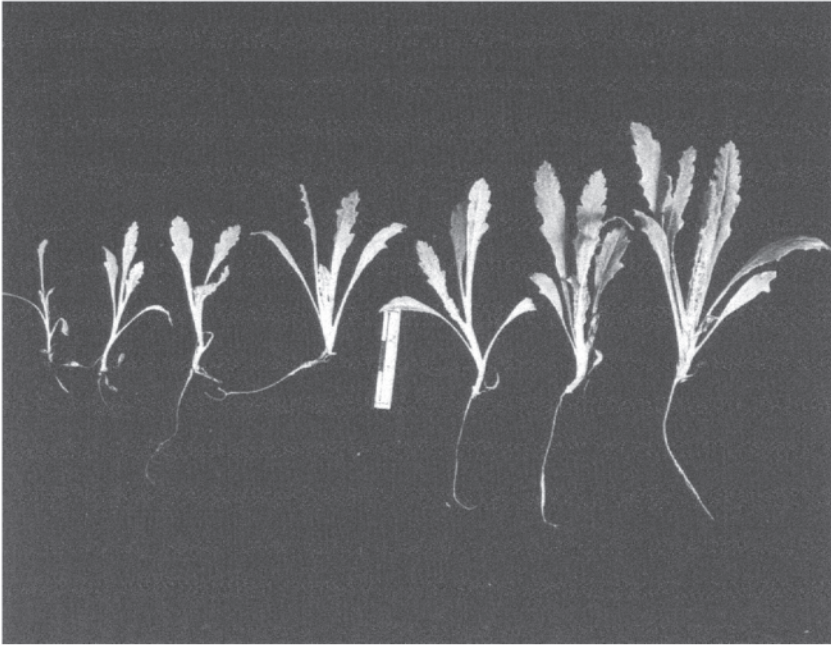
Figure 3 Rosette stage of *P. somniferum*
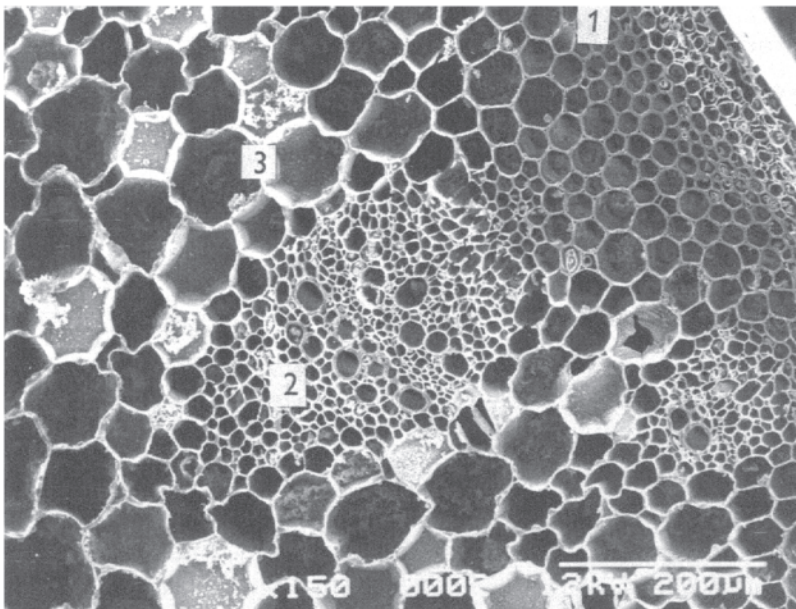
Figure 4 Transversal section of stem *(P. somniferum):* 1, chlorenchyma; 2, vascular bundles; 3, interfascicular parenchyma

The most attractive cells in the phloem are the laticifers. Their diameter is the same as that of the xylem vessels, but the cell wall of the laticifers is thin and no secondary thickening exists (Figure 5).

1.2.2 Leaf

The leaf of the opium poppy is thin and glabrous. Its margin may be serrated or biserrated (Figure 6), differing in different subspecies and cultivars. The leaf apex is acute. Leaves are more or less amplexicaulous with a cordate leaf base; they are arranged spirally on the stem, the phyllotaxy may be 2/5 or 3/8.

The upper epidermis may be papillous; the epidermal cells are straight walled, covered with a waxy cuticle. The mesophyll is bifacial with usually two layers of palisade parenchyma and several layers of spongy parenchyma. The venation of the leaves is pinnate, lateral veins form a reticular network. Vascular bundles are collateral and laticifers are associated with the phloem. The differentiated cells of the lower epidermis have sinuous walls and thick cuticles. The leaves of *Papaver somniferum* are hypostomatal. The development of the stomata is mesoperigenous (Kidwai, 1972); the stomata are anomocytic, slightly sunken.

1.2.3 Pedicel

When the apical meristem enters the generative state, the uppermost internodium elongates and it becomes curved under the developing flower bud (Figure 7). At the
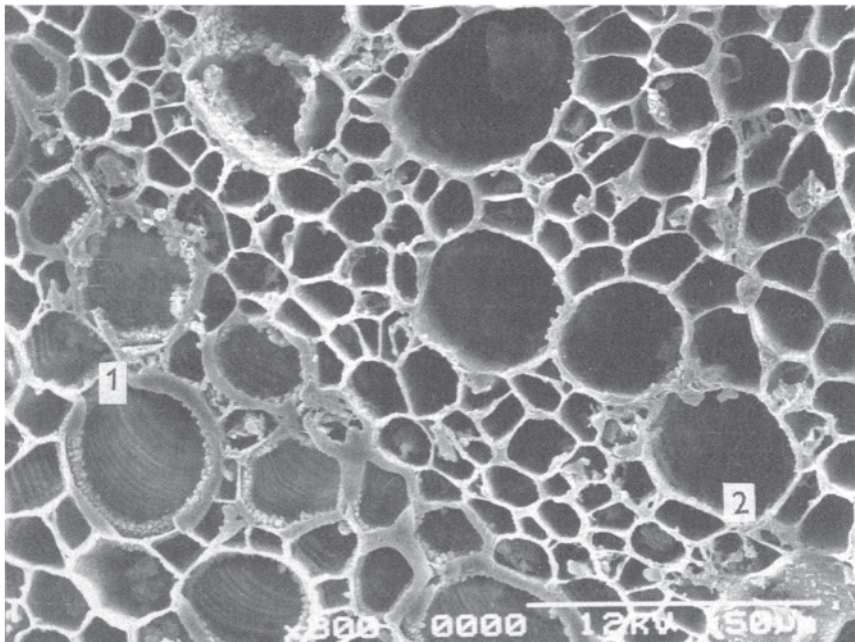
Figure 5 Collateral vascular bundle (transversal section): 1, xylem vessel; 2, laticifer
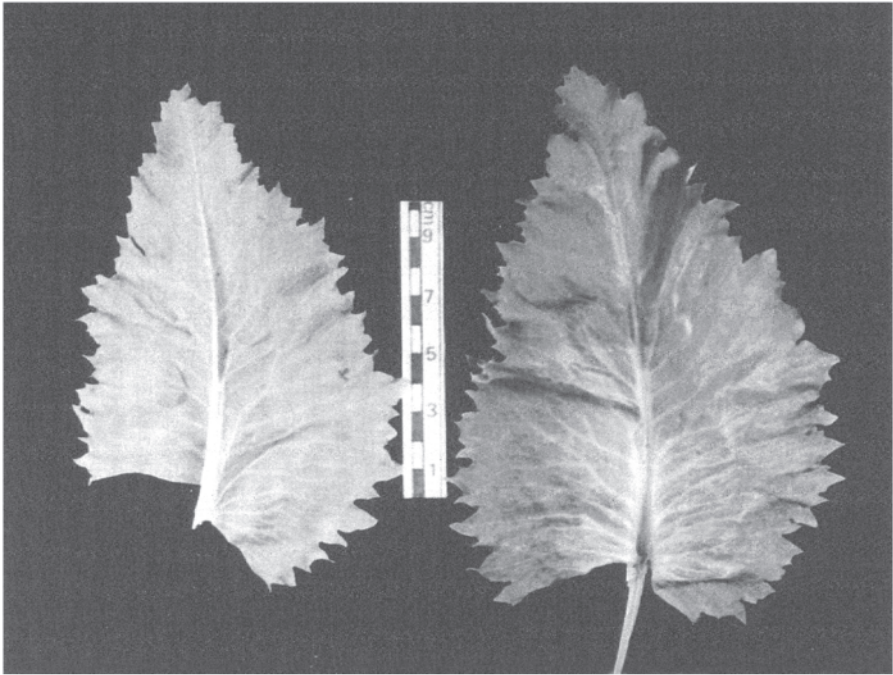
Figure 6 Leaves of *P. somnifenum*

flower opening stage it becomes erect and remains in this position during the ripening of the capsule. The anatomical structure of the pedicel is almost the same as that of the stem, the only main difference being quantitative—in the pedicel there is less mechanical tissue and the number of vascular bundles is also less than in the stem.

2 GENERATIVE ORGANS

2.1 Flower

2.1.1 Development of the Flower (Sárkány and Szalay 1964)

The first step in the development of the flower is the appearance of the two sepal initials on the base of the hemispherical apical meristem in an opposite arrangement. Triangular sepals with a wide base are positioned on the apical dome laterally surrounding the axis. The sepals overgrow the apical dome, consisting of the calyx.

The initials of the four petals emerge under the calyx. They are arranged in two whorls in a decussate arrangement. The development of the petals is slower than that of the sepals.

The anthers develop from four initials of two whorls. The 'indefinite' number of anthers is the result of the multiplication of these initials.

Figure 7 *P. somniferum* at budding stage

Numerous carpels emerge along a ring as a plate-shaped structure at the central area of the apical meristem (Fedde, 1936).

After this early developmental state begins the parallel growth and differentiation of the floral parts. Inside the more or less oval floral bud, enveloped by the calyx, the carpels form a 'cup' with placentae on their inner surface. The upper margin of the carpels form the sessile, discoid stigma, its lobes growing centripetally close to the ovary. The stamens differentiate into filaments and anthers with two bilocular thecae. Following differentiation, in parallel with the processes of microsporogenesis and microgameto-genesis in the locules of anthers and in the ovules, all floral parts show an intensive increase in size.

2.1.2 Characterisation of the Slower

The flowers of *P. somniferum* are radially symmetric and have a heterochlamydeous perianth. The calyx has a protective function and the colour of

the corolla attracts pollinators. The number and arrangement of floral parts is described by the floral diagram (Figure 8) and the floral formula which is $K_2 \, C_{2+2} \, A_{2n+2n} \, G_{(2-\infty)}$ (Fedde, 1936; Alexander, 1952).

At the opening of the floral bud the sepals are shed. The arrangement of petals in the vegetative bud (vernation) is crumpled (Weberling, 1992). After the sepals fall, the petals unfold as a result of intensive growth (Figure 9). The petals fall off within the next two days. The stamens persist for a short period after the abscission of the petals, then they shrink and dry out.

2.1.3 Morphology and Anatomy of Floral Parts

2.1.3.1 Perianth

The sepals are green in colour, their anatomical structure resembles that of the leaves, but the mesophyll is homogenous, composed of 4–10 isodiametric parenchyma cell layers. The venation of sepals consists of 7–10 main veins with collateral vascular bundles. In the phloem the phloem parenchyma is the dominant tissue, with the sieve tubes and companion cells being less well developed. Phloem fibres exist. The laticifers can be easily recognized, forming an arch in the phloem.

The four petals are decussate, arranged in two whorls. The petals of the outer whorl are larger and overlap the two inner ones. The petals are thin in cross section. The outer transversal wall of the epidermis is more or less convex and is covered with a cuticle. Stomata exist. The mesophyll involves 7–10 parenchymatic cell layers, with reticulate, weakly developed venation. The xylem consists of mainly tracheids; in the
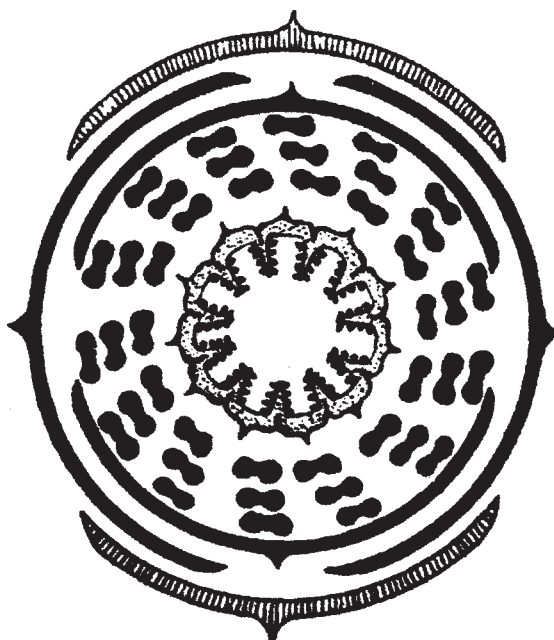
Figure 8 Floral diagram of *P. somniferum*
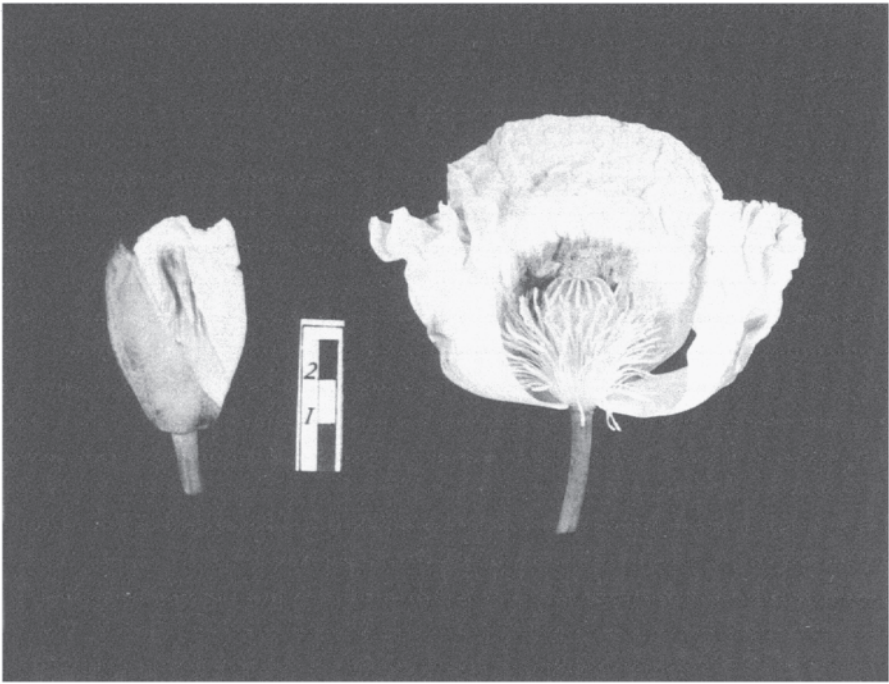
Figure 9 Flower of *P. somniferum*

phloem there are only sieve cells. Laticifers also exist (Kapoor, 1995). The colour of the petals is variable: white, pink, red, etc. in some cases with a dark basal spot.

2.1.3.2 Androecium

The numerous stamen are hypogynous and are inserted on the ring-like receptacle. The filaments are long, more or less flat and terminate at the basal area of the anthers. The anthers comprise two thecae which enclose the pollen sacs.

The filament is covered by epidermis. Stomata and cuticle are present. The vascular elements are arranged in a single concentric bundle, where in the central xylem part tracheids can be found and in the ring-like phloem sieve cells are present.

The loculamentum of the anther has a bilayered wall (exothecium and endothecium). In the loculamentum the sporogenous tissue is covered by the tapetum. The maturation of spores is followed by the longitudinal dehiscence of thecae.

2.1.3.3 Gynoecium

The ovary is globular, its situation is superior and it is connected to the pedicel by a thin gynophore. The style absent, so the sessile plate-like stigmata is on top of the ovary. Stigmatic rays are fused together at their edges. The result of this fusion are the stigma lobes. In *Papaveraceae* family the stigma lobes develop from a pair of rays

of two neighbouring carpels (commissural stigmas). The pollen-receptive papillate surface is situated along the united margin of the stigmatic lobes. *Papaver somniferum* has dry stigma. The vascular supply resembles that of the perianth.

The gynoecium of *Papaver somniferum* is paracarpous, i.e. the margins of fused carpels do not reach the centrum of the ovary on the ventral side, they only form centripetal projections called septa. The septa bear the placentae over their whole surface. The placentae are triangular in transversal section. Their tissue is loosely arranged parenchyma which contains a great number of amyloplasts.

The numerous ovules develop in rows on the placentae covering the surface of the septa. In the ovule, as a result of meiotic division, four haploid macrospores emerge. After disintegration of the three outer cells of the linear tetrad, the innermost haploid cell—the chalazal macrospore—develops into the embryo sac. The ovules are covered with two integuments.

2.2 Fruit

The characteristic fruit type of *Papaver* genus is the unilocular capsule, developing from a multicarpellate paracarp gynoecium (Figure 10). The shape of the capsule is variable: ovate, obovate, clavate, globose, etc. It is supported by a neck. At the top of the capsule is the stigmatic disc. The number of stigmatic rays is the same as that of the carpels. Under the stigmatic disc there are pores dehiscing apically (Gunn, 1980). During the development of the fruit, the ovary wall turns into the pericarp with three parts: the exocarp, mesocarp and endocarp.
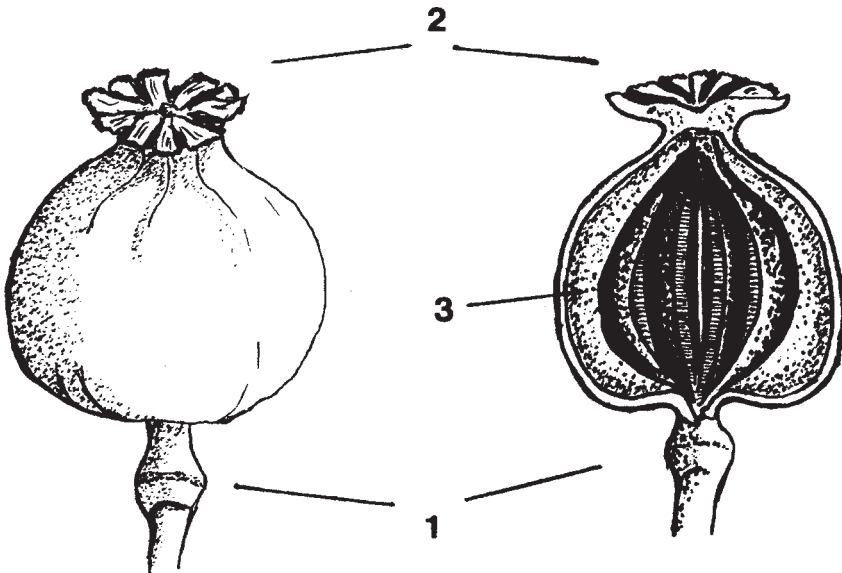
Figure 10 Capsule of *P. somniferum*. 1, receptacle; 2, stigmatic disc; 3, septum

The outer epidermis, covered by a thick cuticle, consists of polygonal cells with thick and frequently pitted walls (Brückner, 1982). The stomata are not sunken. The hypodermis is collenchymatous during maturation of the capsule and becomes sclerified on ripening. The epidermis and hypodermis together are called the exocarp.

The cells of the parenchymatic mesocarp contain chloroplasts and a highly branched vein system (Figure 11). The number of vascular bundles in the capsule is the same as that of the carpels. They form two branches: toward the mesocarp (valve bundle) and toward the placenta (placental bundle). The valve bundles are collateral and form a rich network of bundles in the mesocarp (valve traces). The laticifers of this network (Figure 12) are the main source of latex and their density increases in parallel with the growth of the capsule. The placental bundles are marginal, situated at the fusion of the carpels near to the placentae, and may be amphicribral in structure. The lateral branches (traces) of the placental bundles supply the ovules. When the traces pass into the ovules, no laticifers are present in the vascular elements. The laticifers terminate near the chalaza (Fairbairn and Kapoor, 1960). A characteristic feature of *Papaver* fruit is that the dorsal bundles are absent (Kapoor, 1973). The endocarp is a unicellular layer of tangentially elongated cells.

Following intensive growth, the capsule enters the ripening period. Anatomically this means an increase of lignification in the phloem parenchyma of the placental bundle, in the endocarp and in the ground parenchyma of the capsule and pedicel.
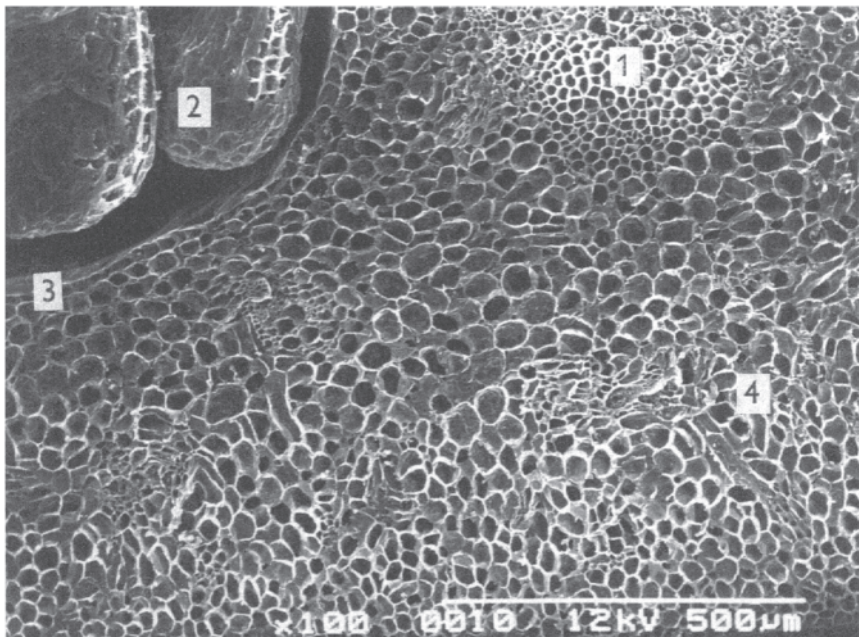
Figure 11 Transversal section of the fruit wall: 1, placental bundle; 2, seeds; 3, endocarp 4, network of valve traces
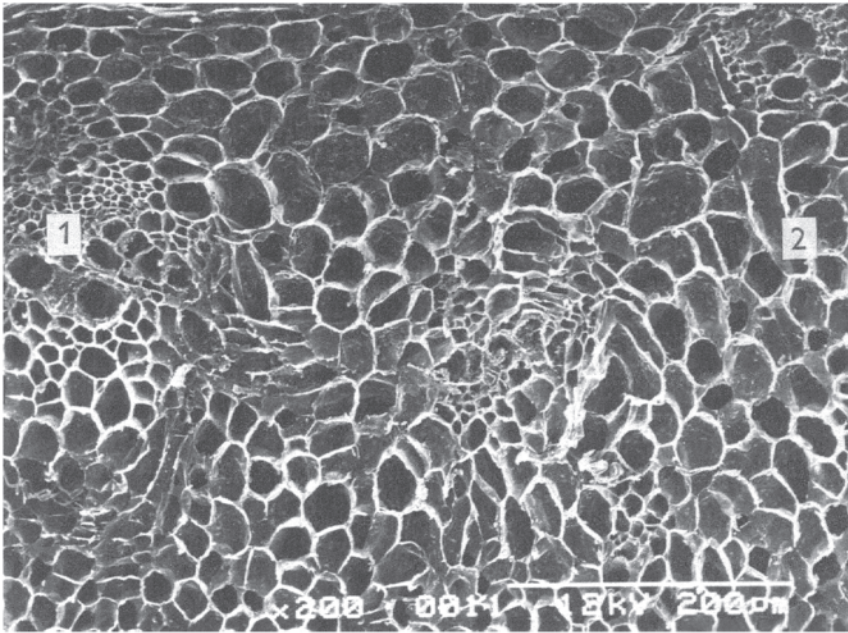
Figure 12 Network of valve traces with laticifers:1, valve trace; 2, laticifer

2.3 Seed

2.3.1 Characteristics of Seed

The seeds are narrowly or broadly reniform (Figure 13), about 1mm in length and 0.6 mm wide (Röder, 1957). The colour of the seeds is very variable; they may be white, cream, yellow—brown, red—brown, red—lilac, blue, blue—grey, grey, dark blue or almost black (Swarbrick and Raymund, 1970). The colour of the seeds may even vary inside a single capsule (Schijfsma *et al.* 1960).

Seeds develop from subcampylotropous bitegmic, crassinucellate ovules (Corner, 1976). The seed coat consists of six cell layers (Figure 14). From the outer integument develops the outer epidermis with a waxy cuticle, a crystalline layer with calcium-oxalate crystal sand and a fibrous layer with conical cells of thick cellulosic walls (Figure 15). A parenchymatous layer with thin-walled cells, a layer with cells of reticulate cell wall thickening and pigment-rich protoplasm, and finally a very thin parenchymatic layer originate from the inner integumentum (Brückner, 1983). The anticlinal wall of the outer epidermis is more or less waxy and bold (Figure 16), forming a pattern of quadrangular or irregular fields (Gunn, 1980). The periclinal wall shows special cuticular ornamentation (Röder,1957; Fritsch, 1979; Mihalik *et al.*, 1995) and this characteristic, together with the shape of the anticlinal walls, is of diagnostic value (Figure 17). The cuticle is inseparable from the outer seed coat.
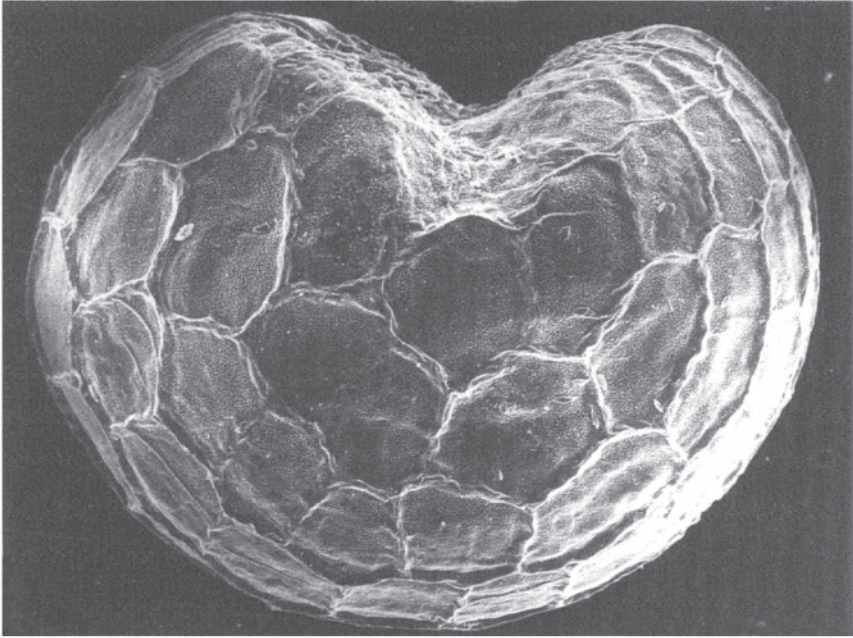
Figure 13 Seed of *P. somniferum*
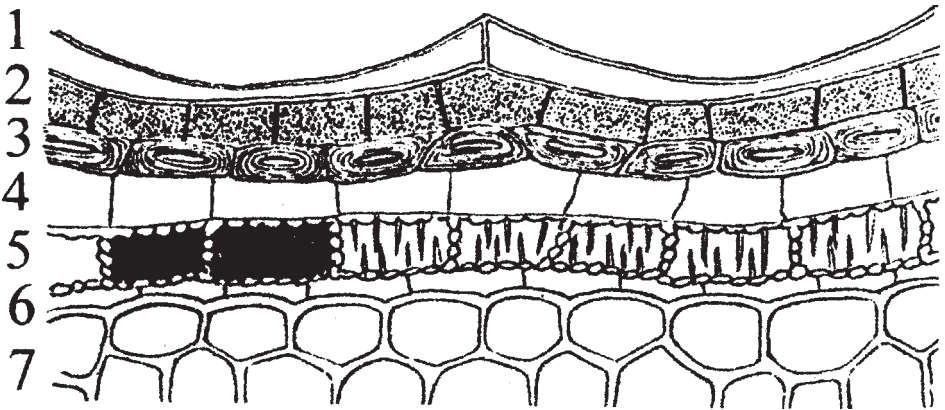
Figure 14 Schematic drawing of seed coat layers of *P. somniferum*. 1, outer epidermis; 2, crystal cells; 3, fibrous layer; 4, parenchyma layer; 5, cells with reticulate cell wall thickening; 6, inner parenchymatic layer; 7, endosperm (Fedde, 1936)

The curved endosperm is soft and fleshy and contains aleurone grains and oil. The cell wall of the endosperm is thin.

The white linear embryo is small, basal, axile, about half the length of the seed. Cotyledons are parallel and entire (McClure, 1957). The placental traces involve
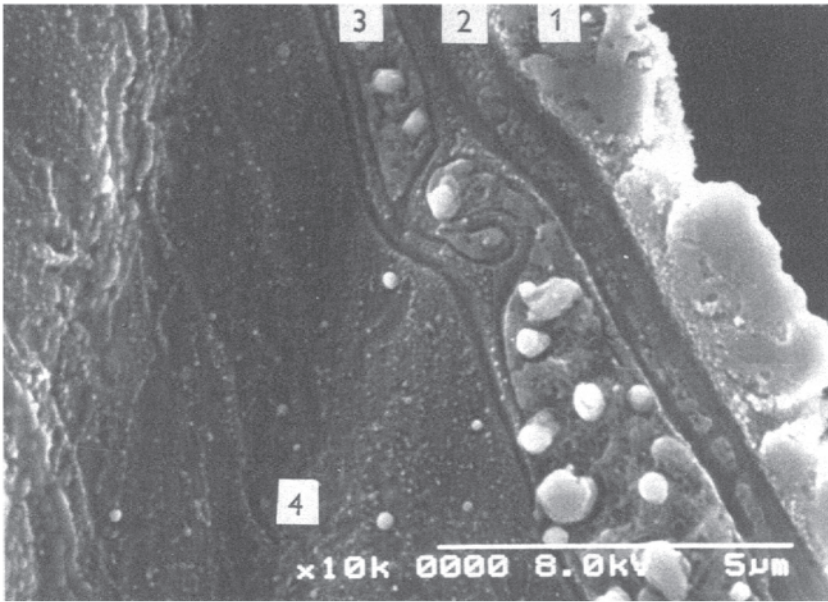
Figure 15 Seed coat layers developing from the outer integument (transversal section): 1, waxy cuticle; 2, outer epidermis; 3, crystal layer; cells with calcium oxalate crystal sand; 4, fibrous layer
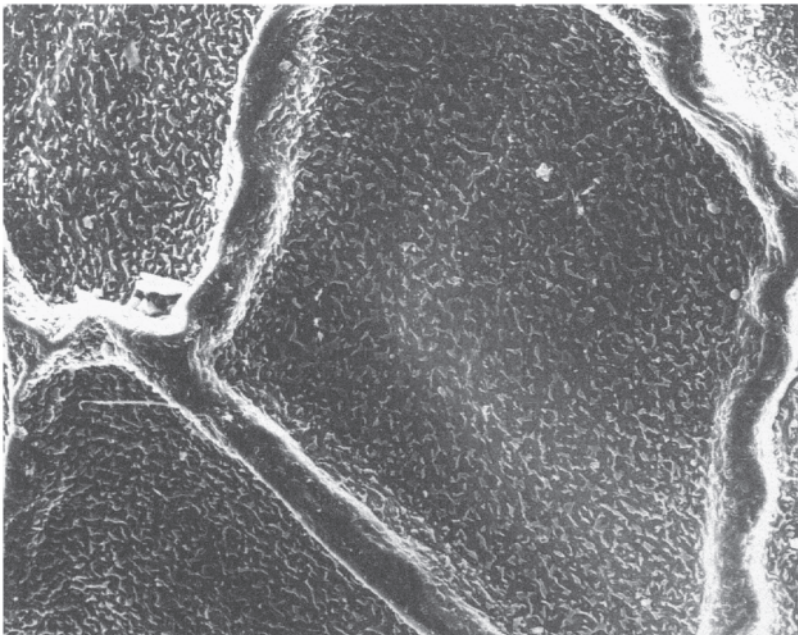
Figure 16 Surface of outer epidermis of seed coat *(P. somniferum)*
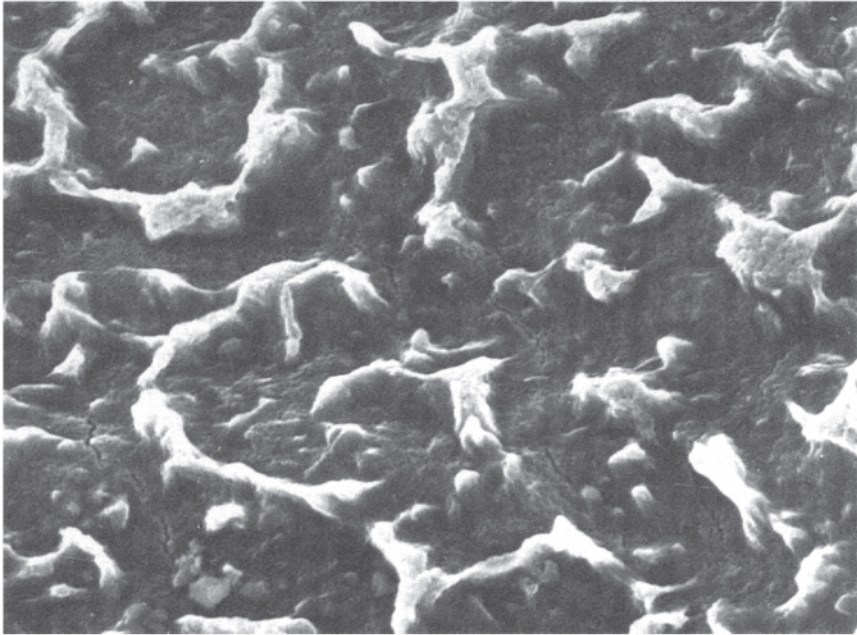
Figure 17 Cuticular ornamentation of outer epidemis *(P. somniferum)*

laticifers but they do not extend into the seed. The procambial strands of the embryo do not contain laticiferous elements—they appear only at a definite state of differentiation.

2.3.2 Germination

The opium poppy has epigeal germination. Following the appearance of the root, the epicotyl elongates, elevating the two lanceolate cotyledons, about 1 cm in length, above the soil surface (Figure 18).

3 STRUCTURE AND DEVELOPMENT OF LATICIFERS

3.1 General Characteristics

Laticifers are cells or series of connected cells containing latex of different composition (Esau, 1977). The first descriptions of laticifers in the capsules of *P. somniferum* were reported by Tschirch and Oestrele (1900) and Fedde (1936). In the genus *Papaver* the laticifers are articulated, i.e. they develop from single vertical files of parenchymaric cells as a result of absorption of their end walls. Lateral anastomoses occur between the neighbouring laticifers. These anastomoses may be complete, resulting in laticifers with increased diameter, or they may only be partial, in which case protrusions (bulges) emerge on the lateral walls of the adjacent laticifers and at the common surface of protrusions (oval orifices) which serve the anastomoses (Fairbairn and Kapoor, 1960).
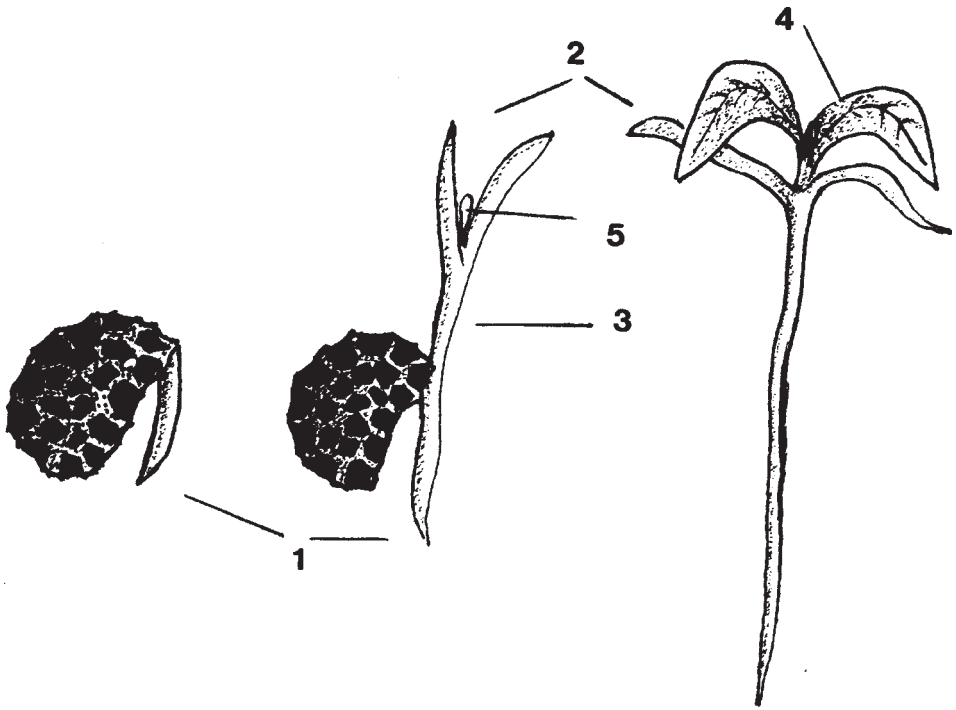
Figure 18 Germination of *Papaver* seed: 1, radicle; 2, cotyledon; 3, hypocotyl; 4, leaf; 5, leaf bud

Laticifers always occur in association with the phloem of all organs of *Papaver* with the exception of the seeds. The existence of laticifers in the filaments of the stamens only became clear in 1976, when Nessler and Mahlberg observed them using optical and electron microscopy.

3.2 Fine Structure

The laticifers of *P. somniferum* can be distinguished from the other cells by the large, irregularly shaped vesicles that are located in the cytosol. The cytoplasm also contains all the organelles of an ordinary plant cell. The ellipsoid and structurally intact nucleus is located peripherally. Mitochondria have their normal size and form. Plastids in laticifers do not contain starch, and their membrane structure resembles that of etiolated plastids in parenchyma cells. The endoplasmic reticulum is mainly rough in the early stages of development, but smooth areas are also visible. The cytosol also contains some dark unidentified particles (Thureson-Klein, 1969). Dickenson and Fairbairn (1975) described a rare organelle covered by a double membrane, with lipid inclusions and tubular structures. The function of this particle is unknown. The vesicles in the cytoplasm are covered by a unit membrane and exist in two principal

forms. The two forms never exists simultaneously in the same laticifer and Dickenson and Fairbairn (1975) regarded them as different developmental stages. The juvenile form can possibly be characterized by osmiophilic granules which adhere to the exterior of the vesicle membrane. In the older form the boundary membrane has one or more osmiophilic 'caps'.

Griffin and Nessler (1989) separated vesicles in terms of their content and mechanical stability. The cell-specific major latex protein (MLP) is deposited in fragile vesicles and isoquinoline alkaloids were detected in vesicles which were more resistant to centrifugal forces and osmotic stress.

3.3 Development of the Laticifer Vesicles

There are two models which explain vesicle formation in laticifers. The formation of vesicles from endoplasmic reticulum was suggested by Sárkány *et al.* (1964), Thureson-Klein (1969) and Nessler and Mahlberg (1976). In the early stages of laticifer differentiation, rough endoplasmic reticulum accumulates along the periphery of the plasma membrane and alternates with smooth endoplasmic reticulum. Vesicles detach from localized dilation of both smooth and rough endoplasmic reticulum and these small particles develop into larger 'capped' vesicles concentrated along the surface of the laticifer. Finally enlargement of the vesicles ceases and they become arranged irregularly in the cytoplasm.

According to the second model the vesicles originate from the central vacuole of the laticifer initials (Griffin and Nessler, 1989). The first step of this process is the filling of the central vacuole with different storage products. At this stage the future laticifer is indistinguishable from the other parenchyma cells. The only peculiar characteristic is the existence of main laticifer protein (MLP) in the vacuole, as the first sign of the further differentiation. As the vacuole grows, it begins to branch and separate into smaller vacuoles containing different storage products. This model suggests that vesicles do not arise as individual particles but are rather built from the previously existing central vacuole.

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3. PHYSIOLOGICAL-ECOLOGICAL ASPECTS

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1 PHASES OF GROWTH AND DEVELOPMENT

1.1 Characteristic Phases of Development

The length of the vegetation period of poppy depends on the ecotype, cultivar characteristics, climatic conditions and sowing time (although both the so-called 'autumn-sown' and 'spring-sown' ecotypes can in fact be sown at various terms as a consequence of the adaptability of these species (Bemáth and Tétényi, 1982)). According to their morphological and physiological features, both types produce an optimum yield only under appropriate climatic conditions and with the use of appropriate agrotechnical methods. However, 'autumn-sown' and 'spring' poppy belong to the same species and they have similar characteristic developmental phases during ontogenesis. The main difference is found in the length of the vegetation period under European conditions. The vegetation period of spring poppy lasts 120–160 days and that of the winter poppy 250–270 days. However, the actual length of the vegetation period shows large differences country by country, as shown in the crop calendar compiled by Gordon (1994) which lists 120–165 days in India, 135–250 days in Afghanistan and 178–205 days in Pakistan. Such differences are also observed in other regions of the world, for instance 150 and 240 days are reported to be necessary for the vegetation period in Mexico.

In spite of the above mentioned differences six development stages can be observed in the course of poppy development (Morász, 1979). The first stage of ontogenesis of poppy is called *the embryo or dormant stage,* which practically means that the seeds are in the pre-germinating stage. The length of this stage can last several years, since poppy preserves germination ability for 4–6 years and this can be further prolonged by storing the seeds at 4°C in a gene bank.

The next phase of ontogenesis is characterized by germination of the seeds. The *germination phase* lasts from rupture of the tesla until the appearance of the first leaves. Under optimal conditions the duration of this period is 15–20 days. Germination of the spring varieties can begin at temperatures of 2–3°C, but the optimum is 7–10°C. Spring poppy seeds germinate very badly or do not germinate at all over 20°C. The optimum germination temperature of the winter poppy, however, is around 15–20°C (Dobos and Bernáth, 1985). Germination also requires proper soil humidity, which is generally assured in open fields after sowing in spring. In this case the seedlings appear on the soil surface after 10–14 days. If the soil is cracked, the seeds have great difficulty in germinating; however, under disadvantageous circumstances an increased number of seeds can be sown, which then aid each other in germinating.

The third phase of poppy development is the *leaf rosette stage*. This lasts from the appearance of the first leaves until the emergence of the flowering shoot and the formation of generative organs. This is the longest ontogenetic period of the poppy. Under average weather conditions it lasts for 50–60 days for spring varieties, and 180–220 days for winter varieties. The duration of this stage is significantly influenced by local climatic conditions and by the cultivation techniques used, e.g. nutrient supply, sowing and thinning time. If the poppy seed is sown late (e.g. in Central Europe it is in April), the leaf rosette period becomes shorter, which has an unfavourable effect on the development of flowers and capsules, and may thus produce lower yields. Hot and dry weather conditions may have the same effect. In this period the optimal weather conditions are 12–14°C average temperatures and moderate precipitation. The number of leaves also depends on the above mentioned factors, as well as cultivar-specific characteristics.

"The next stage of ontogenesis of poppy is known as the *elongation of internodes and branching*. This stage lasts from the beginning of shooting until blossoming of the main axis and is generally about 21–30 days. The optimal weather conditions are 16–18°C average temperature and moderate precipitation. The taking up of water and nutrients is most intensive in this period.

Blossoming and the formation of capsules and seeds is the next developmental stage. On average this lasts for 20–30 days, depending on the number of lateral branches and the flowers on them. The flowers are open for 24 h, the petals being shed two days later. A further 10–14 days are needed after the shedding of flowers on the main and lateral branches until the capsules and seeds reach their final shape and size. Their growth is then terminated but the colour of the capsules is still green. During flowering, sunny, dry and warm (18–20°C) weather is favourable. After flowering (until so-called opium ripeness) more rain but warmer conditions are necessary.

The last stage of poppy development is the *ripening of the capsules and seeds* which takes 15–25 days, depending on the climatic conditions. During this period the leaves fall off, the capsules obtain their characteristic colour and the seeds will separate from the septum. Poppy seeds are ripe for harvesting when the capsule gives a rattling sound and when the foliage is dry and the stems are brittle. The optimal weather for this phase is warm (temperatures above 20°C), dry and sunny conditions. In rainy weather the appearance of fungi and leaching out of the alkaloids may cause severe problems.

1.2 Accumulation of Alkaloids During the Life Cycle

According to the majority of data in the literature, alkaloids are found in all parts of the plants expect the seed (Choudhary and Kaul, 1981). However, in some cases the presence of very small amounts of alkaloids in the seed has been proved. Analysing a commercial sample, Grove *et al.* (1976) found 0.5–1.7 ppm free morphine and 0.1–0.5 ppm codeine in the seed. More recently Hasegawa *et al.* (1992) analysed the morphine and codeine content of poppy seeds available in the Japanese market and proved the presence of these constituents at levels of 7.3–60.1 µg/g morphine and 6.1–29.8 µg/g codeine. The presence of these two alkaloids was also analysed in several kinds of food (bread and dumplings) on which poppy seeds were used for

decoration (0.39 to 4.85 g/piece). In contradiction, some other experts considered these concentrations were the result either of the presence of immature seeds in the sample or that the seed used was contaminated with latex and powder which originated from the powder of the capsule wall and the placenta. Based on the findings of Fairbairn and El-Masary (1968) it was concluded that bound forms of alkaloids may occur in the seeds and these may be metabolized into smaller alkaloid-like substances during germination.

However, much larger differences have been noted in alkaloid accumulation during the vegetation cycle. It was observed that seedlings do not accumulate any alkaloids in the radicle. From the anatomical point of view there is a structural possibility for the accumulation of alkaloids when the laticiferous vessels appear at the opening phase of the cotyledon. However, the actual accumulation starts some weeks later, after the formation of the first two leaves. The results of biotechnological investigations support the theory that the formation of alkaloids is closely connected to the differentiation processes. The accumulation of alkaloids in callus cultures is related to the degree of tissue differentiation (Kamo and Mahlberg, 1988).

There is a close correlation between the growth and development of poppy and the accumulation of alkaloids, in parallel with compositional changes. This was first reported by Vágujfalvi and Tétényi in 1967 and supported afterwards by more detailed investigations (Vágujfalvi, 1968; Tétényi, 1970; Tétényi and Vágujfalvi, 1965). Although, the plant origin and the actual climatic conditions are known to have a remarkable effect on the accumulation processes, it was noted that the level of alkaloids increases continuously during the life cycle. Two optimum points of accumulation were pointed out: before bud formation and at the start of the flowering. The interesting phenomenon that in the course of the vegetation cycle the thebaine/ morphine ratio shifted towards a higher morphine proportion was also reported.

This type of morphinane accumulation has been proved under controlled conditions in a phytotron (Bernáth, 1985, 1986). Investigating two cultivars of different origin ('Kompolti M' (Hungarian) and 'Reading' (English)) under low (8 klx) and high (16 klx) light intensities, the interaction of the ecological conditions and chemotype was determined. As shown in Figure 1, the level of morphinanes in the leaves of both cultivars increased starting at the 50th day after germination and ending at the green capsule stage. The effect of light is manifested in the more accelerated accumulation of alkaloids. During this period of the vegetation phase the accumulation level of alkaloids increased 3–4 times. The proportion of morphine—in harmony with the findings of Vágujfalvi (1968)—changed in the meantime, and its relative mass increased compared to the total alkaloid content. The importance of light intensity on this ratio of different alkaloid compounds proved to be significant: the relative mass of morphine was higher under the low illumination level in both cultivars. Interestingly, it was also observed—in agreement with the earlier results of Tookey et al. (1976)— that the qualitative character of leaf alkaloids measured at the end of the flowering phase showed much similarity to the composition of the developing capsules.

Investigations concerning alkaloid accumulation in the capsule have both theoretical and practical importance. According to Bunting et al. (1963) and Schröder (1965), maximum morphine accumulation in the capsule was reached 42 days after flowering. There is a subsequent decrease in the alkaloid level which was explained in different
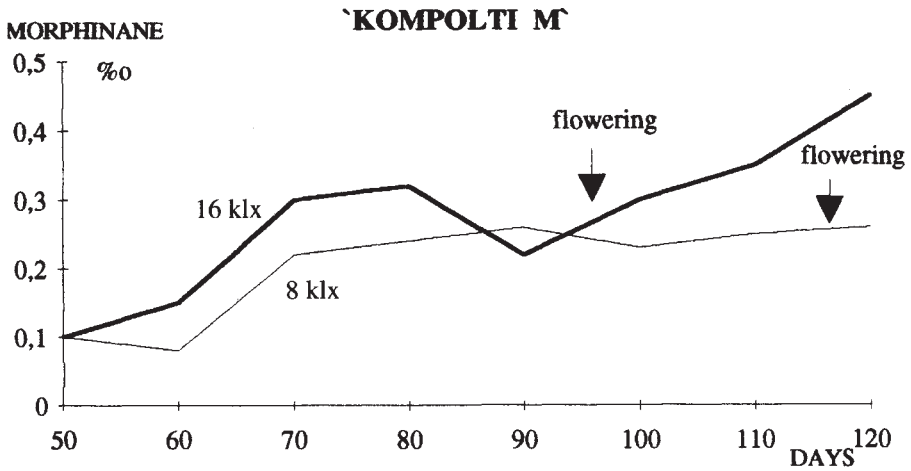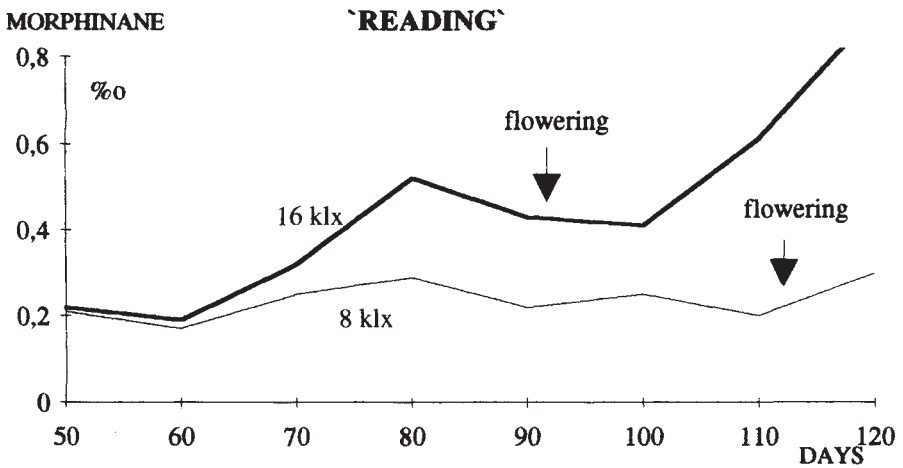
Figure 1 Changes of accumulation of morphinane alkaloids during the development of poppy cultivars ('Kompolti M' (Hungarian) and 'Reading' (English)) under low (8klx) and high (16klx) light intensity (Bernáth, 1985)

ways, such as the effect of weather conditions, infection by fungi, enzymatic degradation, etc. Hofman and Menary (1984b) studied the effect of leaching on the alkaloid content of capsules and concluded that in the absence of leaching the morphine percentage reaches its maximum 42 day after flowering and remains constant thereafter. With the occurrence of leaching in 14-day old capsules the morphine

percentage increased as a result of the dry matter loss in the leached capsule. However, when the capsules were leached much later (42–70 days after flowering) there was a significant decrease in the morphine percentage.

According to chemical analyses carried out at different developmental stages of the capsule it was proved that accumulation processes which occur in the capsule wall and those in the placenta are not identical (Bernáth, 1989). As demonstrated by the data shown in Figure 2 the alkaloid content of the placenta reaches a maximum about 16 days after flowering, before the seeds become coloured. In the capsule wall alkaloid accumulation continues and even increases for an additional eight days. After that time, simultaneously with a dramatic decrease in the water content, 20 to 30% losses in alkaloid contents of the capsule wall and the placenta were observed. The accumulation processes in the capsule wall and in the placenta thus differ significantly, and this is also reflected in compositional changes. Traces of narcotine and narcotoline were found in the placenta and not in the capsule wall. The differences in both accumulation level and composition suggest the presence of some kind of accumulation gradient in the direction of capsule wall—placenta—seed. The transport of primer substances (for seed ripening processes) is promoted over this gradient, while alkaloid translocation is hindered. This is demonstrated by the fact that the maximum alkaloid content in the capsule wall is 23.1%, in the placenta it is 14.9% and only 3–4% alkaloid content can be observed in the early stages of seed development and this level practically disappears with seed colouring.

The decrease in alkaloid content as a result of leaching at the final stages of capsule maturation was also proved in our experiments, in agreement with the results of Hofman and Menary (1984b). The leaching experiments showed the opposite tendency to the above mentioned accumulation gradient of alkaloids: larger amounts were extracted from the capsule wall, a moderate amount from the placenta with practically
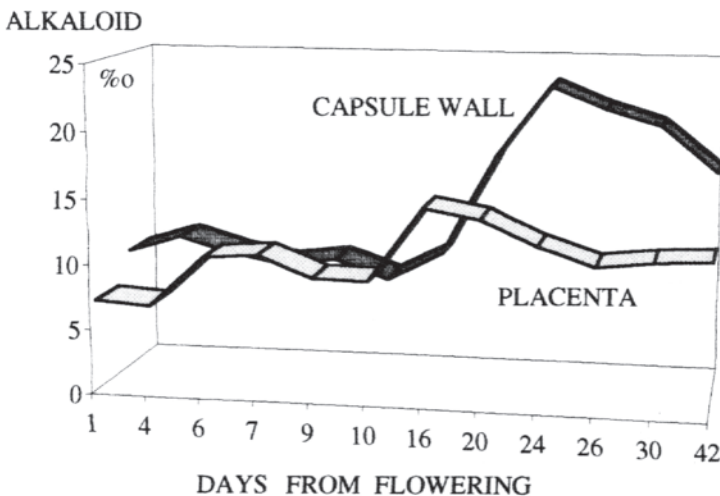
Figure 2 Changes of alkaloid content in the developing capsules of poppy characterized by the changes measured in the placenta and capsule wall (Bernáth, 1989)

no effect on the seeds. This is an important observation from a practical point of view in that the quantitative decrease is followed by compositional changes. Morphine and codeine show the highest solubility and about 60–80% could be leached. During the same 'extraction' time only half the codeine and a few per cent of narcotine and narcotoline could be extracted. Other sources of alkaloid decomposition have been described. Infection by *Alternaria alternata* decreases the dry matter content by 12% and the morphine by 48%. In the case of infection by *Embellisia* spp. 30% of the total morphine was decomposed (Hofman and Menary, 1984a).

2 PHYSIO-ECOLOGICAL REGULATION OF DEVELOPMENT AND PRODUCTION

2.1 Light

2.1.1 Photoregulation

Even in the initial stages of development, light may play an important role, although—in contrast with *P. bracteatum*—this has not been proved in the case of germination of *P. somniferum* (Bare *et al.*, 1978). However, the importance of light in the onset of flowering has been confirmed. Gentner *et al.* (1975) demonstrated, by experiments carried out in a phytotron, that the need for long days is a characteristic phenomenon of the species, although the need for a particular length of inductive period may vary depending on the origin of the variety. The data of Gentner *et al.* (1975), supplemented with the results of our investigations (Bernáth and Dános, 1983) are shown in Table 1. It is obvious that a 15–16 h light period induces flowering in all the varieties investigated. The role of a shorter photoperiod is more ambiguous: although some varieties failed to flower with a shorter period of illumination, this inhibition can be

Table 1 Flowering responses of *Papaver somniferum* to day-length in experiments of Gentner *et.al.* (1975) and Bernáth and Dános (1983)

Country of origin	Photoperiodic induction by light (h)							
	8	10	11	12	13	14	15	16
Iran (UN)	—	—	—	—	—	—	+	+
Pakistan (UN)	—	—	—	—	—	—	+	+
Turkey (UN)	—	—	—	—	—	—	+	+
Yugoslavia (UN)	—	—	—	—	—	—	+	+
Norway (UN)	—	—	—	—	—	0	0	0
Afghanistan (UN)	—	0	0	0	0	0	0	0
Thailand (UN)	—	0	0	0	0	0	0	0
'Reading' (England)	—	0	0	0	0	0	0	0
'Kék Duna' (Hungary)	—	0	0	0	0	0	0	0

[-] No data available.
[+] Positive reaction justified by Gentner *et al.* (1975).
[0] Positive reaction justified by Bernáth and Dános (1983) on 16klx intensity.

overcome by increasing the light intensity. A light intensity of 16 klx or higher is capable of compensating for the development-inhibiting effect of short day length. Generative differentiation was successfully induced under strong light intensity even in a 10-hour light cycle for certain Indian, Thai, Afghan and temperate zone varieties ('Kék Duna' (Hungarian) and 'Reading' (English)). This supports the assumption made by Gentner *et al.* (1975) that in photoregulation the so-called high-energy response plays a role, rather than phytochromes.

The interaction of day length and light intensity is also clear in the regulation of growth. On the basis of investigations carried out in a phytotron (Bernáth and Tétényi, 1979), plant growth is rather intensive under long day conditions, using high 32 klx illumination. In that case 85–95 days are required from the start of germination until the opening of the main flowers. As a result of the decreased light intensity (16 klx) the period needed for blooming becomes longer, taking about 100 days. Using low illumination (8 klx) or short day conditions (l0h illumination cycle) the development phase of the plants becomes restricted and the vegetative phase continues for 130 days or longer.

2.1.2 Effect of Light on Dry Matter Production

Few studies on the incorporation of $C_{14}O_2$ in poppy (Paul and Bassham, 1977) or on seasonal variations of photosynthetic activity (Rustembekov and Arginbaev, 1974) have been reported. On the basis of these investigations the photosynthetic activity of selected cultivars is known to be much higher than wild species. Experiments carried out under controlled conditions have indicated that the extent of dry matter production depends on both the intensity and duration of illumination (Bernáth and Tétényi, 1979). This phenomenon can also be observed in the development of vegetative and generative organs. A correlation between light intensity and mass of vegetative organs under controlled conditions has been proved (Bernáth and Tétényi, 1979)—increasing the light intensity from 8 klx to 32 klx, the dry mass production of 'Kék Duna' (Hungarian) cultivar trebled (Figure 3). This increase was followed by the changes in the organ ratio: the relative amount of stem became higher with increased light duration. In the case of capsule formation, the 8 klx light intensity in the short day cycle had an adverse effect on seed setting—only empty capsules were formed (Figure 4). The optimum capsule dry mass was obtained at 16 klx illumination. The 32 klx light intensity seemed to be higher than required for optimum yields: both the capsule mass and the ratio of seeds decreased.

2.1.3 Effect of Light on Alkaloid Accumulation

The formation of alkaloids is closely related to the primary processes, the special branches of photosynthesis, as expressed in the work of Bills (1978). Clarifying the relationship between light and alkaloid formation is extremely difficult under open field conditions, because of the complexity of the natural systems being studied. Thus, the majority of statements in this respect (Shuljgin, 1969; Morász, 1979) should be considered as observations rather than conclusions. Tookey *et al.* (1976) were the first to note changes in the alkaloid spectrum under controlled conditions in a phytotron.

DRY MASS
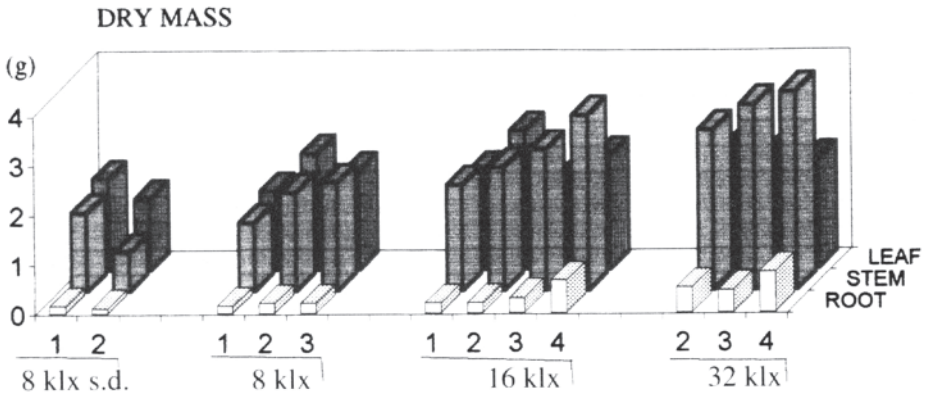
Figure 3 Effect of light conditions (intensity, day length and their combination) on dry matter production of vegetative organs of poppy. Light treatment before vegetative differentiation of apex: 1,4 klx short day; 2,8 klx short day; 3,8 klx long day; 4,16 klx long day

CAPSULE MASS
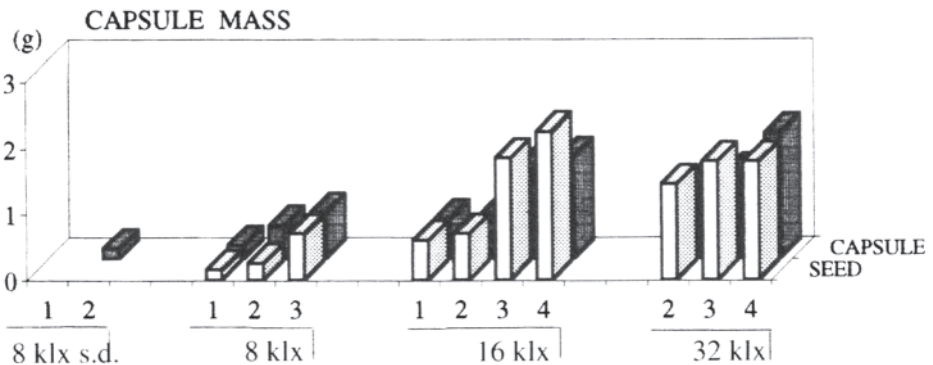
Figure 4 Effect of light conditions (intensity, day length and their combination) on the dry matter production of poppy capsules. Light treatment before vegetative differentiation of apex: 1,4 klx short day; 2,8 klx short day; 3,8 klx long day; 4,16 klx long day

In phytotron experiments (Bernáth *et al.*, 1988) comparing *P. somniferum* cultivars of different origin ('Reading' (English), 'Kék Duna' (Hungarian), 'Afghanistan'—UNL55, 'India'—UNL146, 'Thailand'—UNL55) some aspects of alkaloid accumulation which were affected by light were clarified. The special effect of light was proved for all the cultivars studied. The accumulation of alkaloids became more intensive under relatively high illumination levels (16klx) as a universal phenomenon (Table 2). In parallel with that intensification, the ratio of alkaloid compounds substituting two and three methyl groups (thebaine, codeine) also increased. The investigation carried out by Bernáth and Tétényi (1979) covered a much wider range

Table 2 Alkaloid content of dry-capsules of five cultivars measured in plants grown under different conditions (Bernáth *et al.,* 1988)

Alkaloid ‰	'Kék Duna'	'Reading'	'Indian'	'Afghan'	'Thai'
Long day+12 klx (Phytotron)					
Morphine	6.6	2.8	2.4	2.6	1.7
Codeine	0.0	0.0	0.2	0.3	0.0
Thebaine	0.1	0.0	0.0	0.0	0.0
Papaverine	0.0	0.5	0.0	1.0	1.5
Narcotine	0.0	0.0	0.0	1.0	0.5
Total	6.7	3.3	2.6	4.9	3.7
Short day+16 klx (Phytotron)					
Morphine	7.3	3.0	5.8	3.7	3.5
Codeine	0.5	0.8	0.2	0.8	1.1
Thebaine	0.7	0.1	0.3	0.0	0.1
Papaverine	0.7	0.4	0.0	1.3	1.3
Narcotine	0.8	0.0	0.0	0.9	0.3
Total	10.0	4.3	6.3	6.7	6.3
Open field conditions:					
long day+high light (Hungary)					
Morphine	7.5	3.8	3.3	5.6	3.3
Codeine	0.1	0.9	0.1	0.5	0.1
Thebaine	0.3	0.0	0.2	0.1	0.1
Papaverine	0.0	1.3	0.0	2.4	2.0
Narcotine	0.2	1.2	0.2	2.7	1.2
Total	8.1	7.2	3.8	11.3	6.7

of light intensities (between 8 and 32klx) and led to a similar conclusion. In the case of Hungarian ('Kompolti M') and English ('Reading') cultivars it was also proved that the faster development and increase of dry matter production influenced by light variations was accompanied by more intensive biosynthesis and accumulation of alkaloids. Whilst the increase of total alkaloid content as a result of increasing light intensity seems to be a general tendency, cultivar differences occur in the ratio of components (Figure 5). In the case of 'Kompolti M'—at the low light intensity level resulting in low capsule weight and low total alkaloid content—the morphine provides 100% of the morphinanes; thereafter its ratio decreases under more favourable conditions accompanied by a more intensive alkaloid biosynthesis and accumulation. The first significant accumulation of codeine occurs at 16 klx, but more significant accumulation can be seen at 32 klx. The accumulation process in the English cultivar ('Reading') shows a different picture. The amount of morphine increases to a limited level, whilst the biosynthesis and accumulation of codeine occurs continuously. This phenomenon can be explained by the biosynthetic pathway of alkaloids. The acceleration of the total alkaloid formation under the influence of light variations, which was detected in the case of morphinane, phtalid-isoquinoline and benzylisoquinoline alkaloids, can be explained by the intensification of precursor flow and methylation processes. Similarly, the low alkaloid content at low light intensity
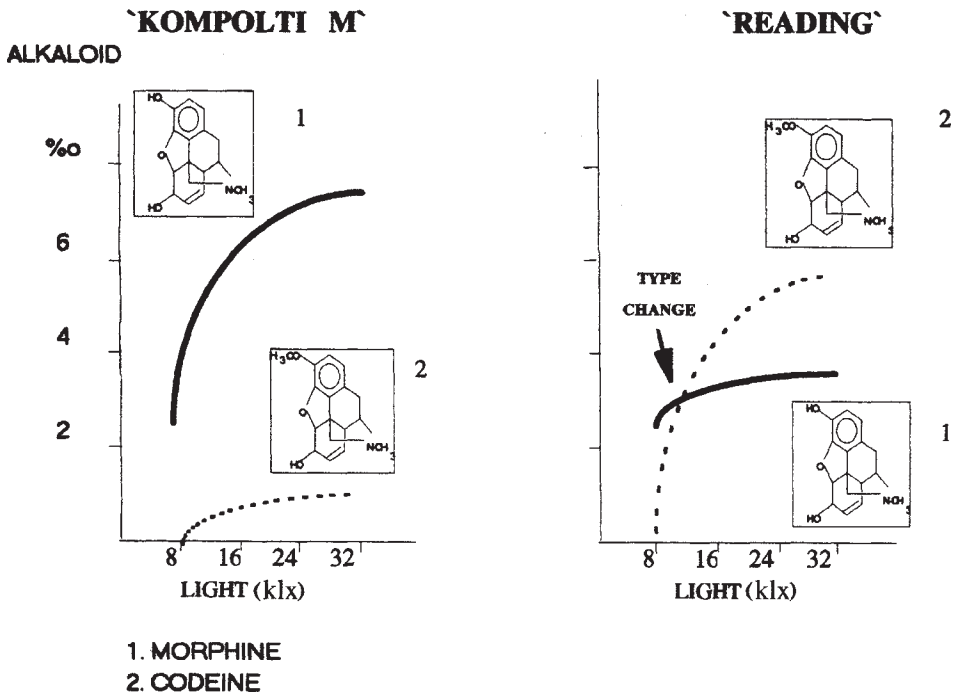
Figure 5 Ecological and chemotaxonomical differences in morphine-codeine saturation curve of cultivars 'Kompolti M' and 'Reading' generated by simultaneous increases in capsule mass and light (Bernáth and Tétényi, 1979). 1, Morphine; 2, Codeine

seems to be interpretable by the absence of precursors and by the inhibition of methylation. The proportional changes of components can be considered as a specific process and the reason for them can be searched for in the characteristic demethylation pathway and in the catalysing enzyme system of morphinanes. The precursors of the morphine group are formed in small amounts at low illumination and are able to demethylate without any difficulties almost up to morphine. On the contrary, a larger amount of precursors can be partially demethylated when the overall alkaloid biosynthesis is promoted by light. In the case of certain cultivars these changes show a different picture which can be appreciated from a chemotaxonomic point of view.

2.2 Temperature

2.2.1 Thermoregulation

Temperature plays an important role in the regulation processes, even at the germination phase. This may be manifested in the blockage (Bare *et al.,* 1978) or induction of germination, or in influencing the whole course of germination. Practical research is usually concerned with the determination of the minimum temperature required for germination or its maximum range (Földesi, 1992). A modern thermogradient

germinator was used by Bare *et al.* (1978) to determine the temperature dependence of seed germination of *Papaver somniferum* variety of Afghan origin. It was found that this variety germinated between a minimum of 8°C and a maximum of 35°C. Other investigations (Dobos and Bernáth, 1985) have indicated that the germination of *Papaver somniferum* varieties and their requirements reflect their earlier adaptation processes. In concurrence with the conclusions of McNaughton and Harper (1964), who studied the germination biology of *P. dubium, P. hybridum, P. rhoeas* and *P. argemone,* the germination of the 'wild' types needs special circumstances. In the case of these species cold treatment (5°C for 2–7 days) or variation of the night/day temperature regime may increase the germination power and the percentage of germinated seeds. The 'Spanish' populations taken from the UN collection showed a 'wild' characteristic from another point of view (Figure 6). The seed had a very low germination power and the inhibition of germination at low (no germination at 5°C) and relatively high temperature regimes (25–30°C) was also noted. Comparison of the germination characteristics of spring ('Kék Duna' (Hungarian)) and autumn types ('Ankara' (Turkish)) shows another aspect of adaptation. The optimum range for the spring variety is between 10 and 25°C, but this shifts to 15–30°C for the variety with an autumn—spring cycle. The higher heat sensitivity of the spring variety is also apparent— a temperature of 30°C causes partial inhibition of germination, while 35°C results in total inhibition. Interpreting this form of adaptation, spring varieties have a protective mechanism to prevent themselves from germinating at higher temperatures and are capable of rapidly initiating their physiological processes in early spring.

The thermoregulation of later stages of development has received less attention. However, the importance of temperature during the later phases has been confirmed by both practical observations during cultivation and a certain amount of research carried out on this subject. Larcher (1975) mentions the sensitive response of *Papaver*

(a)
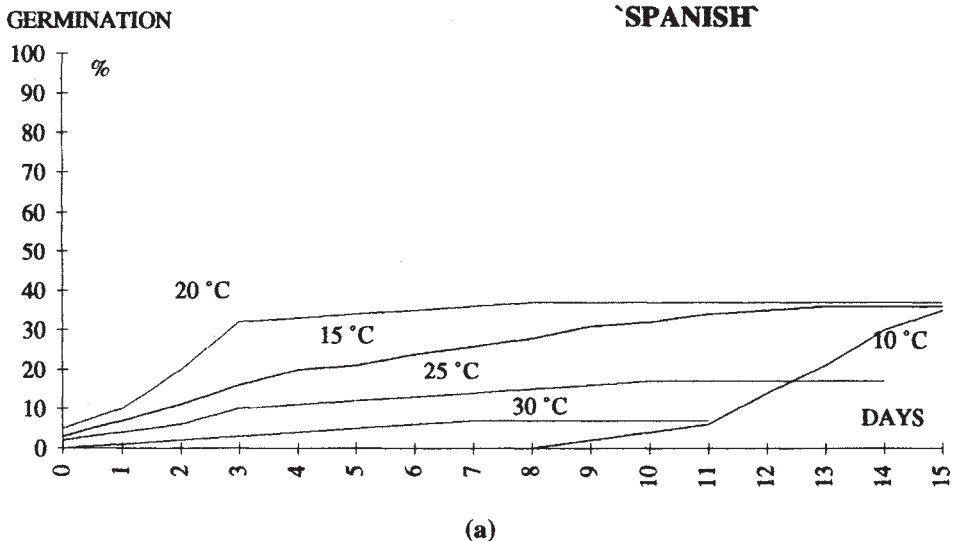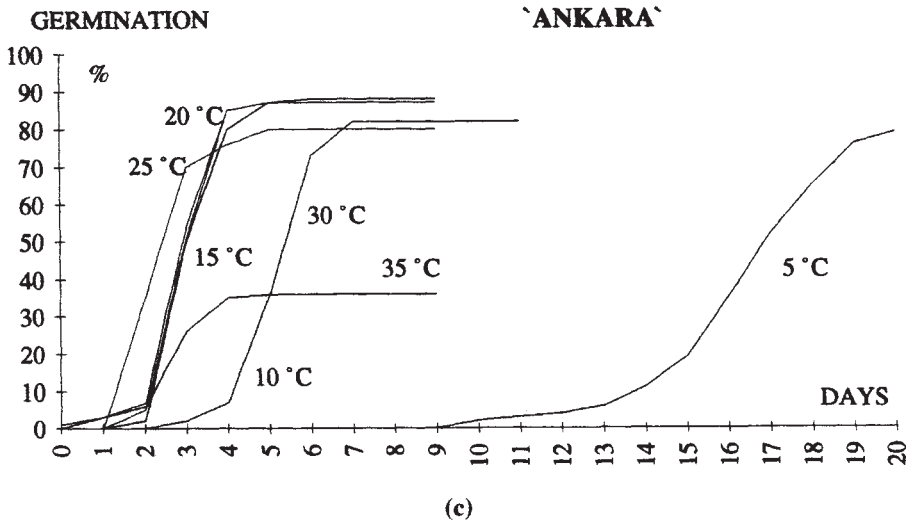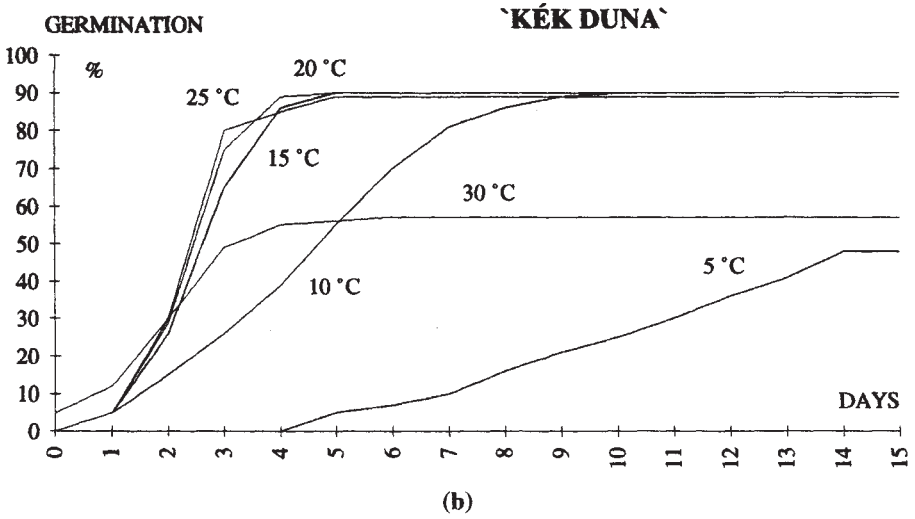
Figure 6a

Figure 6 Germination characteristics of poppy cultivars of different origin characterized by examples of spring ('Kék Duna' (Hungarian)), autumn ('Ankara' (Turkish)) and 'wild' ('Spanish') types (Dobos and Bernáth, 1985)

somniferum to day/night temperature as a physiological example: according to the measurements quoted, a 15/10°C rhythm is the optimum. Morász (1979), in evaluating cultivation experiences in the temperate zone, stated that the particular temperature optima for each developmental phase in terms of mean values are: 12–14°C at the rosette stage; 16–18°C from shooting to the end of flowering; and 21–23°C from

flowering to maturity. Földesi (1978) emphasized the fact that in the early stages of development, an air temperature above the optimum accelerates development, resulting in shortening of the rosette phase, early flowering and a consequently lower yield. This development-accelerating effect of temperature was confirmed under controlled conditions (Bernáth and Tétényi, 1981). Under an experimental programme simulating warm conditions, which may occasionally occur in Hungary (day temperatures raised from 12.5°C to 26°C until the end of vegetation) the period required to start the flowering stage was shortened by 10–15 days. Simultaneously, the plant height was reduced by 10–15 cm, while the average capsule mass halved.

2.2.2 Reaction to Extreme Temperature Conditions

The tolerance response given by the plant to extreme temperature values is also important from the theoretical and practical point of view. El-Hakim (1976) found that under Egyptian conditions *Papaver somniferum* has only limited tolerance to heat. With respect to the lower limit of temperature tolerance, Popov *et al.* (1975) reported that varieties of Eurasian origin could not tolerate temperatures lower than— 7 or—8°C. However, materials classified as being part of the Anatolian group are able to survive considerably lower values. Comparison of varieties representing different growing regions of Northern, Central and Southern Europe in long-term experiments confirmed the wide range of ecological adaptation in Hungary as well (Bernáth and Tétényi, 1982). The varieties 'P-360' (Bulgarian) and 'Ankara' (Turkish)—both classified as 'winter' or 'autumn-sown' varieties—can be expected to overwinter in Central Europe with a reliability of 80–90%, while 'spring' varieties cultivated in wide ranges in Poland, Romania, Hungary, Slovakia or selected ranges in the UK ('Reading') are most sensitive to frost damage and they can overwinter in only one or two cases in a ten-year period. However, the extension of the vegetation cycle by the use of autumn sowing and wintering has had a general favourable effect on the development and dry matter production and has resulted in a fivefold increase in productivity. Similarly the shortening of the vegetation cycle (spring sowing) seems to be unfavourable for the production of both spring and autumn cultivars.

2.2.3 Effect of Temperature on Dry Matter Production

The effect of temperature on dry matter production is connected with both thermoregulation and growth promoting processes and it is difficult to separate it from the interaction of other ecological factors. Experiments carried out under controlled conditions (Bernáth and Tétényi, 1981) indicate some possibilities to determine the effects of temperature from this complex system of factors. 'Kompolti M' (Hungarian) and 'Reading' (English) cultivars were raised on a low-temperature programme (with a day/ night rhythm increasing from 12.5/7.5°C to 18.5/11.5°C) and under warm conditions (with maximum values of 26.0/16.0°C). The result of the experiment is summarized in Table 3. From the data it could be concluded that both cultivars reached maximum height under low-temperature conditions. This was the result of longer growth periods effected by the cold. The prolonged growth caused 10–15 days delay in flowering. The average number of capsules was also less in the

Table 3 The effect of low and high temperature on height and capsule production of poppy in phytotron (Bernáth and Tétényi, 1981)

	Height (mm)	Capsules/ plant (piece)	Mean mass of capsules (g)		Dimension of main capsules (mm)	
			Capsule	Seed	Diam.	Length
'Kompolti M'						
– low temperature	1302	1.15	0.91	0.59	24	31
– high temperature	1182	1.38	0.69	0.53	22	27
'Reading'						
– low temperature	1171	1.03	1.58	1.13	37	37
– high temperature	1038	1.62	1.11	0.07	31	33
L.s.d.	62	0.31	0.21	0.29	2	3

'High temperature': day/night rhythm increasing from 12.5/7.5°C to 26.0/1 6.0°C.
'Low temperature': day/ night rhythm increasing from 12.5/7.5°C to 18.5/11.5°C.

low-temperature experiment: 1.15 capsules per plant were formed on 'Kompolti M' and 1.03 capsules were formed on 'Reading' compared to 1.38 and 1.62 capsules in the high-temperature regime respectively. However, the capsules formed at low temperatures were larger and heavier (by about 10–53%) than those of the high-temperature programme. Seed formation was also promoted in the cold-temperature cycle.

2.2.4 Effect of Temperature on Alkaloid Accumulation

Earlier investigations (Dános, 1968; Hotyn and Novikova, 1968) confirmed that the quantity of morphinane alkaloids in the capsule increases under hot and dry cultivation conditions. Based on data gathered by experts of the UN Narcotics Laboratory (1975) the determinant effect of temperature was also reported.

Experiments carried out in a climatic chamber made it possible to evaluate this correlation numerically (Bernáth and Tétényi, 1981). Two varieties of differing chemical composition ('Reading' and 'Kompolti M') were compared using different temperature and illumination regimes (Figure 7). For both varieties the total and morphinane alkaloid production was greater under warm conditions (when the temperature had been increased from 12.5/7.5°C to 26.0/16.0°C maximum value continuously) and under intensive illumination (32 klx). However, there is a characteristic change in the ratio of the alkaloid components: cold conditions combined with poor light intensity led to a greater proportion of morphine, while warm conditions with strong light intensity resulted in more intensive accumulation of components of higher demethylation levels (codeine, thebaine). The extent of these changes, both in terms of the accumulation level and qualitative character, were strongly determined by the characteristics of the different cultivars. In 'Kompolti M' the demethylation of codeine and thebaine to morphine is only slighdy limited even in the presence of intensive alkaloid metabolism induced by heat and light, while in

(a)

(b)
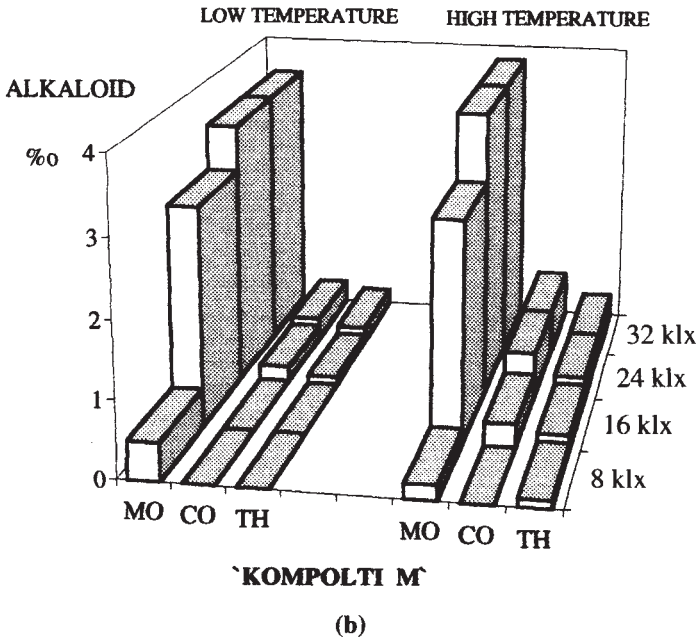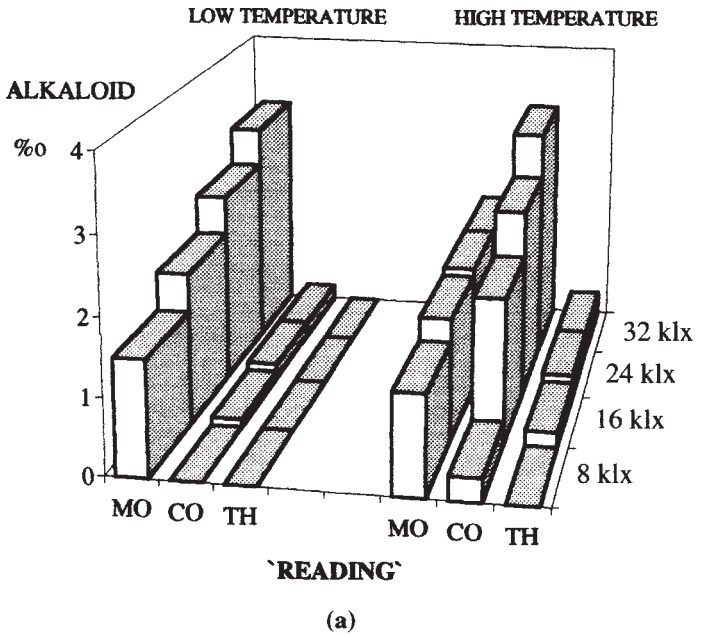
Figure 7 Morphinane spectrum of dry capsules of 'Kompolti M' (Hungarian) and 'Reading' (English) cultivars under the pressure of light—temperature interaction (Bemáth and Tétényi, 1981)

'Reading' the accumulation of morphine is limited to a particular extent and the accumulation of codeine starts to intensify. This chemism-dependent reaction of the cultivar 'Reading' was proved and charactered by the saturation equation calculated to its codeine content *(Y):*

$$Y_{\text{'low temperature'}} = 0.5(1-e^{0.69269-0.000076X}),$$

$$Y_{\text{'high temperature'}} = 6.0(1-e^{0.92604-0.000129X}).$$

These equations show that the enzymatic demethylation of codeine proceeds more rapidly at low temperatures, while at high temperatures the demethylation process is inhibited and codeine becomes saturated at a higher concentration projected onto 6.0 mg/g dry matter.

The above described changes in the formation and accumulation of the alkaloids can be interpreted based on the knowledge gained from the biosynthesis of poppy alkaloids. Considering the parallel changes in plant growth and development, dry matter production and alkaloid accumulation affected by light and temperature, the following can be concluded.

(i)   Environmental circumstances which regulate universal plant processes also stimulate or inhibit alkaloid formation. Thus, an increase in light intensity and higher air temperatures accelerate the accumulation of alkaloids generally due to an intensification of precursor flow and methylation processes; this is indicated by a quantitative increase in all alkaloid groups universally.

(ii) Within the morphinane group general stimulation of alkaloid metabolism also promotes the accumulation of individual alkaloid components. In the majority of the chemotaxa the dominance of morphine can be regarded as a general phenomenon, while the accumulation of codeine and thebaine depends rather on the environment. In the presence of a limited quantity of precursors (under poor light intensity and cold conditions) the characteristic demethylation process of the morphinane group (via thebaine—codeine—morphine) seems to be undisturbed. Under high light intensity and warm conditions, when there is an intensive flow of precursors, after saturation of the morphine level, first codeine, then thebaine start to accumulate in considerable quantities. The saturation level of morphine may have a chemotaxonomic value. Some varieties are able to increase their demethylation processes significantly, while other cultivars can only do this to a lesser extent.

2.3 Water

2.3.1 Effect of Water on Growth and Development

Studying the water balance of *Papaver somniferum* Penka (1968) found that the varying water requirement of the species is based on the changes of their transpiration rate during the vegetation cycle. Two critical phases were pointed out by Penka—initiation of the stems and the bud flower formation stage. Turkhede and Rajat De-Singh (1981) studied the production of *Papaver somniferum* in India from the aspects of stress caused by water deficiency. It was proved in these experiments

that such stress applied at the rosette stage had the most intensive inhibition on growth and development. The rosette stage was also qualified as a critical phase by Lyakin (1977) in the Soviet Union and Schneider and Kuzminska (1975) in Poland. When the degree of water saturation of the soil was reduced from 60% to 30–40% in the rosette stage there was a significant decrease in both capsule and seed yield. Yadav *et al.* (1982) also regarded a 60% saturation level as optimum. Comparing the reaction of three cultivars in a phytotron, a correlation between water supply and temperature was established (Bernáth, 1985). On the basis of the results, the optimum soil saturation level is considered to be dependent on the temperature conditions (Figure 8). Under a 'warm' temperature regime even a soil saturation level as high as 90% promotes the development of plants and higher capsule production is characteristic. Using a 'cold' temperature programme, all three cultivars ('Kompolti M', 'Ankara', 'Reading') preferred the lower water supply. The maximum capsule mass (4.9–10.1 g per individual) was formed at 70% water saturation universally.

There is a practical and theoretical importance of investigations carried out on the water balance of ripening capsules. From the results of the investigations of Bunting *et al.* (1963), LaughHn (1977) and Nash (1980), it is considered that some kind of water translocation begins in the capsules after flowering which is completed at full maturity. In the fourth week after flowering the water content of the placenta is 84–85%, that of the capsule wall is 64–74% and that of the seed is 31–37%. By the end

Figure 8a

"LOW TEMPERATURE"

CAPSULE (g)

WATER SATURATION (%)

(b)
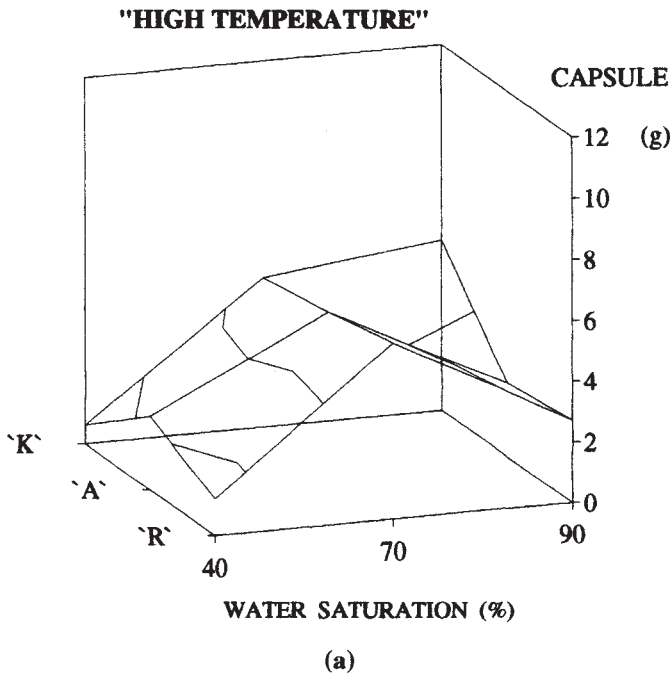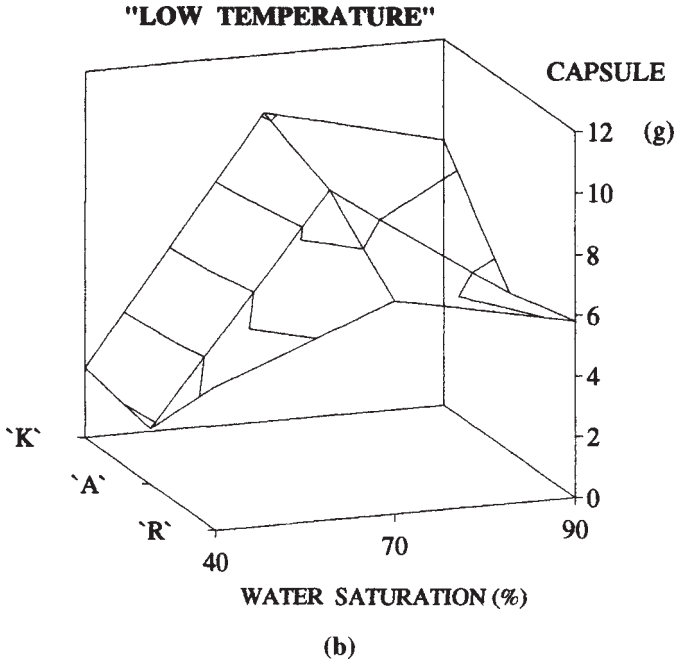
Figure 8 Effect of water supply on the dry matter production of poppy cultivars of different origin ('K', 'Kompolti M'; 'A', 'Ankara'; 'R', 'Reading') under 'high' and 'low' temperature programmes (Bemáth, 1985). 'High' temperature: day/night rhythm increasing from 12.5/7.5°C to 26.0/16.0°C 'Low' temperature: day/night rhythm increasing from 12.5/7.5°C to 18.5/11.5°C

of the eighth week, however, these differences disappear and a balance is achieved at a volume of around 10%. Prokovjev *et al.* (1981) made a detailed investigation on the cell physiological background of this phenomenon. Studying osmotic pressures he found that an osmotic gradient exists in the capsule as a result of the different sugar and potassium ion concentrations. A study of the dry matter accumulation in different parts of the capsule clarified the ripening processes in other aspects (Bernáth, 1989). It was proved, checking twelve development-ripening stages of the capsule, that the dry substance production of the capsule wall and placenta reaches its maximum in the 'glassy' stage of the seed buds (the sixth or seventh day after flowering). The mass of dry substances of the seed increases until the seeds become coloured, up to the 24th or 25th day after flowering. This is the stage when the water content of the capsule starts to drop dramatically and this continues until the start of seed separation from the placenta (30–42 days after flowering).

2.3.2 Effect of Water on Alkaloid Accumulation

The well known relationship between water conditions and dry matter production suggests that the water supply may also determine the alkaloid production. This statement is supported by the work of Turkhede and Rajat De-Singh (1981) who reported that water deficiency acting as a stress factor in the rosette stage reduced the opium yield, though the ratio of the different alkaloid constituents was practically unchanged. In the opinion of Tomar *et al.* (1990) the greatest reduction in seed and husk yields (29.4% and 32.5% respectively) were obtained with water stress imposed at bud and early capsule stages. This is complemented by the statement of Yadav *et al.* (1982) and Hotyn and Novikova (1968) who reported that a favourable precipitation supply increases the opium and morphine yields. With respect to the alkaloid level, most of the examples show a negative interaction of precipitation. According to the work of Dános (1968) rainy weather after flowering and seed setting reduces the alkaloid content. The results of experiments carried out under controlled conditions (Bernáth, 1985) give some explanation of the uncertainty experienced with respect to the alkaloid level. It was proved that a water saturation level around the optimum range (70–90%) has no affect on the accumulation of alkaloids. However, providing a low water supply (40% water saturation) a temperature-dependent reaction occurs. If the shortage of water is accompanied by high temperatures the alkaloid level decreases as a result of the overall damage to physiological processes, which is also expressed in a smaller dry matter production.

In summary, according to recent investigations the decrease of alkaloid content in ripening capsules is related to weather conditions, especially to the amount of precipitation. However this phenomenon has only slight physiological aspects.

2.4 Nutrition

2.4.1 Macroelements

The results of practical experience of the effect of nutrient requirements of *Papaver somniferum* have been summarized by Dános (1968) and Morász (1979). Contradictions in the relatively large amount of data available in the literature drew attention to the need for more exact physiological examinations. In a pot experiment Naumova and Seberstov (1972) found that the maximum capsule production and the highest alkaloid content were both obtained at a 2:2:1 ratio of nitrogen:phosphorus:potassium. An excess of phosphorus in relation to nitrogen was found to lead to a decrease in the internal nitrogen concentration accompanied by a lower alkaloid content. Nitrogen supply has been reported to be favourable around maximum levels by Nowacki *et al.* (1976), whereas others have emphasized the advantage of low nutrient doses for alkaloid production (Georgeta and Oltea, 1977). The relationship between nutrients and morphine content can be characterized as an optimum effect (Schröder, 1966). However, the dry matter production, rather than the morphine content, can be modified by variations in nutrient supply (Földesi, 1978).

To eliminate contradictions it became necessary to carry out more exact experiments. Laughlin (1978) studied the uptake of labelled mono-calcium phosphate and found that phosphorus appeared in the leaves on the 50th day after sowing. In a

sand culture Costes *et.al.* (1976) determined the role of different forms of nitrogen, of phosphate and of the Mg^{2+}, Ca^{2+} and Na^+ cations in growth, development and alkaloid content. Their results can be summarized as follows.

(i)   Nitrogen has the strongest physiological effect and the use of the nitrate is the most efficient.
(ii) In agreement with other literature references Ca^{2+} accelerates growth and development and promotes the formation of alkaloids.
(iii) Without actually having any significant effect on the processes of growth and development, the Na^+ cation has a favourable influence on alkaloid accumulation.
(iv) The physiological effect of phosphorus and Mg^+ did not prove to be critical from the point of view of alkaloid production.

However, the role of nutrient supply cannot be strictly separated from the complex system of external factors; the light intensity, for instance, affects the assimilation of nutrients due to its direct influence on photosynthesis. The changes in the capsule and seed production of the Hungarian variety ('Kék Duna') under controlled conditions provide a clear illustration of this interaction (Bernáth and Tétényi, 1986). As shown in Figure 9, at low light intensity, not only a high dose of nutrients (18–0 mg N/week, 3.7 mg P/week, 18.0 mg K/week) but also a medium dose (12.0 mg N/week, 2.5mg P/week, 12.6 mg K/week) proved to be a statistically high value. By contrast, at an illumination of 16 or 32 klx the same doses of nutrients resulted in response equivalents to an optimum curve. A certain difference could be observed in the optimum for capsule and seed yields: the optimum shifted towards medium nutrient supply (N2) for seed production and a high dose of nutrients (N3) for capsule production.
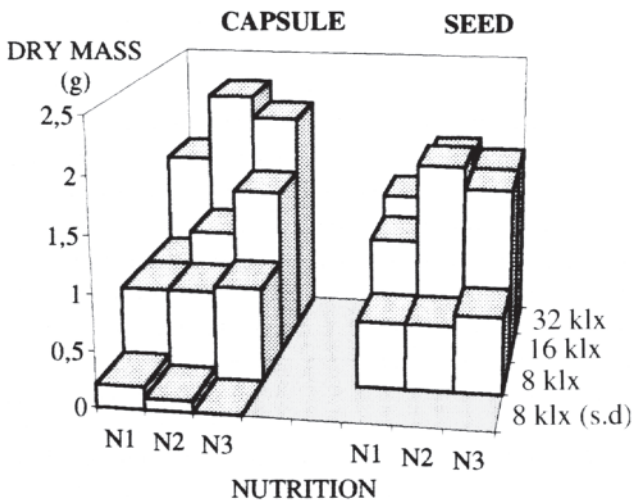
Figure 9 Interaction of nutrition and light on seed and capsule production of poppy (Bernáth and Tétényi, 1986). N1, 6 mg/week/plant; N2, 12mg/week/plant; N3, 18mg/week/plant. (All dosages doubled from rosette stage and trebled after bud formation

The accumulation of alkaloids also showed a characteristic change as a function of light and nutrient supply. This is demonstrated by the modification of the levels of two alkaloid components of the morphinane group, as shown in Figure 10. It was found that at both low light intensity (8 klx or short-day conditions) and high light intensity (32 klx), the nutrient supply level had hardly any influence on the accumulation of alkaloids. By contrast, at a light intensity of 16 klx the influence of nutrients was significant and the quantity of both co-alkaloids was maximum for N3. Thus, the effect of nutrition can be justified if there are no other limiting factors. Under the given experimental conditions, low or excessively strong illumination inhibited or promoted alkaloid formation to such an extent that the tolerance response expected for the nutrient level failed to appear.

2.4.2 Microelements

The importance of microelements has been realized for some time. Boron (Bergman, 1979) is transported in the plant by means of the transpiration flow and is mainly concentrated in the tips and edges of the leaves, since deficiency symptoms are chiefly observed in young plant organs. Deficiency may arise at various soil concentrations of boron. Changes in the concentration of the soil solution (drought) or an increase in the pH value of the soil may result in deficiency symptoms even when the soil reserves appear to be sufficient, thus causing inhibitions in development and growth: 9×10^{-5}mg/gB concentration in the soil at 6.9 pH value may lead to complete destruction of the poppy plant. The boron concentration measured inside the plant gives a good indication of the degree of deficiency. At an internal concentration of 19×10^{-3}mg/g etiolation of the growing tip is observed, while at $6-8 \times 10^{-3}$mg/^{g}, severe deficiency symptoms appear on the leaves and aberrated flowers are formed. The
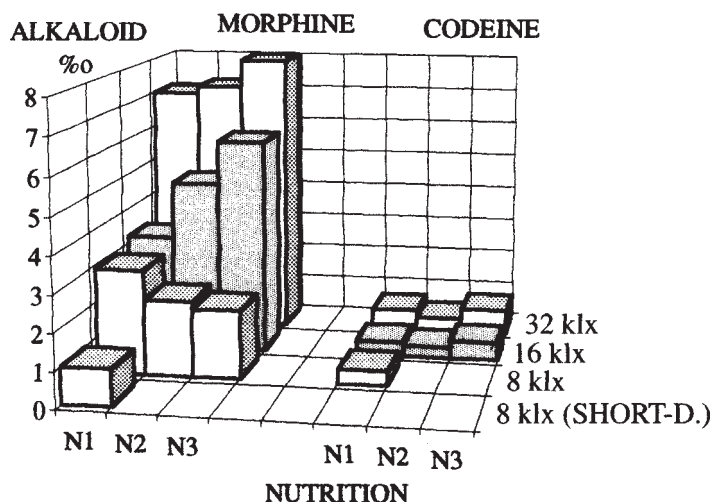
Figure 10 Effect of nutrient-light interaction on the accumulation of two main alkaloids of morphinane group (Bernáth and Tétényi, 1986). week/plant; N2,12 mg/week/plant; N3,18mg/week/plant. (All dosages doubled from rosette stage and trebled after bud formation)

advantage of boron treatment was proved under Australian conditions (Laughlin, 1979). Using 2kg B/ha dosage under field conditions both the capsule and seed production was much higher. In a pot experiment, Gorgiev and Hot (1982) examined the effect of excess boron. The result showed that when the concentration in the leaves rises above 60×10^{-3} mg/g there is a 10–38% reduction in morphine content and a 3.5–27.1% reduction in dry matter production. In another pot experiment, similar results were obtained by Seberstov and Arsuhina (1966), who found that the optimum for capsule production and morphine production fell into the same range of boron supply. These observations indicate that the critical physiological range between adequate boron supply and excessive boron is very narrow.

The physiological effect of molybdenum is discussed in the work by Seberstov and Arsuhina (1966). In a pot experiment, as for boron, an optimum point was justified for both dry matter and morphine production. Further detailed studies on molybdenum and other microelements are required in order to clarify the physiological background fully.

The effect of Mn, Fe and Zn combinations was studied by Anwar *et al.* (1993) in Indian cultivation conditions. The morphine, codeine and thebaine contents in the latex were higher when using these elements in moderate dosages; however the latex yield reduced as a result of high application levels.

2.5 Biotic Factors

The intra-specific competition of *Papaver somniferum* is manifested through the growing area required for individual plants. The optimum number of plants varies both with the year and with the cultivation region. The actual intensity of competition is also influenced by the universal physiological processes of the plant which are modified through nutrient supply, water, weather conditions, etc. Such a modified physiological state is generated by growing both autumn-sown and spring-sown poppies at different spacings (Bernáth and Tétényi, 1982). The overwintering variety ('P-360' (Bulgarian)) produces a significantly higher number of capsules and seeds per plant on a larger growing area over an average of three years than on a smaller one (Table 4). For spring varieties these differences in individual production were only found in the form of tendency—the advantage of a larger growing area being more moderate. This can definitely be attributed to the fact that in the spring cycle the physiologically determined lower production capacity can be achieved even in a denser stand, so there is not as much competition between the individuals. These differences are also obvious with respect to alkaloid production per plant. In the spring cycle there is only a slight change of morphine production depending on the growing area. In an autumn—spring cycle, however, the increased competition which develops on a 20×20 cm^2 growing area has an unfavourable effect on the yield of morphinane alkaloids; when compared to a larger area, the morphinane production of individuals reduced to almost half. The unfavourable effect of a high plant density on latex yield was also proved by Bhandari *et al.* (1989) who studied Indian poppy cultivation. These responses were attributable to a decrease in the number of fully developed capsules per plant.

Inter-specific competition must be expected between poppies and weed species present

Table 4 Mean capsule and seed production per plant of autumn ('P-360') and spring ('Kék Duna') standard cultivars in their own vegetation cycle regarded as optimum under Hungarian conditions, using small and large spacings between plants (Bernáth and Tétényi, 1982)

Cultivar and spacing (cm)	Mean values over three vegetation cycle (1977–1980)					
	Mass of capsules (g/plant)	Yield of capsules (g/m^2)	Mass of seeds (g/plant)	Yield of seeds (g/m^2)	Number of capsules per plant	Mass of single capsule (g)
'P-360'						
50 × 20	7.23	72.3	9.01	90.1	2.76	2.62
20 × 20	3.33	83.2	5.00	125.0	1.63	2.04
'Kék Duna'						
50 × 20	3.31	33.1	4.58	45.8	2.61	1.27
20 × 20	2.38	59.5	3.06	76.5	2.18	1.09

in the poppy agro-system in both autumn—spring and spring vegetation cycles. The competitive ability of poppy is extremely poor, as indicated by the fact that a relatively complex technological system or large amount of manual work must be applied in order to overcome weeds (Földesi *et al.*, 1980). If the weed spectrum is not suppressed, the development phases of the poppy are shortened and the plants begin to flower at a height of 10–12cm without the development of the characteristic rosette stage, leading to the formation of capsules of 3–10mm size.

2.6 Complex Physio-ecological Regulation of the Alkaloid Production

Physiological analysis of the poppy makes it obvious that its growth and development is influenced by a complex system of ecological factors and the plant response to this system is also a complex one generating various interactions. Experiments carried out under controlled conditions have made it possible to characterize these interactions mathematically. The model which was calculated by analysing data from one of the Hungarian *Papaver somniferum* cultivars ('Kompolti M') is illustrated in Figure 11.

On the basis of the determination coefficients calculated, alkaloid production is determined to be around 43% by the intensity of primary processes, i.e. by the quantity of dry matter accumulation. Alkaloid levels and changes in organ ratio are responsible for approximately 22–27%, while the alkaloid composition has practically no influence on production. Taking these into consideration, it is obvious that any factor which generates a high tolerance response in dry matter production will also have a special importance in relation to alkaloid production. The four main environmental factors examined (light, temperature, nutrients and water) have an approximate 80% determinative effect in this respect. Light and water supply, in particular, can be emphasized for their greater effectiveness. From a practical point of view, water supply is one of the essential factors which can be regulated agronomically.
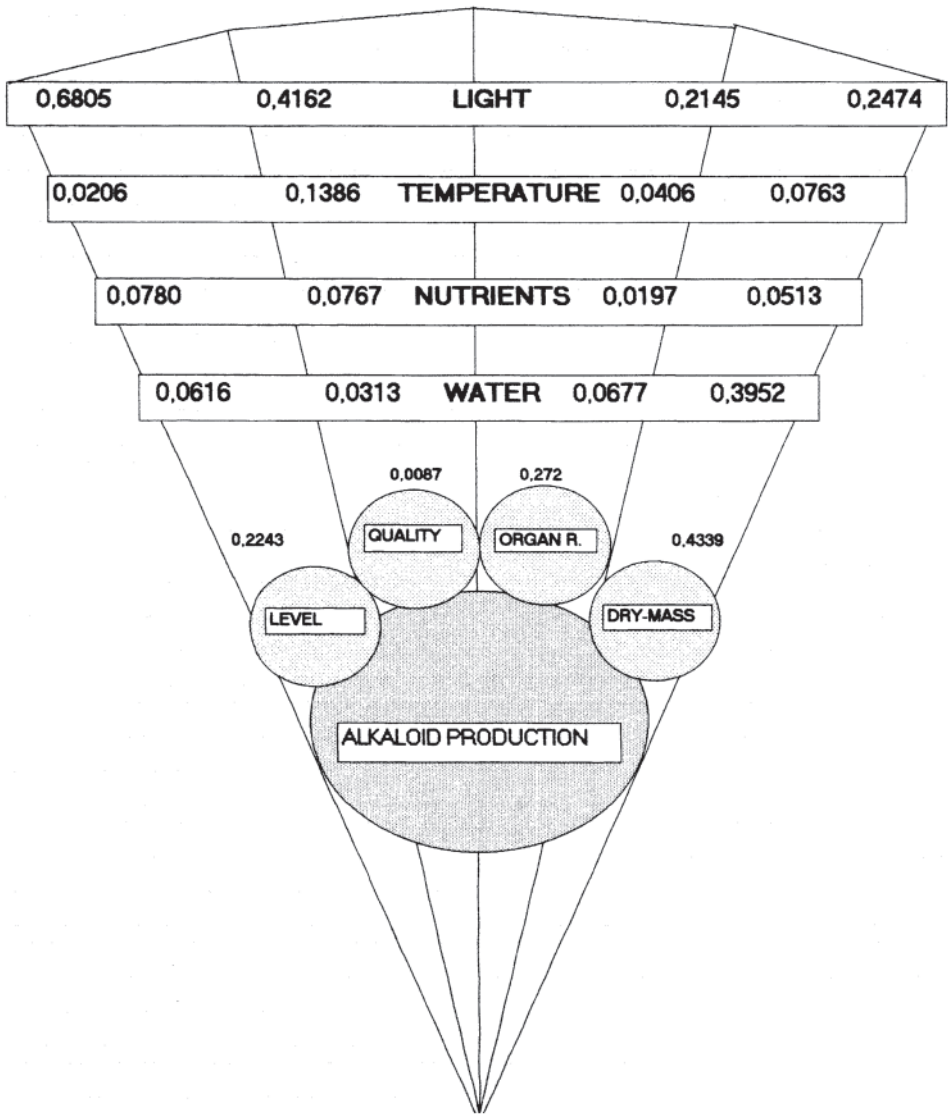
Figure 11 Alkaloid production model of *Papaver somniferum* (cv. 'Kompolti M') affected by environmental conditions. The direction and effectiveness of action are characterized by determination coefficients (Bernáth, 1986)

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4. GENETICS AND BREEDING OF *PAPAVER SOMNIFERUM*

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1 INTRODUCTION

The opium poppy *Papaver somniferum* is a multipurpose crop which is used as a medicinal or ornamental plant, as well as a source for seeds and seed oil. Morphine, codeine, thebaine, narcotine and papaverine are the most important alkaloids produced by the plant and are exploited by the pharmaceutical industry as analgesics, antitussives and anti-spasmodics.

The plant is a herbaceous annual with a distinct vegetative phase, characterized by numerous large pinnatisect leaves spreading horizontally and a reproductive stage (80–100 days after sowing) during which flowering stems with drooping buds are formed. Capsule maturation coupled with a high concentration of alkaloids is reached 110–150 days after sowing.

The divergent and long history of domestication and breeding of *P. somniferum* has resulted in the development of several different land races, chemotype varieties and cultivars adapted to various uses and climatic conditions. Cultivation of the plant therefore covers a wide geographical area from Bombay to Moscow in the Northern hemisphere and Tasmania in the Southern hemisphere (Krikorian and Ledbetter, 1975).

2 CYTOGENETTCS AND REPRODUCTIVE SYSTEM

Papaver somniferum ($2n=22$) is a member of the genus *Papaver,* which includes some 100 species and is affiliated to the section *Mecones* comprising five species, among which *Papaver setigerum* ($2n=44$) is a close relative and probably the ancestor of the opium poppy (Hammer and Fritsch, 1977). The karyotype of *Papaver somniferum* as described by Kaul *et al.* (1978) consists of mostly sub-terminal chromosomes: two pairs of very long chromosomes (8.65–9.37 μm); four medium pairs (7.50–7.87 μm); and three pairs of short chromosomes (6.37–6.75 μm). Chiasma frequency in meiosis was studied by Patra and Chauhan (1988). Failure of chromosome pairing during meiosis and the formation of unbalanced gametes have been reported; at low temperature laggards and asymmetric distribution of the chromosomes were observed at meioses (Yamazaki, 1936).

Based on cytological studies of the inter-specific hybrid between *P. somniferum* and *P. setigerum,* Hrishi (1960) reached the conclusion that *P. setigerum* consists of three different genomes named A, B and C with 11, 3 and 8 chromosomes respectively. The chromosomes of genome A pair well during meiosis with the chromosomes of *P. somniferum* while those of genome C remained unpaired indicating the allopolyploid nature of *P. setigerum*. Cytological studies of the hybrids between *P. somniferum* and *P. bracteatum* (2n=14) and *P. orientale* (2n=28) from the section *Oxytona*, led to the conclusion that *P. somniferum* has no homologous chromosomes with the species of the section *Oxytona* (Yasui, 1937). The formation of bivalents observed by Ojala and Rousi (1987) in hybrids of *P. somniferum* and the polyploid species of the section *Oxytona,* namely *P. orientale* and *P. pseudo-orientale* might result from autosyndesis pairing in the genomes of the polyploid species as reported by Yasui (1936) and Milo *et. al.* (1986) in inter-specific hybrids of these species.

2.1 Breeding System

Papaver somniferum is considered to be a predominantly self-pollinating species with various rates of out-crossing depending upon variety and environmental factors; large colourful flowers with numerous stamens and large amounts of pollen attract insects, especially bees; the transfer of pollen from one flower to another might also be performed by wind (Patra *et al.,* 1992). The rate of out-crossing is increased in some varieties by anatomical and or physiological features such the presence of a waxy layer on the stigma which has to be pierced (by an insect for example) in order to enable fertilization to take place (Bhandari, 1990) or the existence of protandry when another dehiscence occurs before the stigma is receptive. Nyman and Hall (1976) reported 9% cross-pollination between plants with low and normal alkaloid contents. In European varieties the rate of out-crossing varies from 15 to 40% depending on the frequency of pollinators (Morice and Louarn, 1971). A wide range of out-crossing in Indian varieties has also been reported: 0–70% depending on flower colour (bees prefer white flowers to purple ones) and population size of pollinators (Khanna and Shukla, 1983).

The capsule size and shape of the poppy vary to a large extent depending upon cultivar and origin (Veselovskaya, 1976). Based on the width to length ratio, five different shapes have been identified: oval, broad oval, orbicular, flat and conical. The two first shapes are common in Indian varieties, whereas the last three shapes are frequently found in European poppies. It therefore seems that capsule size is related to geographical distribution and is also associated with seed yield: the orbicular and conical shapes being higher seed yielders than flat or oval capsules (Veselovskaya, 1976).

2.2 Male Sterility

Male sterility, either genic or genic—cytoplasmic, is widely used for the commercial production of hybrid seeds in several crops. Natural occurrences of male sterility in *P. somniferum* have not been reported. However, sterile male plants were obtained in the M_1 generation following irradiation of poppy seeds with 10 and 20 kR gamma rays (Singh and Khanna, 1970). Male sterility was also observed in the F_2 generation

of the inter-specific hybrid between *P. somniferum* and *P. setigerum* (Hrishi and Hrishi, 1960) and a linkage between flower colour and pollen sterility was also detected in this study. Male sterility can thus be induced in poppy by mutagenic treatments or generated by inter-specific crosses; however, in order to be exploited in hybrid seed production, its genetic nature and stability have to be determined.

3 INHERITANCE OF AGRO-MORPHOLOGICAL CHARACTERS

Genetic variations in different agro-morphological traits of economic importance, i.e. plant height, days to flowering, number and weight of capsules, seed and latex yield, have been studied in various populations. The heterogeneity of the plant material used in the different studies limits the comparison of the results and generalization of the conclusions (Saini and Kaicker, 1987). However, several authors have emphasized the predominance of additive variance in the genetic control of several characters of the plant (Kandalkar *et.al.,* 1992; Shukla, 1992). Singh and Khanna (1975) observed an important additive genetic component for the control of most of the agro-morphological characters in opium poppy. Similar results were reported in a population of 80 half sibs families and in another F_2 population derived from 50 crosses between 100 individual plants (Srivastava and Sharma, 1987a). In a diallel cross between ten elite varieties of opium poppy, Khanna and Shukla (1989) also found important additive effects for the genetic control of agro-morphological traits. Depending upon the population studied, dominance and over-dominance were involved in the inheritance of plant height and seed weight (Hlavackova, 1978). In a study of 24 parents from European varieties and collections, dominance effects were noticed for various agro-morphological traits (Dubedout, 1993). Shukla and Khanna (1992) found a non-additive gene action for earliness. Maternal effects have been observed for earliness, seed weight and morphine content (Khanna and Shukla, 1989), and for characters such as plant height, flowering period and number of capsules (Sharma *et al.,* 1988).

3.1 Inheritance of Disease Resistance

Several diseases like powdery mildew, root rot and leaf blight occur in poppy. There are almost no reports on resistance in this crop. Some varieties are known to be more susceptible than others to leaf blight *(Helminthosporium papaveris)*. Poppy crops suffer heavily from mildew caused by *Peronospora arborescens*. Locally collected strains were found to be susceptible to downy mildew in a field trial conducted at three locations in India (ICAR, 1989; Pandey and Nigam, 1988). One line was found to be more tolerant to this pathogen (Sharma *et al.,* 1991). It was observed that plants with varying degrees of laceration expressed some level of tolerance to downy mildew. However, none of the genetic stocks available in India is completely resistant.

3.2 Inheritance of Flower Colour

The poppy is distinguished by its beautiful flowers that bloom in various colours, from pure white to red, pink and violet. A recessive mutation which is very common in ornamental varieties is known as 'double petal' in which most of the anthers are

transformed into petals. All cultivated poppies *(P. somniferum)* have big flowers compared with the tetraploid species, *P. setigerum*. A similar trend is found in section *Oxytona:* the tetraploid species *P. orientale,* is much smaller than the diploid *P. bracteatum* (Goldblatt, 1974).

The petal colour is genetically stable and is not affected by environmental changes. Several studies on the genetic control of flower colour, carried out in the first half of the century, were summarized by Veselovskaya (1976). Two to four genes and a series of multiple alleles (Bhandari, 1989), were reported to control the flower colour. In general, this character is monogenically inherited; darker colours are dominant over white, with some epistatic interactions between the genes controlling the petal colour.

4 INHERITANCE OF CHEMICAL CHARACTERISTICS

Qualitative and quantitative variations have been reported for the profile and/or the content of different alkaloids in *P. somniferum* and *P. setigerum* (Kalman-Pal *et al.,* 1987; Garnock-Jones and Scholes, 1990) (Table 1). A spontaneous low alkaloid mutant was analysed by Nyman and Hall (1976). Its morphine and total alkaloid content were ten times less than in the original variety. Another mutant with a chemotype rich in thebaine and very poor in morphine was also described. These mutations are controlled by single recessive genes, and probably result from blocks along the morphinane biosynthetic pathway, before and after thebaine synthesis.

Alkaloid production is controlled by the genotype of the plant and by environmental factors. The important influence of climatic factors during plant development on the spectrum of alkaloids has been reported by several authors (Bernáth *et al.,* 1988; Ghiorghita *et al.,* 1990). As for agronomic traits, the heritability estimates for morphine content are variable, depending upon the population under study. Medium heritability estimates were reported for morphine content in Indian (Khanna and Shukla, 1986) and European varieties (Morice and Louarn, 1971; Dubedout, 1993).

The variation in alkaloid content is partly additive and partly dominant (Lal and Sharma, 1991); similar observations were made by Srivastava and Sharma (1987a) on a population of 80 half sibs families. However, narrow-sense heritability for morphine content is low, 0.13 (Srivastava and Sharma, 1987b) or moderate, 0.22 (Lal and Sharma, 1990) indicating an important dominant component of the genetic variation in this characteristic.

Table 1 Range of contents of the major alkaloids (as percentage of dry latex) of *P. somniferum, P. setigerum* and their Fl and F2 hybrids (data summarized from Khanna and Shukla, 1986)

Species	Morphine	Codeine	Thebaine	Narcotine	Papaverine	Total
P. somniferum	13.1–16.1	2.9–4.4	0.9–2.0	4.8–7.3	0.0–0.4	25.3–27.1
P. setigerum	3.6–11.2	2.1–2.6	1.5–2.3	—	4.2–8.1	11.6–18.0
F1	4.3–14.4	1.3–6.5	1.9–11.2	0.0–1.3	2.9–8.0	19.1–27.3
F2	6.3–20.3	Trace–5.6	Trace–14.7	0.0–12.1	0.7–9.2	23.6–32.2

Heterosis has been reported in *P. somniferum* for agronomic and chemical characteristics; in European seed varieties, the yields of capsules and seeds of F_1 hybrids are generally superior to the mean parental yields, and often to the best performing parent (Mirczulska, 1967; Hlavackova, 1978; Sip *et al.,* 1977; Dubedout, 1993). In opium poppy, Saini and Kaicker (1982) reported 52.8% heterosis for the yield of capsules, and 22.7% for the seed yield; 43.6% heterosis was found for the opium yield. The results for morphine content of the capsules are inconsistent but, in hybrids, the morphine content is often intermediate between the parental values (Dubedout, 1993; Sharma and Singh, 1983; Morice and Louarn, 1971). However, marked heterosis for morphine content was found in some parental combinations (Khanna and Gupta, 1981; Singh and Khanna, 1991a).

4.1 Association Between Characteristics

The relationships within and between agro-morphological and chemical traits have been studied by several authors in various genetic materials. The number of capsules, number of stigmatic rays and dry plant weight were positively correlated at the genotype level with opium, morphine and seed yields (Khanna and Singh, 1975). Among the agronomic traits, Kaicker *et al.* (1975) found a positive correlation between capsule number, size and husk weight; the last two characteristics being positively correlated to opium yield. Similar results were observed by Dubedout (1993) and Shukla and Khanna (1987) who reported a positive correlation among the number of capsules, their yield and the seed yield; the time of flowering was positively related to the weight of the capsules. In another report, Khanna and Shukla (1991) found an association between white latex and papaverine content. Dubedout (1993) found no correlation between the agromorphological characteristics and the content of the morphinane alkaloids of the capsules; among the alkaloids, the morphine content was positively correlated to the thebaine and codeine contents.

5 BREEDING

The existence of substantial variations in the available gene pool of a species is necessary for any successful breeding programme. Several independent studies on the evaluation of the genetic variation in the cultivated germplasm of *P. somniferum* reached the conclusion that only a limited variation prevails in Indian genetic stocks (Singh and Khanna, 1991b; Sharma *et al.,* 1992) and European stocks (Dubedout, 1993) for most agronomic and chemical traits. This is related to the narrow genetic base of genotypes with common ancestry. The genetic and breeding aspects of opium poppy were investigated more intensively in Europe in the early 1960s (Hlavackova, 1959; Dános, 1965; Andersson and Loof, 1966) and during the past decade in India (Singh *et al.,* 1995; Sharma and Singh, 1983). The breeding objectives in these regions were different. In European countries, the yields of poppy straw, seeds and seed oil were the predominant targets, whereas in India—the world's largest producer of opium—the latex yield and morphine content are very important characteristics in breeding. Moreover, the different climatic conditions and cultural practices in Europe and India have directed the breeding of diverse cultivars with different photoperiod

requirements, plant height, lodging and disease resistance, latex yield and morphine content.

Varietal, mass and pure line selections have been applied by several breeders of opium poppy for the development of improved cultivars (Singh *et al., 1995*; Sharma and Singh, 1983). However, the most widely used method which has produced several commercial cultivars is the pedigree selection by which, through hybridization between parents with different desirable characteristics, lines combining most of them are developed. The pedigree method has been used successfully for increasing the yield of capsules (Taranich, 1974), opium and seeds (Khanna and Shukla, 1989), the morphine content and the lodging resistance (Lörincz, 1978). This method, however, markedly reduces the genetic variability and contributes to narrowing the genetic basis of the cultivated germplasm. Substantial progress has been achieved during the last 30 years in France, where the yield of morphine has increased from 4.5 kg/ha in 1961 to 10.5 kg/ha in 1991. This has been achieved mainly through genetic and, to some extent, agro-technical improvements of the morphine content of capsules. During this period, the yield of dry matter has not changed significantly (Dubedout, 1993). The lack of correlation between the alkaloid content and the yield of dry matter reported by several authors (Morice and Louarn, 1971; Dubedout, 1993), offers the possibility of improving the plant species for both criteria.

Recurrent selection ensuring the renewal of a larger genetic basis than the pedigree method has been suggested for the improvement of the agronomic and chemical characteristics of opium poppy (Sharma and Singh, 1983; Dubedout, 1993). The production of synthetic varieties increases the genetic basis of the population (Heltman and Silva, 1978).

5.1 Exploitation of Heterosis

Substantial amounts of heterosis have been observed for morphine and seed yield, as well as for most of their components (see above). The implementation of these results into the production of commercial high-performing hybrid cultivars is, however, hampered by the lack of a genetic system promoting cross-pollination. Genic—cytoplasmic male sterility is the most appropriate and widely used system in several crops for the production of hybrid cultivars.

Induced male sterile mutants have been obtained in plant populations of opium poppy, either by irradiation with gamma rays (Singh and Khanna, 1970), or by inter-specific hybridization (Hrishi, 1960), but these mutants were not characterized. The latter approach is most promising for detecting interactions between nuclear genomes and foreign cytoplasm, and sorting out combinations of plasmons and nuclear genes governing male sterility and fertility restorer genes (Kaul *et al., 1978*).

In the absence of male sterility, self-incompatibility can be used for the production of hybrid seeds. The high number of seeds obtained from a capsule (5000–20000) makes the opium poppy very suitable for hybrid seed production. The use of hybrid cultivars in this crop is the most potent and rapid breeding strategy for combining several desirable characteristics from different parents and for exploiting the considerable amount of heterosis reported for morphine and seed yields. Intensive efforts for the building up of appropriate inbred stocks with high general and specific

combining ability, to which male sterility will be introduced, are needed for the successful use of commercial F_1 hybrids.

5.2 Mutation and Polyploid Breeding

Spontaneous and mutagen-induced mutants have been reported in *P. somniferum*. Such mutants can be used directly as new cultivars: the 'Soma' variety was released from a spontaneous mutant in the variety "Indra" (Nyman, 1978). More frequently, the mutants have been used as parents in breeding programmes (Chauhan *et al.*, 1987). Spontaneous mutants with a low morphine content or with a high thebaine content were isolated by Nyman and Hall (1976). In addition, several spontaneous mutants with altered floral morphology were identified (Sharma and Singh, 1983); these mutants are recessive and inherited monogenically.

Gamma-rays at doses of 10 and 20 kR were used on seeds of opium poppy (Khanna and Singh, 1975). In the M_1 generation several mutations affecting different characteristics of the plants, i.e. male sterility, opium less, high morphine yield, and high number of capsules per plant, were obtained. Treatments with chemical mutagens such as ethylene amine (0.05%) and nitroso-ethyl urea (0.02%) were also assayed and these produced mutants with increased morphine content in comparison with the control population. Induced dwarf and early flowering mutants were also reported by Nigam *et al.* (1990) who applied 10 to 80 kR gamma-ray treatments to the seeds. A combined treatment of gamma-rays and EMS was effective in enhancing mutation frequency (Patra and Chauhan, 1990; Chauhan and Patra, 1993).

The occurrence of biochemical mutants following mutagenic treatments indicates the good potential of this approach in altering the alkaloid profile of plants. A codeine chemotype in which the demethylation to morphine is blocked would be most valuable both to the pharmaceutical industry and for the prevention of the illegal use of morphine. So far, no codeine-only producing mutant of *P. somniferum* has been reported. However, in several medicinal and aromatic plants, specific hereditary metabolic blocks have been induced along biosynthetic pathways (Levy, 1982). The thebaine-rich mutant reported by Nyman and Hall (1976) strengthens this possibility in *P. somniferum*.

Experimental mutagenesis is also promising for improving the agronomic characteristics of the plant and has been achieved in several crops. In various plant species, the content and/or yield of secondary metabolites has been increased by the use of polyploidy (Mears, 1980). In *P. somniferum*, tetraploid and triploid plants were found to have a higher morphine content and to yield a higher number of capsules per plant than the diploid plants from which they were induced (Andreev, 1963); triploid plants performed best for these characteristics. Similar results were obtained by Milo *et al.* (1987) in *P. bracteatum*. The polyploid plants were later in flowering and their seed setting was very poor, especially in triploids. Polyploidy therefore seems of little use for breeding for high seed yield, but it might be considered for increasing morphine yield in the opium poppy.

5.3 Selection Criteria

The selection process is at the basis of all breeding strategies and therefore a judicious choice of the most appropriate selection criteria is crucial for the efficiency of the

breeding work. The most important characteristics for an ideotype plant in opium poppy and the genetic sources for their availability have been identified (Khanna and Gupta,.1981; Singh *et al.,* 1995). Latex yield in India, or poppy straw in Europe, and the yield of alkaloids, are the ultimate targets in breeding of the opium poppy. Based on a study of a large collection of land races from different geographical regions in India, Sharma *et al.* (1981) proposed a desirable plant type featuring dwarf stature (for lodging resistance), early flowering and a high number of as big as possible capsules.

The selection index is the most efficient method when at least two characteristics are measured. Considering the association between different characteristics in 14 varieties and their F_2 generations, Kaicker *et al.* (1975) suggested that the best simple selection criterion could be the opium yield, whereas a multi-character index should involved days to flowering, plant height, capsule and leaf number and capsule husk weight. In another study, Bhandari and Gupta (1991) suggested that latex yield could be improved by selecting for capsule volume and a reduced number of capsules per plant.

Recently, Dubedout (1993) elaborated on a selection index based on a study of 24 European varieties and their hybrids. This index takes into consideration the heritability and correlation coefficients of different components of the morphine yield. The most important criterion is the total yield of morphine equivalents—defined as 100% of the morphine content, plus 96.9% of the codeine content, plus 91.6% of the thebaine content. Accordingly, the selection index proposed by Dubedout (1993) is: I=3(yield of morphine equivalents)+3 (content of morphine equivalents)+2 (morphine content)+ 1 (weight of a capsule). This index sets a high priority for the morphine content and considers the morphinane alkaloids thebaine and codeine.

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III.   CHEMISTRY-BIOCHEMISTRY OF POPPY

1.   CHEMICAL STRUCTURES OF ALKALOIDS

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1 INTRODUCTION

Since the isolation of morphine by Sertürner in 1805, 44 alkaloids have been isolated from the opium poppy. The chemistry of poppy alkaloids has been the subject of several reviews (Kühn and Pfeifer, 1963; Pfeifer, 1971; Santavy, 1970, 1979) and many books have also been devoted to this topic (Ginsburg, 1962; Henry, 1949; Small and Lutz, 1932). The purpose of this chapter is to present the isolation and structure elucidation of poppy alkaloids. Syntheses of the alkaloids and important chemical reactions will also be discussed.

2 BENZYLISOQUINOLINE ALKALOIDS
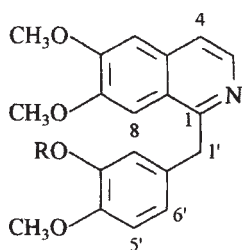
R = CH₃ papaverine
R = H   palaudine
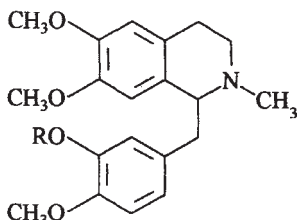
R = CH₃ laudanosine
R = H   laudanine
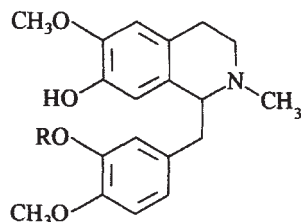
R = CH₃ codamine
R = H   reticuline

The group of 1-benzylisoquinoline alkaloids includes both fully aromatic compounds, e.g. papaverine, and the 1,2,3,4-tetrahydro derivatives, such as laudanosine. Tetrahydrobenzylisoquinoline alkaloids contain a chiral carbon centre at position C-1 and the alkaloids of poppy belong in the S series. It is of interest that some racemic alkaloids have also been isolated. Reviews on the benzylisoquinoline alkaloids have been published (Burger, 1954; Deulofeu *et al.,* 1968).

2.1 Papaverine

The isolation of papaverine from opium (Barbier, 1947; Ramanathan and Chandra, 1981) or poppy heads (Bognár *et al.,* 1967a, 1969; Gorecki and Bognár, 1968; Hodková *et al.,* 1972; Poethke and Pechmann, 1959) has been studied by a number of authors.

Papaverine was the first opium alkaloid whose structure was elucidated mainly by means of oxidative degradation. The structure has been confirmed by numerous syntheses, and since papaverine has achieved therapeutic importance as an antispasmodic, commercially useful methods of synthesis are of importance. The industrial synthesis of papaverine has been reviewed (Lukiyanov *et al.,* 1972). Nevertheless, there is ongoing interest in the synthesis of this alkaloid (Kozikowski and Ames, 1980). Papaverine has been prepared by alkylation of 6,7-dimethoxy-l-chloro-isoquinoline with a corresponding 74% yield (Taylor and Martin, 1972), or by modification of the Pomeranz—Fritsch reaction (Hirsenkorn, 1991), which is a convenient new procedure.

Papaverine can be reduced to 1,2,3,4-tetrahydropapaverine (Craig and Tarbell, 1948; Stenlake *et al.,* 1974). This compound has been detected in the opium poppy by a carrier dilution method involving the use of radiolabelled N-norreticuline (Brochmann-Hanssen *et al.,* 1971a).
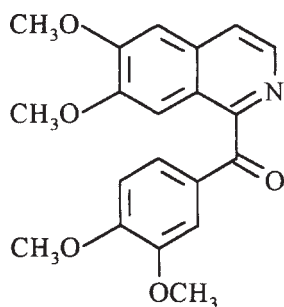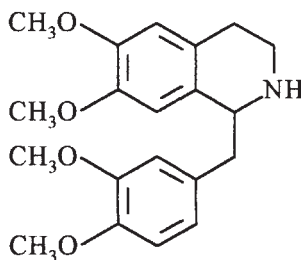
papaveraldine   tetrahydropapaverine

These studies indicate that tetrahydropapaverine is the principal immediate precursor of papaverine. Mild oxidation of papaverine yields papaveraldine and several by-products (Postaire *et al.,* 1987). Papaveraldine has been isolated from poppy capsules in the re-crystallization mother liquor of papaverine (Hodková *et al.,* 1972). It was also detected in Indian opium (Ramanathan, 1963). It was considered to be an artefact formed by the oxidation of papaverine during separation of the alkaloids. In chloroform solution, papaverine decomposes in sunlight to a mixture of products, e.g. papaverine N-oxide, papaveraldine and papaverinol (Pfeifer *et al.,* 1972). The anion derived from papaverine and sodamide in liquid ammonia has been alkylated to give 1'-substituted compounds (Buzas *et al.* 1985). Papaverine can be acylated or alkylated at position C-6' (Wiegrebe *et al.,* 1968). Further 6'-substituted papaverine derivatives (hydroxymethyl, nitro and halogeno) have also been prepared (de Lera *et al.,* 1987; Klivényi *et al.,* 1977; Pavelka and Kovar, 1976).

Papaverine can be dimerized (linkage of C-6' carbons) by oxidation with vanadium oxytrifluoride in trifluoroacetic acid (Kupchan *et al.*, 1973).

The conformation of papaverine in solution has been studied by nuclear magnetic resonance (NMR) spectroscopy (Marsaioli *et al.*, 1978; Pohl and Wiegrebe, 1965; Rae and Simmonds, 1987). Detailed mass spectral (Ohashi *et al.*, 1963; Tatematsu and Goto, 1965) and X-ray studies (Reynolds *et al.*, 1974) have also been performed.

2.2 Palaudine

A new alkaloid—palaudine—was isolated from opium (Brochmann-Hanssen and Hirai, 1968). It is a 3'-O-demethylated papaverine whose structure was confirmed by synthesis. Evaluation of the PMR spectra of papaverine and palaudine was also reported. Palaudine has been detected in green poppy (Proksa *et al.*, 1979). The formation of palaudine was reported to occur on the partial O-methylation of 6,4'-dimethyl-papaveroline with diazomethane (Buchs and Brossi, 1981) or on the selective O-demethylation of papaverine (Brossi and Teitel, 1970).

2.3 Reticuline

The isolation of (±)-reticuline (and (+)-reticuline) from opium was described in the 1960s (Brochmann-Hanssen and Furuya, 1964a,b; Brochmann-Hanssen and Nielsen, 1965). The structure of (±)-reticuline was confirmed via the PMR spectrum and by its methylation with diazomethane to (±)-laudanosine. Reticuline was isolated in the phenolic fraction of poppy plants collected in the state of opium ripeness (Proksa *et al.*, 1979) and in poppy pods (Gaevskii *et al.*, 1976; Mamochkina *et al.*, 1976). Martin *et al.*, (1967) detected (-)-reticuline in fresh budding plants and seedlings of *Papaver somniferum* L. On exposure of such plants to $^{14}CO_2$ they observed the incorporation of radioactivity into (-)-reticuline and thebaine (Martin *et al.*, 1967). Reticuline was also detected in poppy plants by means of radioimmunoassay (Wieczorek *et al.*, 1986).

The structure and configuration of (+)-reticuline were elucidated by transformation into L(+)-laudanosine on treatment with diazomethane. Oxidative degradation also provided evidence of the structure of reticuline (Battersby *et al.*, 1965b).

A synthesis of 1,2-dehydroreticulinium chloride was devised and it was demonstrated that the latter compound is incorporated efficiently into the morphinane alkaloids. Moreover, 1,2-dehydroreticulinium ion proved to be a natural product in *Papaver somniferum* (Borkowski *et al.*, 1978). (±)-Reticuline has been shown to be a precursor of the morphine alkaloids in *Papaver somniferum* L.

It was demonstrated that (S)-reticuline is converted to (R)-reticuline via oxidative attack at the chiral centre (De-Eknamkul and Zenk, 1990; Loeffler *et al.*, 1990). The R enantiomer is not susceptible to oxidation and its internal cyclization yields salutaridine which has the 9R configuration.

Following recognition of the biosynthetic importance of reticuline, its synthesis was thoroughly investigated by Bischler-Napieralsky ring closure (Chan and Maitland, 1966; Jackson and Martin, 1966a; Jain, 1962; Kunimoto, 1961).

The optical isomers of reticuline were prepared by resolving O,O'-dibenzyl-(±)-reticuline with O,O-dibenzoyltartaric acid and debenzylating the products by

treatment with hydrochloric acid, when (+)- and (-)-reticuline hydrochlorides were obtained (Battersby *et al.,* 1965c).

An efficient synthesis has been elaborated for racemic and optically active N-norreticuline and reticuline (Rice and Brossi, 1980). The Bischler—Napieralsky method was used with unprotected diphenolic intermediates. Moreover, 2´-bromotartanilic acids proved to be extremely suitable for the resolution of (±)-N-norreticuline. The enantiomers of reticuline were readily prepared from N-norreticuline.

Two methods have been devised for the synthesis of reticuline, both starting from intermediates of an industrial papaverine synthesis. The first method made use of a regioselective ether cleavage of 3,4-dihydropapaveraldine (Dörnyei *et al.,* 1982). The other procedure, which involves partial O-demethylation of homoveratronitrile and separation of the isomers, offers a new approach to N-norreticuline (Szántay *et al.,* 1981). The second method also affords an enantioselective synthesis of R(+)-N-norreticuline. A new synthesis of (±)-reticuline has been reported in which a Reissert-type intermediate was used (Kerekes *et al.,* 1978).

(R)-Reticuline, (R)-laudanosine and (R)-tetrahydropapaverine have been obtained in high optical purity through asymmetric hydrogenation of the corresponding 1,1'-didehydro-N-acylderivatives (Noyori *et al.,* 1986).

Reticuline has been synthesized from the appropriately substituted styrene epoxide, by means of the Pomeranz-Fritsch ring closure (Hirsenkorn, 1990). An asymmetric total synthesis of (+)-reticuline was accomplished via chiral formamidines in five steps (Meyers and Guiles, 1989). Similarly, an asymmetric synthesis of reticuline was achieved from L-DOPA using 1,3 asymmetric induction (Konda *et al.,* 1975b).

Reticuline is of crucial importance in the biosynthesis of poppy alkaloids. Interest in studies of the role played by reticuline as a precursor of numerous alkaloids in plants has led to the synthesis of several radioactively labelled reticulines (Battersby *et al.,* 1964, 1965c; Borkowski *et al.,* 1978; Brochmann-Hanssen *et al.,* 1971c).

The oxidative coupling of reticuline can yield various products, e.g. morphinandienones (salutaridine), aporphines (isoboldine) and protoberberines (scoulerine). These reactions will be discussed in connection with the syntheses of the individual alkaloids.

The biotransformation of (±)-reticuline yielded aporphine (isoboldine) and protoberberine (coreximine and scoulerine) alkaloids. Incubation of reticuline with rat liver homogenate at 37°C resulted in the accumulation of alkaloids (Kametani *et al.,* 1977c). The oxidative coupling of (+)-reticuline with cuprous chloride and oxygen in pyridine was studied as an enzymatic model. The main product was (+)-corytuberine (28%), but (+)-isoboldine (8%) and pallidine (6%) were also isolated (Kametani *et al.,* 1977a).

The production of radicals during the oxidation of reticuline has been studied by means of electron spin resonance (ESR) spectroscopy (Hewgill and Pass, 1985).

The NMR and mass spectra (MS) of isoquinoline alkaloids (reticuline and laudanosine) have been studied in detail (Janssen *et al.,* 1990; Madhusudan *et al.,* 1985).

The absolute configuration of reticuline was elucidated (Battersby *et al.,* 1965a) via its optical rotatory dispersion (ORD) spectrum.

2.4 Laudanosine

S-(+)-Laudanosine is a minor opium alkaloid which is only rarely detected (Ayyangar and Bhide, 1988; Kleinschmidt, 1959; Machovicova *et al.,* 1977). As a consequence of the origin and composition of laudanosine, it was suggested early on that this alkaloid is N-methyltetrahydropapaverine. (±)-Laudanosine was prepared in excellent yield by the reduction of papaverine methosulphate with sodium borohydride (Bentley and Murray, 1963a). The absolute configuration of natural (+)-laudanosine was determined by chemical correlation with S-(-)-N-norlaudanosine, the configuration of which was established by oxidative degradation (Corrodi and Hardegger, 1956). S-(-)-N-norlaudanosine was converted into S-(+)-laudanosine by N-methylation. These results are in agreement with the conclusions previously proposed on the basis of the correlation of its rotational shifts in different solvents with those of S-(-)-α-phenylethylamine (Bentley and Cardwell, 1955).

The absolute configuration of (+)-laudanosine was recently determined by means of NMR spectroscopy with the use of a chiral shift reagent (Jankowski *et al.,* 1982).

Several methods have been described for the synthesis of laudanosine. Intramolecular acylation between two molecules of 3,4-dimethoxyphenylacetic acid afforded a keto acid which proved to be a starting material for a new synthesis of (±)-laudanosine and 1,2,3,4-tetrahydropapaverine (Elliot, 1970, 1972). Laudanosine has been prepared in high yield from 6,7-dimethoxy-2-methyl-3,4-dihydro-isoquinolinium bromide by reaction with 3,4-dimethoxybenzyl bromide and zinc (Shono *et al.,* 1983), and in low yield by reaction with 3,4-dimethoxybenzyltrimethylsilane and caesium fluoride in dimethylformamide (Takano *et al.,* 1982). Racemic laudanosine was resolved with the aid of mandelic acid.

Asymmetric reductions of 3,4-dihydropapaverine to norlaudanosine were reported and the S antipode was formed in moderate optical yields (Archer *et al.,* 1971; Yamada *et al.,* 1983). S(+)-Laudanosine was prepared from the methyl ester of L-DOPA via the Pictet—Spengler reaction exploiting asymmetric induction (Konda *et al.,* 1975a). The R antipode was also synthesized by this procedure (Konda *et al.,* 1977).

The synthesis of R-laudanosine was reported by the asymmetric alkylation of 6,7-dimethoxytetrahydroisoquinoline (Coppola, 1991; Gawley and Smith, 1988; Gottlieb and Meyers, 1990). Another enantioselective synthesis of (+)-laudanosine was described from dopamine and R-(+)-glycerylaldehyde (Czarnocki *et al.,* 1986).

Laudanosine undergoes C-l-N cleavage on the action of cyanogen bromide (Kerekes and Gaál, 1980) or chloroformic esters (Kametani *et al.,* 1976a; Lee and Wiegrebe, 1986). Stereoisomeric quaternary salts were prepared from laudanosine with alkyl halides (Kóbor *et al.,* 1969; Stenlake *et al.,* 1981); their structures were studied by means of NMR spectroscopy and X-ray crystallography (El-Sayad *et al.,* 1982). (±)-Laudanosine was converted to O-methylflavinantine in high (53%) yield by electro-oxidative cyclization (Miller *et al.,* 1971), but the aporphine alkaloid glaucine was isolated in the reaction of laudanosine with vanadiumoxytrifluoride (Hartenstein and Satzinger, 1977; Kupchan and Kim, 1975).

The oxidation of laudanosine N-oxide with potassium chromate resulted in N-norlaudanosine (Bentley and Murray, 1963b). L(+)-Laudanosine was obtained by sodium—liquid ammonia fission of tetramethylmagnolamine or by the resolution of

(±)-laudanosine. Under specific conditions laudanosine methiodide can revert to the original base when it is boiled with ethanolamine (Tomita and Takano, 1960) or on the action of lithium aluminium hydride in tetrahydrofuran (Tomita and Ibuka, 1962).

The structure of laudanosine has been studied in detail by NMR spectroscopic (proton and ^{13}C) methods (Janssen *et al.,* 1990; Singh *et al.,* 1978) and by mass spectrometry (Madhusudan *et al.,* 1985; Tomita *et al.,* 1966). Its absolute configuration was corroborated by means of CD and ORD methods (Craig *et al.,* 1966; Kametani and Ihara, 1968)

2.5 Laudanidine and Laudanine

(-)-Laudanidine and its racemic form laudanine were isolated from opium by Hesse in the nineteenth century. It has been detected and re-isolated from opium by several authors (Brochmann-Hanssen and Furuya, 1964b; Brochmann-Hanssen *et al.,* 1965; Genest and Belec, 1967; Kleinschmidt, 1959). Laudanidine was also recently isolated from green poppy (Proksa *et al.,* 1979). The treatment of (-)-laudanidine with diazomethane yields (-)-laudanosine which indicates its structure and absolute configuration. On the other hand, the oxidative degradation of laudanidine or its ethyl ether confirmed the location of the phenolic hydroxy group. The synthesis of laudanine was achieved via the Bischler-Napieralsky reaction; the 3'-hydroxy group was protected with a benzyl group (Ferrari and Deulofeu, 1962; Frydman *et al.,* 1958; Miller *et al.,* 1973). Laudanine has also been obtained by heating racemic laudanosine with concentrated hydrochloric acid at 100°C.

NMR studies on laudanidine have been performed (Janssen *et al.,* 1990) and the absolute configuration of laudanidine has been established from its ORD and CD spectra (Craig and Roy, 1965; Craig *et al.,* 1966).

2.6 Codamine

(+)-Codamine is a minor opium alkaloid whose optical activity had not been determined before re-isolation (Brochmann-Hanssen *et al.,* 1965). It was isolated from opium and characterized via its PMR and IR spectra. The methylation of codamine with diazomethane yielded (+)-laudanosine, and the oxidative degradation of codamine ethyl ether elucidated its structure and absolute configuration. The alkaloid has also been isolated from poppy pods (Mamochkina *et al.,* 1976).

A variation of the synthesis of racemic codamine by Bischler—Napieralsky ring closure led to an improvement (Billek, 1956; Onda, 1954)—an O-benzyl protective group was employed during the Bischler—Napieralsky reaction. N-Formylnorcodamine was also prepared and reduced with LiAlH$_4$ to (±)-codamine.

(±)-Codamine was obtained by the reduction of protopapaverine methiodide with sodium borohydride. Enantiomers of codamine were prepared by resolution of O-benzoyl-(±)-codamine with O, O'-dibenzoyltartaric acids (Cassels and Deulofeu, 1966).

The synthesis of (±)-O-benzylcodamine has been reported and it was resolved with O, O'-dibenzoyltartaric acids. O-Debenzylation afforded natural codamine (Battersby *et al.,* 1968). Codamine was obtained by the Eschweiler—Clarke N-methylation of the corresponding 1-benzyltetrahydroisoquinoline (Kametani *et al.,* 1968).

Codamine is a useful starting material for the syntheses of aporphines. These oxidative cyclizations can be performed with lead tetra-acetate (Hara *et al.*, 1976a; Hoshino *et al.*, 1974, 1975) or vanadium oxytrifluoride (Kupchan *et al.*, 1976; Miller *et al.*, 1973).

Spectroscopic studies (NMR and UV) have been applied to characterize and identify codamine (Cassels and Deulofeu, 1966; Hoshino *et al.*, 1974).

3 MORPHINANE ALKALOIDS

The chemistry of morphine alkaloids has been surveyed in several books (Bentley, 1954; Ginsburg, 1962) and reviews (Bentley, 1971; Blaskó and Cordell, 1988; Holmes, 1952; Holmes and Stork, 1952; Stork, 1960; Stuart, 1971; Szántay *et al.*, 1994).

3.1 Morphine and Codeine

R = CH$_3$ codeine
R = H morphine

R = CH$_3$ thebaine
R = H oripavine

neopine
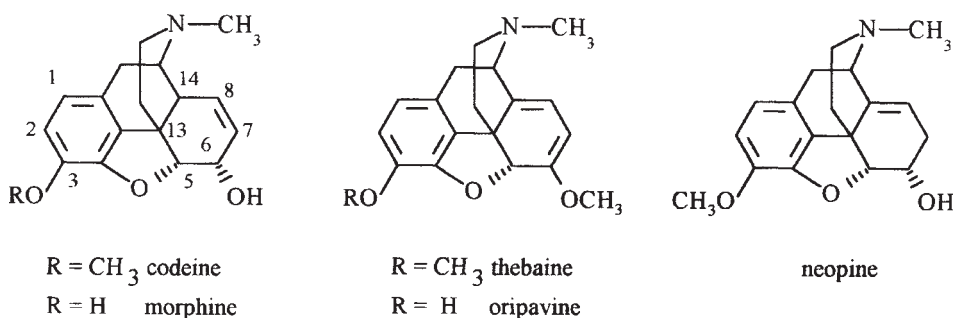
In spite of the fact that more than 60 species or lower taxonomic units of the genus *Papaver* have been studied, the presence of morphine has been detected in only two species from the section *Mecones* only, i.e. *P. somniferum* L. and *P. setigerum* DC. Morphine was isolated as a minor alkaloid from the mature capsules of *Papaver* decaisnei Hochst (Slavik, 1980). Nevertheless, the presence of morphine has been demonstrated in other plants, such as hay and lettuce (Hazum *et al.*, 1981). Morphine was also recently detected in mammalian tissues (Hosztafi and Fürst, 1995).

Morphine has five carbon chiral centres at positions 5(R), 6(S), 9(R), 13(S) and 14(R), and this structure is responsible for the familiar opioid pharmacological effects. The stereochemistry of morphine has been established mainly through chemical degradation. Since it has been clearly established that morphine and its methyl ether, codeine, are members of the same stereochemical series, much of the evidence relating to the configurational relationships in codeine or its degradation products (used primarily because of their greater stability as compared to the corresponding unmethylated compounds) will apply equally to morphine. Later Spectroscopic methods corroborated the correct structure. First, X-ray crystallographic studies (Lindsey and Barnes, 1955; Mackay and Hodgkin, 1955) justified the results of chemical degradation and then the conformations of rings C and D were investigated

by NMR (^{1}H and ^{13}C) Spectroscopic methods (Batterham *et al.,* 1965; Carroll *et al.,* 1976; Chazin and Colebrook, 1986; Okuda *et al.,* 1964; Rüll, 1963; Terui *et al.,* 1975). The absolute configurations of morphine alkaloids have been corroborated by means of ORD and CD studies (Bobbitt *et al.,* 1959; Bognár *et al.,* 1975; Weiss and Rüll, 1965), the results being in agreement with earlier data (Bentley and Cardwell, 1955; Kalvoda *et al.,* 1955). The absolute stereochemistry of morphine was elucidated by the performance of numerous total syntheses and biomimetic syntheses (Szántay *et al.,* 1994).

There are numerous reactions for interconversions in the morphine series. Thebaine can be converted to codeine or neopine (Barber and Rapoport, 1976; Gates, 1953; Gavard *et al.,* 1965; Kotick *et al.,* 1980; Weller and Rapoport, 1976), and the O-demethylation of codeine to morphine has also been achieved (Lawson and DeGraw, 1977; Rapoport *et al.,* 1951; Rice, 1977). These reactions mimic the biosynthesis of morphine in the opium poppy. The reverse reactions are also known, i.e. thebaine derivatives can be prepared from codeine (Barber and Rapoport, 1975; Rapoport *et al.,* 1956, 1967; Seki, 1970).

The catalytic hydrogenation of morphine and codeine yields the corresponding 7,8-dihydromorphine (codeine), but codeine (morphine) undergoes rearrangement to dihydrocodeinone (dihydromorphinone) when heated in the presence of noble metals (Gaal, 1962; Rapoport *et al.,* 1950).

Codeine can be oxidized to codeinone by several methods (Findlay and Small, 1950; Rapoport and Reist, 1955). Morphinone has been prepared by silver carbonate oxidation of 3-methoxymethylmorphine, followed by acid hydrolysis (Rapoport *et al.,* 1957). The benzylic (C-10) position of codeine and morphine is also sensitive to oxidation: 10-hydroxycodeine (Rapoport and Stevenson, 1954), 10-hydroxymorphine (Rapoport and Masamune, 1955) and 10-oxomorphine (Proksa, 1984; Proksa *et al.,* 1978) have been isolated.

The acid-catalyzed rearrangements of codeine and morphine yield apocodeine and apomorphine, respectively, these products containing an aporphine skeleton (Granchelli *et al.,* 1980; Small *et al.,* 1940). The mechanism of the morphine-apomorphine rearrangement has been studied in detail (Berényi *et al.,* 1982, 1983).
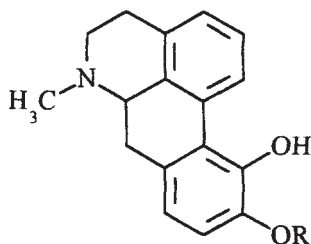
R = H   apomorphine

R = CH$_3$ apocodeine

Morphine and codeine (May and Jacobson, 1977; Whitehouse *et al.,* 1990) have been acetylated and 3,6-diacetylmorphine (heroin) is the most important compound

in this series. Heroin exhibits morphine-like analgesic action, but it leads to a high level of physical dependence.

The methylation of morphine to codeine is a well established industrial process (Heumann, 1958), but numerous other ethers have been prepared. Ethylmorphine (a slight analgesic) and 3-morpholinoethylmorphine (pholcodine, anti-tussive) are employed therapeutically.

N-demethylation is an important reaction of morphine alkaloids which is utilized for the preparation of opioid antagonists. Morphine (codeine) may be N-demethylated by the von Braun reaction with cyanogen bromide (Hageman, 1953). The application of chloroformates is now preferred to cyanogen bromide, and this procedure is widely applied in morphine chemistry (Abdel-Monem and Portoghese, 1972). Morphine(codeine) yields a urethane derivative, which can be converted to a secondary amine. Numerous procedures have been devised for N-demethylation with chloroformates in order to find an efficient method (Brine *et al.,* 1976; Montzka *et al.,* 1974; Rice, 1975). Vinyl and a-chloroethyl chloroformate have proved to be the most effective reagents for this purpose (Olofson and Pepe, 1977; Olofson and Schnur, 1977; Olofson *et al.,* 1977, 1984). Diethyl azodicarboxylate is also a frequently used reagent for the N-demethylation of morphine derivatives (Hosztafi, 1987). It is of interest that normorphine—an intermediate of the morphine metabolism—has been detected in poppy (Miller *et al.,* 1973).

Aromatic ring substitution of morphine (codeine) usually results in a reduction of its analgesic potency. Such derivatives have been prepared in electrophilic aromatic substitution reactions, including halogenation (Bentley and Dyke, 1959; Hosztafi and Makleit, 1994; Singh *et al.,* 1982), nitration (Baggesgaard Rasmussen and Boll, 1958; Gaál and Bognár, 1962), acetylation (Small and Mallonee, 1947), etc.

The phenolic hydroxyl group of morphine derivatives has been eliminated by hydrogenolytic cleavage of phenyltetrazolyl ethers (Bognár *et al.,* 1974; Reden *et al.,* 1979).

6-O-Tosyl (mesyl) derivatives of morphine and codeine were prepared in order to study the nucleophilic substitution reactions. The mechanisms of these reactions were clarified and a great number of semi-synthetic compounds have been produced (Makleit *et al.,* 1977b,c,d), including azidomorphine derivatives which are potent analgesics.

Epimeric morphine (codeine) derivatives and 6-amino-substituted compounds have been obtained by means of the Mitsunobu reaction (Simon *et al.,* 1991, 1992).

3.2 Thebaine

The separation of thebaine has been reported from poppy capsules (Bognár *et al.,* 1967b; Gorecki and Bognár, 1968; Hodková *et al.,* 1972) and from opium (Ramanathan and Chandra, 1980).

The treatment of thebaine with bromine in acetic acid or with N-bromosuccinimide (Conroy, 1955) yields 14-bromocodeinone, which is a useful compound for the preparation of neopine derivatives. Introduction of a 14-hydroxy substituent into a 4,5α-epoxymorphinane nucleus may be accomplished from thebaine by peroxide treatment to give 14-hydroxycodeinone (Hauser *et al.,* 1974; Iijima *et al.,* 1978;

Seki, 1960); (Bentley and Robinson, 1952); (Lutz and Small, 1939). The latter compound is an important starting material for the synthesis of pure opioid antagonists, e.g. naloxone and naltrexone.
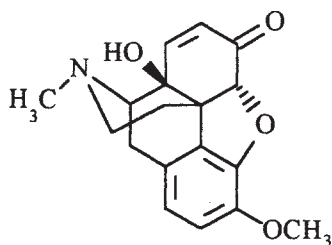
14-hydroxy-codeinone
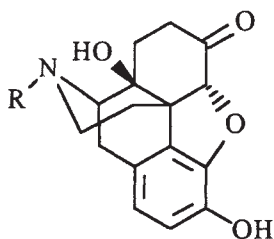
R = CPM   naltrexone
R = allyl   naloxone

Nitration of thebaine either with tetranitromethane (Allen and Kirby, 1981) or with dinitrogen tetroxide (Archer and Osei-Gyimah, 1979; Osei-Gyimah and Archer, 1980) yields 14-nitrocodeinone as a main product.

Thebaine very readily undergoes the Diels—Alder addition of dienophiles, besides forming adducts with p-benzoquinone, maleic anhydride, acrylic acid esters and methyl vinyl ketone. The adduct with methyl vinyl ketone reacts with Grignard reagents to give tertiary alcohols. Unexpectedly, these compounds display a very high analgesic activity, i.e. they are ~1000 times more potent than morphine. O-Demethylation of the C-3 methoxy groups results in a further increase in analgesic potency. There are also derivatives with analgesic activities about 1000–8000 times that of morphine (Bentley, 1971; Lewis, 1974; Lewis *et al.,* 1971).

The reactions of thebaine with alkali metals in liquid ammonia give dihydrothebaine-(Φ and ß-dihydrothebaine (Bentley *et al.,* 1952; Razdan *et al.,* 1978). The latter compound can be prepared from thebaine by reduction with LiAlH$_4$ (Bentley *et al.,* 1969; Schmid and Karrer, 1950). Thebaine forms a variety of products under conditions of catalytic hydrogenation. The detailed mechanisms of these reactions have been studied by several authors (Small, 1955; Szabó and Bognár, 1953). The course of the reduction is markedly dependent upon the type of catalyst and the pH of the solution.

Generation of an anion from thebaine is readily achieved by treatment with n-buthyllithium at—78°C in tetrahydrofuran. This anion can be methylated on C-5 to provide a new procedure for the synthesis of 5-methyldihydromorphinone (metopon), which is a strong analgesic (Boden *et al.,* 1982).

N-demethylation of thebaine (Pohland and Sullivan, 1967) can be accomplished by reaction with diethyl azodicarboxylate (Hosztafi, 1987; Schwab, 1980). It is noteworthy that thebaine forms C-9-N cleaved products with cyanogen bromide or chloroformic esters (Bertgen *et al.,* 1967a,b). Both N-demethylation and a Diels-Alder reaction took place in the reaction of thebaine with two equivalents of diethyl azodicarboxylate (Merz and Pook, 1970).

The rearrangement of thebaine (Channon *et al.,* 1969; Fleischhacker *et al.,* 1968) in strong acids (concentrated HCl or methanesulfonic acid) leads to the formation of morphothebaine derivatives (Granchelli *et al.,* 1977, 1980). In dilute HC1 the rearrangement product is thebenine. Novel rearrangement products have been isolated following the action of acetic anhydride on thebaine (Allen *et al.,* 1984b).
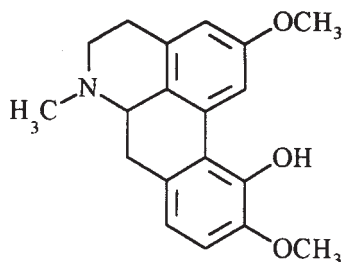
morphothebaine
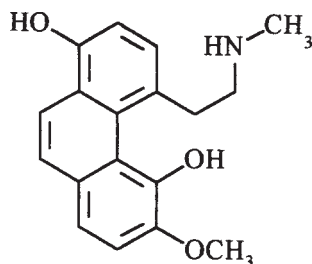
thebenine

Thebaine undergoes rearrangement during its reaction with phenylmagnesium bromide (Bentley and Robinson, 1952). The product, phenyldihydrothebaine exists in four stereoisomeric forms. It has a chiral carbon (originally C-9), and the second centre of asymmetry is the biphenyl system. The structure of phenyldihydrothebaine was confirmed by means of Hofmann degradation reactions and oxidative degradation. Later, the parent compound was prepared by reduction of the iminium salt generated from thebaine and magnesium iodide (Bentley, 1967). The photochemical rearrangement of thebaine (Theuns *et al.,* 1984) yields the same derivative.
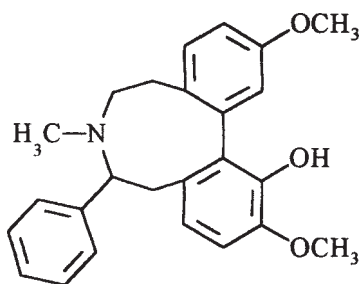
phenyldihydrothebaine

The 16-hydroxy derivative of thebaine has been obtained from opium and characterized by means of UV, IR, NMR and mass spectrometry. It has been isolated from the non-phenolic alkaloid fraction of opium and purified by preparative thin layer chromatography (TLC) and column chromatography. The authors could not exclude the possibility that 16-hydroxythebaine was an artefact produced during the

drying or storage of opium or during the isolation and purification of the alkaloids (Brochmann-Hanssen *et al.*, 1972).

3.3 Oripavine

Oripavine was isolated at levels of 0.1% from the dried capsules of a variety of opium poppies grown in Tasmania (Nielsen *et al.*, 1983). An earlier attempt to detect its presence by the isotope dilution method was unsuccessful (Brochmann-Hanssen and Okamoto, 1980). Spectral (UV, PMR and mass) properties of oripavine have been reported by several authors (Sariyar, 1982; Shaffiee *et al.*, 1977; Slavik and Slavikova, 1994). The synthesis of oripavine was elaborated from morphine. 3-Acetyl-6-O-methylmorphine was oxidized with manganese dioxide to 3-acetyloripavine, which was hydrolyzed to give oripavine (Barber and Rapoport, 1975). 3-O-(*tert*-Butyldimethylsilyl)-oripavine was prepared by the same method and its Diels-Alder reactions were studied (Klein *et al.*, 1990).

3.4 Neopine

Neopine has been isolated from opium (Dobbie and Lauder, 1911; Homeyer and Shilling, 1947) and its separation from codeine can be achieved by fractional crystallization of the sulphate salts. The isolation of neopine from poppy straw by modification of the Kabay-Bognár process has also been reported. Codeine-free neopine was conveniently prepared by re-crystallization of a mixture of hydrochlorides from methanol (Berényi *et al.*, 1986).

Neopine can be prepared from thebaine via neopinone or 14-bromocodeinone (Conroy, 1955; Krausz and Rüll, 1960; Okuda *et al.*, 1965). The reduction of neopinone with $NaBH_4$ is not stereospecific and gives a mixture of neopine and isoneopine. When bulky reducing agents are used, stereoselective reduction to neopine is achieved (Wunderly and Brochmann-Hanssen, 1977). Similarly, the reduction of 14-bromocodeinone or 14-bromocodeine ($NaBH_4$) yields neopine and its isomers. It has been reported that the reduction of 14-chlorocodeine resulted in neopine as a single product (Makleit *et al.*, 1977a). Neopine has also been prepared by the oxymercuration of thebaine (Barber and Rapoport, 1976; Dauben *et al.*, 1979).

The transformations of 6-O-sulfonyl esters of neopine have been studied by several authors (Berényi *et al.*, 1980; Beyerman *et al.*, 1984; Maat *et al.*, 1979; Rapoport and Bonner, 1951).

N-Demethylation of neopine can be performed with diethyl azodicarboxylate (Hosztafi *et al.*, 1980, 1985), whereas O-demethylation yields neomorphine (Berényi *et al.*, 1983; Small, 1947). The double bond of neopine seems to be less reactive, but some reactions (hydrogenation (Horn *et al.*, 1978), hydroboration (Takeda *et al.*, 1969) and oxidation with OsO_4 (Sargent *et at.*, 1958)) have been reported. The spectral properties (NMR, MS and CD) of neopine have been studied in detail (Audier *et al.*, 1965; Batterham *et al.*, 1965; Okuda *et al.*, 1964; Rüll, 1963; Rüll and Gagnaire, 1963; Wheeler *et al.*, 1967).

3.5 Pseudomorphine
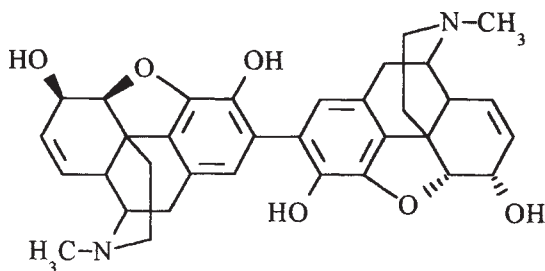
pseudomorphine

Pseudomorphine or 2,2'-bimorphine can be readily obtained by the oxidation of morphine. It has been isolated from opium by several investigators, but at the time it was not known whether it was a natural product or whether it was formed during the processing of opium. Pseudomorphine has been detected in opium (Froemming *et al.,* 1963) and its fast enzymatic oxidation has been demonstrated. In the 1970s, pseudomorphine was re-isolated from poppy pods (Danelyants *et al.,* 1973; Matantseva *et al.,* 1980; Mushinskaya *et al.,* 1971). It was revealed that the crude poppy enzyme fraction converts morphine into pseudomorphine in the presence of hydrogen peroxide. This finding suggests that pseudomorphine is a natural product (Vágújfalvi and Petz-Stifter, 1982). The enzymatic oxidation of morphine (Roerig *et al.,* 1976; Schenck *et al.,* 1965) and pseudomorphine (Froemming *et al.,* 1963) has been studied in detail. The structure of pseudomorphine was confirmed (Bentley and Dyke, 1959) on the basis of its reactions. Spectral studies (IR, UV and NMR) of pseudomorphine have also been performed (Graden *et al.,* 1990; Holcomb *et al.,* 1973). The mechanism of pseudomorphine formation has also been discussed (Yeh and Lach, 1961b).

Pseudomorphine is highly fluorescent (Darwin and Cone, 1980) and numerous sensitive fluorometric assays of morphine in biological material are based on the conversion of morphine to a pseudomorphine derivative (Mulé and Hushin, 1971; Sansur *et al.,* 1972; Takemori, 1968; Yoshimura *et al.,* 1966). Pseudomorphine is a decomposition product of morphine salts and its detection and separation are very important. Chromatography has proved to be a very efficient method for the detection and separation of pseudomorphine. Reference may be made here to studies involving paper chromatography (Miram and Pfeifer, 1958; Yeh and Lach, 1961 a), gas chromatography (Yeh, 1973), thin layer chromatography (Ebel and Rost, 1980; Holcomb *et al.,* 1973; Kupferberg *et al.,* 1964) and liquid chromatography (Beaumont and Deeks, 1982; Jane and Taylor, 1975; Lee, 1984; Nelson *et al.,* 1982; Roksvaag *et al.,* 1980).

3.6 Salutaridine

salutaridine   salutaridinol-I
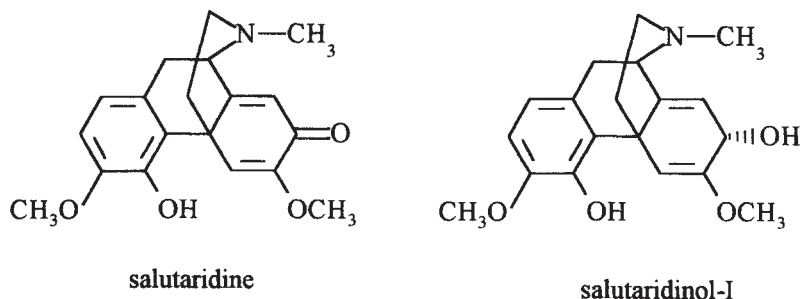
Salutaridine plays an important role as a biosynthetic intermediate in the biogenesis of morphinane alkaloids.

The presence of Salutaridine has been demonstrated in *Papaver somniferum* by means of carrier dilution after feeding with labelled reticuline. Brochmann-Hanssen *et al.*, isolated Salutaridine from opium (Brochmann-Hanssen *et al.*, 1970), and Proksa *et al.*, later found it in the phenolic alkaloid fraction of poppy plants collected at the stage of opium ripeness (Proksa *et al.*, 1979). Salutaridine has also been detected in poppy by means of radioimmunoassay (Wieczorek *et al.*, 1986).

The conversion of Salutaridine to thebaine has been demonstrated to proceed through salutaridinol-I, one of the two epimeric alcohols formed in the reduction of Salutaridine with sodium borohydride. Salutaridinol-I can also be detected in growing poppy capsules (Barton *et al.*, 1965). Recently its structure (7S-salutaridinol) has been revised (Lotter *et al.*, 1992). Salutaridinol-I can be transformed both chemically (acidic treatment) and enzymatically to thebaine (Barton *et al.*, 1965; Lenz and Zenk, 1994). Hydrogenation of Salutaridine was found to give tetrahydrosalutaridinol (Haynes *et al.*, 1968). (±)-Salutaridine has been treated with phenyllithium and methyllithium to yield the corresponding salutaridinols. Treatment of 7-phenylsalutaridinol with acid produced the racemic form of 7-phenylthebaine (Soeiro *et al.*, 1982).

Salutaridine was prepared before it was isolated from *Croton salutaris*. Dihydrothebaine-Φ (prepared from thebaine) was acetylated and the product was then successively oxidized with SeO_2 and MnO_2 to yield O-acetylsalutaridine. Mild alkaline hydrolysis of the latter produced Salutaridine (Barton *et al.*, 1965).

Salutaridine was also prepared by oxidation of the hydroboration products of thebaine (Takeda *et al.*, 1969).

O-Methylation (CH_2N_2) of 6-O-demethylsalutaridine yielded Salutaridine at low levels (Rearick and Gates, 1970). Another simple and efficient method has been developed for me preparation of Salutaridine from 6-O-demethylsalutaridine in four steps (Horvath and Makleit, 1981).

The biomimetic synthesis of Salutaridine from reticuline has been studied in detail. Oxidative p,p' coupling of reticuline yields Salutaridine. Dilution with inactive salutaridine was used to prove the formation of this alkaloid when labelled (±)-reticuline

was subjected to a variety of oxidizing conditions (potassium ferricyanide, manganese dioxide, ferric chloride). When potassium ferricyanide was used, there was a 0.015% yield. The low yield obtained in these experiments was probably due to the fact that salutaridine itself seemed to be more rapidly oxidized than reticuline. On the other hand, salutaridine yielded 1, 1'-bissalutaridine in these reactions. A significant improvement in the biomimetic synthesis of salutaridine was achieved when N-acylnorreticulines (R=CO_2Et; COCF$_3$) were used as starting materials and thallium tris (trifluoroacetate) was employed as the oxidative reagent, resulting in N-acylnorsalutaridines in 16–35% yields (Schwartz and Mami, 1975). The high regioselectivity of the reaction was attributed to the chelating effect of the thallium ion. (±)-N-Ethoxycarbonylnorreticuline was reduced to give epimeric (±)-salutaridinols, and acidic treatment of this mixture afforded (±)-thebaine.

(-)-N-Acyl-6'-bromonorreticulines (R=CO_2Et; COCF$_3$) were subjected to phenolic oxidative coupling with a variety of aryliodoso complexes in dichloromethane. N-Trifluoroacetyl-1-bromonorsalutaridine prepared by this means was transformed to a mixture of 1-bromosalutaridinols, and the latter were converted to 1-bromothebaine. (-)-Codeine was obtained from 1-bromothebaine in two steps (White *et al.,* 1983). A feasible and effective cyclization method was developed for p,o oxidative coupling of N-acylnorreticulines and 6'-halogeno-substituted-N-acylnorreticulines, utilizing lead tetra-acetate or iodosobenzene diacetate in the presence of strong organic acids. The corresponding 1-halogeno-N-acyl-norsalutaridines were formed in 15–58% yields. Salutaridine was obtained by N-methylation of N-norsalutaridine prepared from N-formylnorsalutaridine by acidic deformylation (Szántay *et al.,* 1980). Later, the preparation of salutaridine was also achieved by the direct oxidative coupling of (±)-reticuline. When lead tetra-acetate was used in the presence of trichloroacetic acid, (±)-salutaridine was isolated at a level of 2.7% yield (Szántay *et al.,* 1982), although isoboldine (14%) was also obtained as a major product. Salutaridine was also obtained (1% yield) by the photolysis of 2'-bromoreticuline (Kametani *et al.,* 1971c, 1972).

2'-Amino-O-benzyl-(±)-laudanine was diazotized and the resulting diazonium salt was decomposed thermally (Pschorr reaction) to give (±)-salutaridine in 1.1% yield. The aminobenzylisoquinoline was resolved and the (-)-enantiomer was used for the synthesis of salutaridine. The latter was converted into thebaine by Barton's method (Kametani *et al.,* 1969b). A new total synthesis of salutaridine has been reported (Ludwig and Schäfer, 1986) in which a tetrahydro (1, 2, 10, 11) derivative of salutaridine was an intermediate. Dehydrogenation of the latter compound yielded racemic salutaridine. Salutaridine was also prepared by the palladium(0)-catalyzed cyclization of 2'-bromoreticuline (Wiegand and Schäfer, 1995).

3.7 Somniferine

A dimeric morphinane alkaloid has been isolated from opium poppies grown in Tasmania. Somniferine was obtained from the poppy head. Its structure was confirmed via its PMR and ^{13}C-NMR spectra. Somniferine contains a thebaine and a 14-hydroxymorphinone unit. The linkage is found between C-16 carbon of thebaine
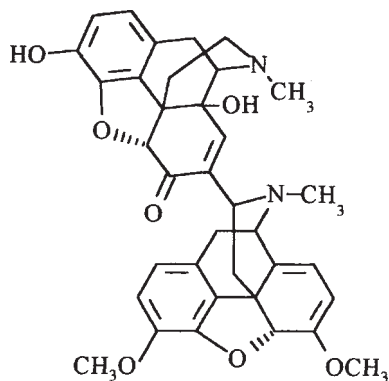
and C-7 carbon of 14-hydroxymorphinone. The methyl ether of somniferine has also been detected in the poppy (Dragar and Bick, 1988).

3.8 Morphine N-oxide and Codeine N-oxide

Diastereomeric morphine N-oxides have been isolated from the polar basic components of *Papaver somniferum* by preparative TLC as has the major isomer of codeine N-oxide. Isolation was performed from fresh capsules. The authentic diastereomeric N-oxides were also prepared from morphine and codeine. The major isomers possess equatorial N-methyl groups. The structures of the N-oxide pairs were elucidated in PMR and MS studies. TLC systems were elaborated to separate the N-oxide pairs (Philipson *et al.*, 1976). It is of interest that the formation of isomeric N-oxides was not observed earlier (Craig and Purushothaman, 1970; Kelentey *et al.*, 1957). The presence of morphine N-oxide was detected in the capsule and stem latex of *Papaver somniferum* L. by means of ^{14}C-labelled morphine as tracer (Gurkan, 1984). A crude poppy enzyme fraction transforms morphine to morphine N-oxide (Vágujfalvi and Petz-Stifter, 1982).

4 APORPHINE ALKALOIDS

Aporphine alkaloids of the opium poppy were earlier isolated from other plants. These alkaloids have the absolute configuration corresponding to the S series, which relates them to (+)-reticuline. Several reviews on the aporphine alkaloids have been published previously (Kametani and Honda, 1985; Shamma, 1967; Shamma and Slusarchyk, 1964).

4.1 Isoboldine

Isoboldine has been isolated from the diphenolic alkaloid fraction of opium. Spectral studies (PMR, MS, UV and ORD) have shown the isolated alkaloid to be identical with synthetic (±)-isoboldine (Brochmann-Hanssen *et al.*, 1967, 1971b, 1973).

(+)-Isoboldine has the 6a-S absolute configuration. The absolute configuration was also elucidated in X-ray (Brown and Hall, 1977) and ORD-CD studies (Kametani *et al.*, 1970).

Reticuline was oxidized with potassium ferricyanide at pH=6 to afford (±)-isoboldine

isoboldine   corytuberine   magnoflorine
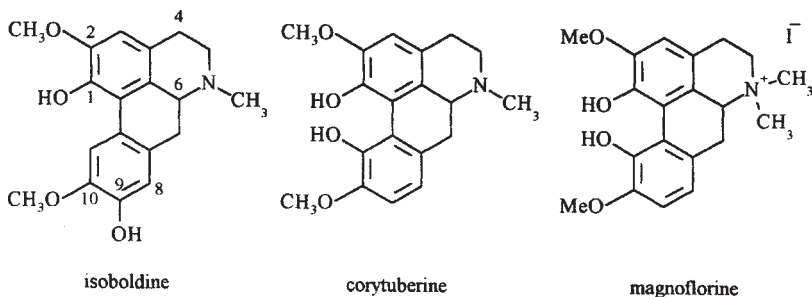
in 5% yield (Chan and Maitland, 1966). The product was identified via its spectral (UV, NMR and IR) properties. A lower (0.5%) yield (Jackson and Martin, 1966a) has been reported in the same reaction, but oxidation of 6'-bromoreticuline increased the yield (2.5%) of isoboldine. In this case, the bromine atom is eliminated. Isoboldine was obtained in low (0.5–5%) yields by phenolic oxidative coupling with various reagents (Ag_2CO_3, $K_4[Fe(CN)_6]$, $VOCl_3$) (Franck et al., 1967; Kametani et al., 1969a, 1971b, 1976b).

Marked improvements have been reported in the oxidative coupling of (±)-reticuline. Isoboldine was obtained at a 53% yield when the oxidation was effected with vanadium oxytrichloride (Schwartz, 1973) and at a 14% yield when lead tetra-acetate was used (Szántay et al., 1980, 1982). The phenolic oxidation of reticuline with cuprous chloride and oxygen in pyridine gives corytuberine and isoboldine as by-products (Kametani et al., 1976b, 1977a). The transformation of phenols to quinol acetates with lead tetra-acetate has been studied in detail. The conversion of quinol acetates with trifluoroacetic acid led to the syntheses of aporphine alkaloids, e.g. isoboldine (Hara et al., 1976b). Photolysis of 6'-bromoreticuline (Gupta and Bhakuni, 1988) in the presence of NaOH resulted in isoboldine in ~ 20% yield. Pschorr cyclization of 6-aminobenzyltet-rahydroisoquinolines likewise afforded isoboldine (Bhakuni and Kumar, 1986). The photolytic Pschorr synthesis of isoboldine has also been performed (Kametani et al., 1971d). Isoboldine has additionally been prepared by the photolysis of the proaporphine orientalinone (Gözler et al., 1986).

The UV and NMR spectra of aporphine alkaloids, including isoboldine, have also been studied in detail (Brochmann-Hanssen et al., 1967; Jackson and Martin, 1966a; Ricca and Casagrande, 1979) and found to provide a useful tool for determination of the structure. The mass spectrum of isoboldine (Brochmann-Hanssen et al., 1967; Jackson and Martin, 1966b) has similarly been investigated.

4.2 Corytuberine

Corytuberine has been isolated from opium (Nijland, 1965) and identified by conversion into magnoflorine.

Corytuberine has been prepared by phenolic oxidative coupling of reticuline (Kametani et al., 1976b, 1977a). The reaction of reticuline N-oxide with cuprous chloride gave corytuberine in high yields (Kametani and Ihara, 1979, 1980). Boron trichloride selectively cleaved the methylenedioxy group of (S)-bulbocapnine methyl

ether and after methylation yielded (S)-corytuberine, which clarified the absolute configuration of the latter alkaloid (Gerecke *et al.,* 1976).

The spectral characteristics of corytuberine (UV, NMR and MS) have been reported (Jackson and Martin, 1966b,c; Slavik and Slavikova, 1984).

4.3 Magnoflorine

The quaternary alkaloid magnoflorine has been isolated from opium (Nijland, 1965); the alkaloids were separated by column (cellulose) chromatography. It has also been isolated from the callus tissue of the opium poppy (Furuya *et al.,* 1972; Ikuta *et al.,* 1974) and from poppy (Brochmann-Hanssen *et al.,* 1971b, 1973). The structure of magnoflorine was elucidated in spectral studies, or by interconversion to other aporphine alkaloids. Magnoflorine results in O,O'-dimethylcorytuberine methiodide on methylation. It is noteworthy that racemic magnoflorine iodide may be resolved by paper chromatography with a buffer solution as a developing solvent. The same resolution was effected on cellulose column chromatography (Tomita and Sugamoto, 1962). Hofmann elimination reactions of magnoflorine iodide (Lu and Tsai, 1988; Tomita and Takano, 1960) have been studied in detail. Magnoflorine yields a Hofmann elimination product on the action of sodium metal in liquid ammonia (Tomita and Fukagawa, 1963). Since magnoflorine is identical to the methiodide of corytuberine, synthetic methods elaborated for corytuberine are suitable for the synthesis of magnoflorine. Detailed PMR and MS studies have been performed on magnoflorine (Dolejs, 1984; Marsaioli *et al.,* 1979; Slavik and Dolejs, 1973). Its absolute configuration was confirmed (Albonico *et al.,* 1966; Djerassi *et al.,* 1963; Ringdahl *et al.,* 1981a) by means of ORD and CD studies.

5 BENZO[C]PHENANTHRIDINE ALKALOIDS

Sanguinarine and its derivatives belong in the group of benzo[c]phenanthridine alkaloids. We use the numbering system of *Chemical Abstracts;* atoms N-13 and C-14 correspond to positions N-5 and C-6 in the commonly used nomenclature. These alkaloids have been reviewed (Simanek, 1985).
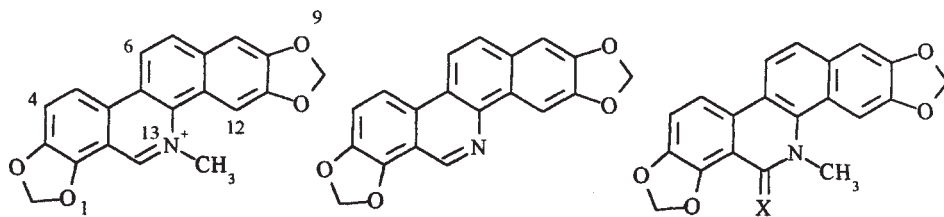
sanguinarine   norsanguinarine   X = H$_2$ dihydrosanguinarine

                                                X = O oxysanguinarine

5.1 Sanguinarine

The presence of sanguinarine has been detected in opium by paper chromatography and paper electrophoresis (Hakim *et al.,* 1961). Sanguinarine was found in the root, stem and leaves of the opium poppy but not in the capsule. Later, this alkaloid was isolated from the callus tissue of the opium poppy (Furuya *et al.,* 1972; Ikuta *et al.,* 1974). Sanguinarine was identified via mass spectroscopy. It has also been detected in a poppy suspension culture (Forche and Frautz, 1981; Songstad *et al.,* 1989).

The structure of sanguinarine was elucidated via transformation reactions. Acetylation of chelidonine and the reaction of O-acetylchelidonine with mercuric acetate yielded sanguinarine. The degradation of sanguinarine and chelidonine with zinc dust gave benzo[c]phenanthridine, as an identical product. The oxidation of chelidonine with mercuric acetate, chromic acid or potassium permanganate led to a mixture of sanguinarine and dihydrosanguinarine (Beke *et al.,* 1958). Reduction of sanguinarine with sodium borohydride gives dihydrosanguinarine, which can be transformed into sanguinarine by treatment with mercuric acetate or with dichlorodicyanobenzo-quinone (Ishii *et al.,* 1984). These reactions were exploited for the purification of sanguinarine (Stipanovic *et al.,* 1972). Sanguinarine is present as an equilibrium mixture of the quaternary iminium form and the nucleophilic solvent adduct in protic solvents. The adduct with the hydroxide ion is called a pseudobase. Sanguinarine readily forms Ψ-cyanide with alkali cyanides; this reaction is important during the identification of benzophenanthridine alkaloids. Re-crystallization of sanguinarine from methanol or ethanol gives rise to 14-alkoxydihydrosanguinarine, and treatment with acetone in alkaline medium yields 14-acetonyldihydrosanguinarine. This compound was isolated from the callus tissue of opium poppy by column chromatography, but it proved to be an artefact of the isolation process (Furuya *et al.,* 1972).

Several methods have been reported for the total synthesis of sanguinarine (Dyke and Sainsbury, 1967; Dyke *et al.,* 1968a,b; Sainsbury *et al.,* 1970; Shamma and Tomlinson, 1978; Smidrkal, 1984).

The skeleton of sanguinarine can be prepared from protopine (Onda *et al.,* 1969, 1971) or from narcotine (Onda and Kawamaki, 1972). A biomimetic synthesis of sanguinarine has been reported from coptisine (Hanaoka *et al.,* 1986). The total synthesis of chelidonine (Cushman *et al.,* 1980; Oppolzer and Keller, 1971; Oppolzer and Robbiani, 1983) is also suitable for the preparation of sanguinarine.

The NMR (^{1}H and ^{13}C) and MS spectra of the alkaloid have been studied (Blaskó *et al.,* 1988; Slavik *et al.,* 1968). The tautomerism of sanguinarine has been studied by spectroscopic (UV and PMR) methods (Hruban *et al.,* 1970; Tolkachev and Lasskaya, 1974).

5.2 Norsanguinarine

Norsanguinarine was isolated first from the callus tissue of the opium poppy (Furuya *et al.,* 1972; Ikuta *et al.,* 1974). Its structure was elucidated via its UV, NMR and MS spectra. It proved to be identical to the authentic sample produced as an intermediate of sanguinarine synthesis (Sainsbury *et al.,* 1970). Norsanguinarine can be prepared from sanguinarine chloride by thermal degradation (Haisova *et al.,* 1973).

5.3 Dihydrosanguinarine

Dihydrosanguinarine has been isolated from the callus tissue of poppy (Furuya *et al.,* 1972; Ikuta *et al.,* 1974). Dihydrosanguinarine is usually an intermediate during the syntheses of sanguinarine *(vide supra)*. A biomimetic synthesis of dihydrosanguinarine was reported from coptisine (Hanaoka *et al.,* 1986).

UV spectral characteristics of dihydrosanguinarine have been reported (Hruban *et al.,* 1970) and NMR (Ishii *et al.,* 1975; MacLean *et al.,* 1969; Onda *et al.,* 1970).

5.4 Oxysanguinarine

Oxysanguinarine has similarly been isolated also from the callus tissue of the opium poppy (Furuya *et al.,* 1972; Ikuta *et al.,* 1974). Oxysanguinarine can be prepared by oxidation of sanguinarine nitrate with potassium ferricyanide in alkaline solution. Sanguinarine φ-cyanide is also a useful starting material for the preparation of Oxysanguinarine (Ishii *et al.,* 1984). It can be reduced to dihydrosanguinarine with lithium aluminium hydride (MacLean *et al.,* 1969). Two alternative methods have been reported for the synthesis of Oxysanguinarine (Shamma and Tomlinson, 1978; Smidrkal, 1984). 6-Iminosanguinarine has been prepared from Oxysanguinarine (Castedo *et al.,* 1981).

6 PAPAVERRUBINE ALKALOIDS
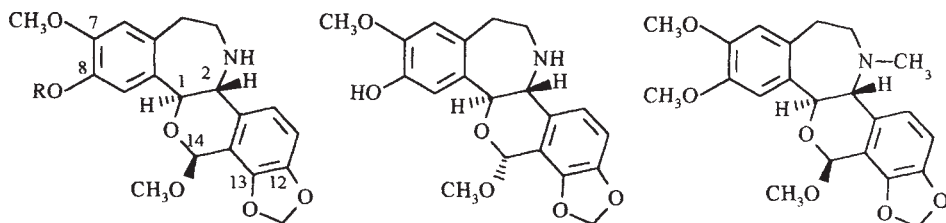
R = H   porphyroxine (papaverrubine D)   epiporphyroxine (papaverrubine C)   glaudine
R = CH$_3$ papaverrubine B

6.1 Porphyroxine (Papaverrubine D)

The isolation of porphyroxine was described by Merck in 1837. This minor alkaloid of opium gives a very intense and characteristic red colour with dilute mineral acids (Awe and Winkler, 1958). The concentration of the alkaloid varies with the source of the opium, and the colour reaction is useful for determining the geographic origin of opium seized in illicit traffic. Although many investigators earlier attempted to isolate the pure alkaloid, it was only successful in 1962. Pfeifer and Teige described the physical properties of porphyroxine and named it papaverrubine D (Pfeifer and Teige, 1962). They found six alkaloids in the genus of *Papaver*, which have similar structures (Pfeifer, 1962; Pfeifer and Banerjee, 1964). Reproducible methods have been elaborated for the isolation of porphyroxine from opium (Genest and Farmilo, 1963; Szendrey,

1968). The structure of porphyroxine was soon elucidated. The precise structure was determined by correlation of the NMR and mass spectra with those of the corresponding rhoeadineisorhoeadine alkaloids (Brochmann-Hanssen and Hirai, 1967; Pfeifer, 1966a; Pfeifer *et al.,* 1965). The structures of rhoeadine-type alkaloids were confirmed mainly by means of chemical degradations (Santavy *et al.,* 1965). By methylation of the secondary nitrogen, papaverrubines can be converted into the rhoeadine (isorhoeadine) alkaloids.

Two independent routes have been reported for the synthesis of rhoeadine alkaloids (Hohlbrugger and Klötzer, 1974; Shamma and Töke, 1973, 1975).

6.2 Papaverrubine B

Papaverrubine B has been isolated from Japanese opium and it proved to be O-methylporphyroxine. Authentic papaverrubine B was prepared by methylation of porphyroxine with diazomethane. Papaverrubine B was characterized by means of spectral (NMR, UV and IR) studies (Hughes and Farmilo, 1965; Pfeifer, 1965, 1966a).

6.3 Papaverrubine C (Epiporphyroxine)

The alkaloid papaverrubine C has been isolated from Japanese opium by means of column chromatography (Hughes *et al.,* 1967; Pfeifer, 1965). Its structure was corroborated by chemical evidence because porphyroxine yielded papaverrubine C on the action of methanolic hydrochloric acid. Detailed NMR. analysis indicates that papaverrubine C is a C-14 epimer of porphyroxine. It was considered that papaverrubine C was an isolation artefact because porphyroxine can readily be isomerized to papaverrubine C. This change is effected on heating of a solution of porphyroxine.

6.4 N-Methyl-14-O-desmethylepiporphyroxine

N-Methyl-14-O-desmethylepiporphyroxine has been isolated from opium and characterized by means of mass and NMR spectroscopy (Brochmann-Hanssen *et al.,* 1968). Its structure was confirmed by chemical conversions to known compounds, e.g. glucamine and N-methylepiporphyroxine. like glucamine, it has a hemiacetal structure, with a thermodynamically stable configuration at position-14, and *trans*-stereochemistry at the B/D ring junction. The new alkaloid may be obtained from N-methylporphyroxine by hydrolysis. There is reason to believe that N-methylporphyroxine, having a less stable configuration at position 14 may occur in the opium poppy. It is therefore possible that N-methyl-14-O-desmethyl-epiporphyroxine may not exist as such in the fresh plant, but may be formed by acid hydrolysis and epimerization during the storage of opium or in the course of the extraction and purification of the alkaloid mixture.

6.5 Glaudine

Glaudine was isolated from opium by means of preparative TLC (Pfeifer, 1964, 1965). Glaudine was found earlier in *Papaver* glaucum as a major alkaloid. It is identical to O,N-dimemylporphyroxine. Its structure was confirmed by spectroscopic (UV, MS

and NMR) studies (Brochmann-Hanssen and Hirai, 1967; Shamma *et al.,* 1968). The absolute configuration of glaudine was elucidated via its the ORD and CD spectra (Santavy *et al.,* 1967; Shamma *et al.,* 1971).

7 PHTHALIDEISOQUINOLINE ALKALOIDS

R = CH$_3$ narcotine
R = H narcotoline

R = CH$_3$ narceine
R = H nornarceine

narceine imide
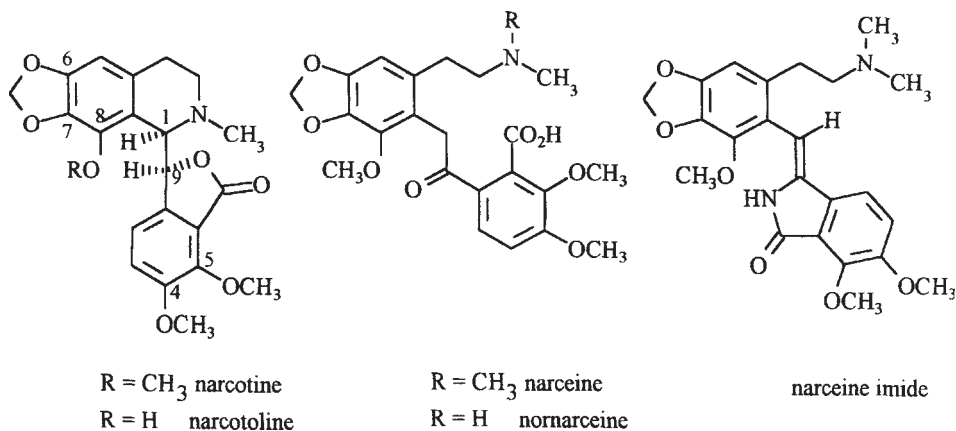
7.1 Narcotine

After morphine, narcotine is the second most abundant alkaloid present in opium. Russian and Indian opium have the highest narcotine contents (12% and 7.7% respectively). Narcotine, as a weakly basic alkaloid, remains insoluble during the extraction of opium with water at pH 5.5. Narcotine can readily be extracted from opium at acidic pH (≈3) with chloroform (Barbier, 1950; Ugai, 1960). The separation of narcotine from Indian opium has been described (Ramanathan and Chandra, 1981). The isolation of narcotine from powdered poppy capsules by the Kabay method has been reported (Bienicki and Rylski, 1956; Bognár *et al.,* 1967b, 1969; Gorecki and Bognár, 1968; Hodková *et al.,* 1972; Poethke and Pechmann, 1959). Gnoscopine has also been isolated from opium, but it was probably formed from narcotine during processing. Treatment of narcotine with oxidizing agents yields cotarnine and opianic acid, but reductive decomposition gives hydrocotarnine and meconin. Hesse found hydrocotarnine in the mother liquor of opium, after morphine isolation, but it was probably a degradation artefact

The Schotten—Baumann reaction of narcotine with ethyl chloroformate yielded N-carbethoxycotamine and meconine (Whaley and Meadow, 1954). The preparation of the chloro-urethane formed by cleavage of the C-1-N bond was reported (Angerer *et al.,* 1992). (+)-ß-Narcotine has been prepared by hydrolysis of the product obtained from the reaction of narcotine with cyanogen bromide (Gaál *et al.,* 1971b). This process involves a specific inversion at chiral centre C-l. Photochemical racemization of a-narcotine has been reported (Kametani *et al.,* 1977b).

(-)-α-Narcotine (1**R**,1'S) was allowed to react with cyanogen bromide in a tetrahydrofuran-water or chloroform-ethanol solvent mixture in the presence of magnesium oxide (Kerekes and Gaál, 1980). The reactions yielded (-)-N-cyano-1(S)-hydroxy (ethoxy)-1,2seco-1'(S)-narcotine. Refluxing of the seco compounds in dilute hydrochloric acid resulted in cyclization, and (+)-ß-narcotine was isolated as main product Reaction of narcotine with sodium alcoholates (Schmidhammer and Klötzer, 1978) results in substitution of the 5'-methoxy group, while the action of aqueous alkalis leads to inversion of the C-l' centre. 5'-O-Demethylnarcotine was also prepared and it was later isolated from poppy (Répási *et al.,* 1993).

The stereochemistry of narcotine (1R 1'S) was elucidated from ORD studies (Ohta *et al.,* 1963b, 1964). Narcotine was reduced with lithium aluminium hydride (Simanek and Klasek, 1971, 1973), yielding a 1'-hydroxybenzylderivative the structure of which was correlated with that of natural laudanosine (Onda and Kawamaki, 1972).

The kinetics and mechanism of the hydrolysis of narcotine have been studied (Pawelczyk and Zajac, 1975).

N-Nornarcotine can be prepared by reacting narcotine N-oxide with ferric citrate (Allen *et al.,* 1984a).

Gnoscopine has been prepared by the Bischler-Napieralsky method. A mixture of α and ß-norgnoscopine was isolated and methylated to α and ß-gnoscopine (Gaál *et al.,* 1971b). This synthesis was re-investigated and the formation of an unnatural regioisomer was detected (Varga *et al.,* 1991).

Narcotine has been synthesized by treating the appropriately substituted N-methyl-3,4-dihydroisoquinolinium salt and the bromophthalide with zinc in acetonitrile (Shono *et al.,* 1983). Narcotine can be prepared from the benz[d]indeno[l,2-b]azepine skeleton (Kametani *et al.,* 1975).

The phthalideisoquinoline ring system of narcotine can be converted to other alkaloids, e.g. benzophenanthridine or protoberberine skeletons (Onda and Kawamaki, 1972; Simanek and Klasek, 1973).

The spectral properties of narcotine (NMR, MS and UV) have been investigated in detail (Janssen *et al.,* 1990; Ohashi *et al.,* 1963; Pinyazhko, 1964; Sangster and Stuart, 1965; Tatematsu and Goto, 1965; Uhrin and Proksa, 1989)

7.2 Narcotoline

The isolation of narcotoline has been reported from opium (Pfeifer, 1957b; Pfeifer and Weiss, 1955) and from poppy head (Baumgarten and Christ, 1950; Bognár *et al.,* 1967a; Tulecki and Gorecki, 1969; Zsadon, 1966).

Narcotoline yields (–)-narcotine on treatment with diazomethane. The structure of narcotoline was corroborated by degradation reactions (Pfeifer, 1957a; Pfeifer and Weiss, 1956; Zsadon and Dezséri, 1964).

Several alkyl ethers (Me; Et) of narcotoline have been prepared by the Rodionov method. Methylation with trimethylphenylammonium butylate yielded a mixture of α- and ß-narcotine and α-gnoscopine (Gaál *et al.,* 1971 a). Gorecki *et al.,* prepared numerous O-alkyl derivatives of narcotoline (R=Et, Pr, benzyl, CH_2CO_2Et) and converted these ethers to homologues of narceine (Gorecki, 1973b,c; Gorecki and

Kubala, 1973a,b). They also studied the degradation of narcotoline ethers to obtain cotarnine derivatives. Several esters of narcotoline were prepared (Gorecki, 1973a). Natural (-)-narcotoline was converted to narcotolinephenyltetrazolyl ether, which yielded (-)-hydrastine on catalytic hydrogenation (Kerekes *et al.,* 1980).

Proton and carbon-13 NMR assignments of narcotoline have been reported (Janssen *et al.,* 1990).

8 NARCEINE-TYPE ALKALOIDS

8.1 Narceine

The isolation of narceine has been reported from poppy heads (Gorecki and Bognár, 1968; Tulecki and Gorecki, 1969) and from opium (Addinall and Major, 1933a). The structure of narceine was indicated by the finding that it could be prepared by heating narcotine methiodide with alkali (Addinall and Major, 1933a,b). Narcotine yields nornarceine enol lactones on derivation with acetic anhydride (Allen *et al.*, 1984a; Koblicova *et al.,* 1981). Blaskó *et al.* studied the Hofmann elimination of α-narcotine methiodide and attempted to isolate the enol lactone. They observed the formation of narceine directly (Blaskó *et al.,* 1982). Acetic anhydride treatment of narceine did not yield the expected enol lactone while the reaction of α- and ß-narcotine methiodides led to the formation of Z- and E-narceine enol lactones respectively.

N-Demethylation of narceine ethyl ester with cyanogen bromide yields a derivative of nornarceine (Vesely *et al.,* 1979). Nornarceine results from the treatment of narcotine with dilute acetic acid. It was isolated from opium, but it is considered to be an isolation artefact. N-Benzylnornarceine was obtained by Hofmann degradation of N-benzylnarcotinium bromide. N-debenzylation of N-benzylnornarceine yielded nornarceine (Klötzer *et al.,* 1972). Nornarceine can be transformed in several steps into a papaverrubine-type derivative (Klötzer *et al.,* 1971). The oxidation of narceine with Ce^{4+} was studied by means of ESR spectroscopy (Dixon and Murphy, 1976).

8.2 Narceinone
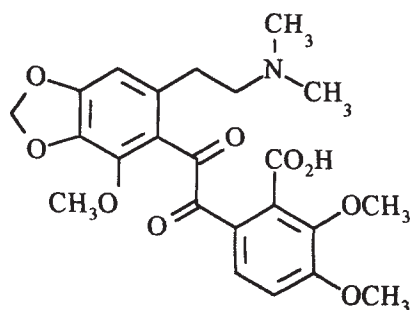
narceinone

Narceinone has been isolated from the unlanced dried capsules of poppy. Its structure was elucidated by comparison with an authentic sample (Chaudhuri and Thakur, 1989). Narceinone can be prepared by air oxidation of narceine in methanolic potassium hydroxide.

8.3 Narceine imide

This compound was prepared earlier from narceine or narceine amide. Hodkova *et al.* isolated a new alkaloid from poppy capsules (Hodková *et al.,* 1972). On the basis of chemical degradation (Trojanek *et al.,* 1975), spectral analysis, and comparison with a synthetic specimen, it was identified as narceine imide. Both (E and Z) isomers of narceine imide have been found in poppy (Proksa and Voticky, 1980). Determination of narceine imide has been performed by HPLC (Proksa, 1981). Nornarceine imide has been prepared from nornarceine by fusion with urea (Proksa, 1986).

Blaskó *et al.,* stated that unless and until the presence of narceine imide is conclusively demonstrated in the crude plant extracts prior to treatment with ammonia, it should be considered an isolation artefact (Blaskó *et al.,* 1982).

9 PROTOBERBERINE ALKALOIDS
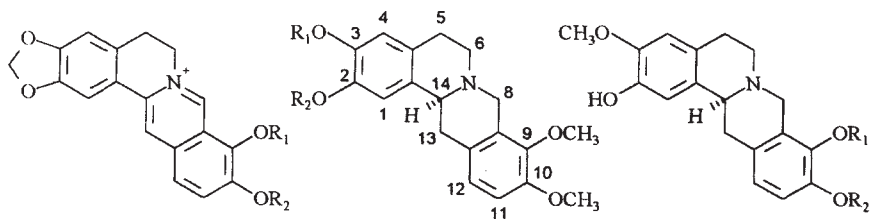
$R_1 = R_2 = CH_3$ berberine
$R_1 + R_2 = CH_2$ coptisine

$R_1 + R_2 = CH_2$ canadine
$R_1 = CH_3, R_2 = H$ isocorypalmine

$R_1 = H, R_2 = CH_3$ scoulerine
$R_1 = CH_3, R_2 = H$ stepholidine

Protoberberine alkaloids of poppy contain quaternary protoberberine salts e.g. berberine and coptisine, or tetrahydroprotoberberine derivatives, for example canadine. These alkaloids bear substituents at C-2, C-3, C-9 and C-10 positions without exception. Protoberberine alkaloids were reviewed earlier in the monograph *The Alkaloids* (Bhakuni and Jain, 1986; Jeffs, 1967).

9.1 Berberine

In a patented process for chromatographic separation, the isolation of appreciable quantities of berberine was described from opium poppies (Ose *et al.,* 1960). Later, negative tests were reported for berberine in several varieties of opium poppy by means of paper chromatography and paper electrophoresis (Hakim *et al.,* 1961).

The detection of berberine in opium poppy was unsuccessful even by a carrier dilution method (Brochmann-Hanssen *et al.,* 1971c). Battersby *et al.,* detected berberine by using radioactively labelled precursors (Battersby *et al.,* 1975).

The evidence indicative of the existence and structures of the ammonium form and the pseudobase form have been elucidated for berberine or coptisine (Beke, 1958). Berberine chloride may be reduced to canadine with a variety of reducing agents. This reduction may be stopped at the dihydroberberine stage. Berberine is unstable in the presence of concentrated alkali yielding oxyberberine. The Cannizzaro reaction of berberine leads to dihydroberberine and oxyberberine, whereas reduction with sodium borohydride gives tetrahydroberberine (Bentley and Murray, 1963b).

Oxidation of berberine with potassium ferricyanide affords a dimer (oxybisberberine), which can be converted to 8-methoxyberberine phenolbetaine. The latter compound rearranges into a mixture of α- and ß-hiydrastines (Moniot and Shamma, 1976). Photooxidation of oxyberberine gives an intermediate ?-lactol, which can be reduced to (±)-ß-norhydrastine (Shamma *et al.,* 1977a). Hydrolysis with concomitant air oxidation of polyberbine, derived from berberine chloride, yields a papaveraldine-like compound (Murugesan and Shamma, 1980). Oxidation of berberine with nitric acid results in berberidic acid as main product (Chinnasamy and Shamma, 1979).

Photo-oxygenation of berberine chloride gives 8-methoxyberberinephenolbetaine, which can be converted to α- and ß-hydrastines (Hanaoka *et al.,* 1977). 8-Methoxyberberine phenolbetaine proved to be a starting material for the synthesis of non-naturally occurring protoberberines (Hanaoka *et al.,* 1982a).

Berberine reacts with nucleophilic anions, e.g. cyanide ion or the anion of acetone, to undergo a C-8 substitution. C-8-Substituted products were formed in the reaction of berberine with dichlorocarbene (Manikumar and Shamma, 1981) and diazoacetic ethyl ester (Göber, 1972).

Degradation of berberine to naphthalene derivatives has been reported on the action of acetic anhydride and sodium acetate (Shamma *et al.,* 1975, 1977b).

Kametani *et al.* (1969c) reported the total synthesis of (±)-canadine, whose dehydrogenation with iodine afforded berberine iodide. Berberine can be prepared by the photochemical reaction of allocryptopine (Dominguez *et al.,* 1967).

The PMR and UV spectra of berberine have been studied (Blaskó *et al.,* 1988; Hruban *et al.,* 1970; Jewers *et al.,* 1972).

9.2 Canadine

Canadine has been detected in Kirghiz opium (Bessonova *et al.,* 1970). It has also been detected in poppy in tracer experiments (Battersby *et al.,* 1975). Canadine is readily oxidized by atmospheric oxygen to berberine and it is doubtless that berberine is a constituent of all plants which contain canadine. The synthesis of canadine was reported in the Mannich reaction of 2'-bromobenzyltetrahydroisoquinoline followed by O-methylation (Kametani *et al.,* 1969c, 1971a). Canadine can be prepared from hydrastine with the appropriately substituted homophthalic anhydride (Cushman and Dekow, 1979).

The synthesis of canadine has been reported from the corresponding isochromanone (Narasimhan *et al.,* 1981, 1983). The asymmetric synthesis of canadine has also been described (Pyne, 1987).

Hydrogenation of ophicarpine acetate yields canadine (Ohta *et al.,* 1963a) which clarifies the absolute configuration of the latter substance.

Canadine undergoes C-8-N cleavage on the action of cyanogen bromide (Albright and Goldman, 1969; Rönsch, 1974; Sallay and Ayres, 1963).

Numerous papers have appeared that deal with the NMR and MS spectra of canadine (Chen and MacLean, 1968; Hughes *et al.,* 1976; Janssen *et al.,* 1990; Richter and Brochmann-Hanssen, 1975).

9.3 Coptisine

Coptisine has been detected in opium by means of paper chromatography (Hakim *et al.,* 1961).

The structure of coptisine was elucidated via its reduction to (±)-stylopine. The reverse reaction was also performed, i.e. the oxidation of stylopine yields coptisine.

(±)-Stylopine has been prepared by several cyclization methods (Bradsher and Dutta, 1961; Dai-Ho and Mariano, 1987). Racemic stylopine was obtained through an isochromanone derivative (Narasimhan *et al.,* 1983). Stylopine and canadine were prepared by Bischler—Napieralsky cyclization of the appropriate isoquinolin-3-ones (Yasuda *et al.,* 1987).

Rhoeadine can be converted to coptisine via rhoeageninediol in several steps (Klasek *et al.,* 1968). The photochemical formation of coptisine has been described from protopine (Dominguez *et al.,* 1967).

Coptisine can be converted to a spirobenzylisoquinoline derivative (Hanaoka *et al.,* 1982b). The UV and NMR spectra of coptisine have been measured (Hruban *et al.,* 1970; Jewers *et al.,* 1972). The pseudobase formation of coptisine was studied by means of UV and NMR spectroscopy (Simanek *et al.,* 1976).

9.4 Scoulerine

The isolation of this alkaloid from opium has been reported. Scoulerine is a weak base and can be extracted at pH 1.5 It has been purified by preparative TLC and its structure elucidated via its NMR and MS spectra (Brochmann-Hanssen and Nielsen, 1966b).

Three routes have been elaborated for the synthesis of scoulerine. Two of these employ the Bischler—Napieralsky reaction. In the third, N-norreticuline is resolved, and the (+) and (-) enantiomers are used for synthesis of the enantiomers. (-)-Norreticuline can be converted into a separable mixture of (-)-scoulerine and (-)-coreximine by employing formaldehyde in the Mannich reaction. Since the absolute configuration of (-)-norreticuline is known, the latter reaction confirms the configuration of scoulerine (Battersby *et al.,* 1966). Scoulerine has been prepared from the bromo-substituted tetrahydro-isoquinoline via the Mannich reaction with formaldehyde, followed by the debromination of 12-bromoscoulerine (Bhakuni and Kumar, 1983; Kametani and Ihara, 1967). Dibenzylscoulerine has been prepared by

Bischler—Napieralsky cyclization of the corresponding lactam (Pandey and Tiwari, 1979).

Treatment of (±)-reticuline N-oxide with ferrous sulphate in methanol afforded a mixture of (±)-scoulerine and (±)-coreximine (Kametani and Ihara, 1979).

The NMR and MS spectra of scoulerine have been studied (Cashaw *et al.*, 1976; Chen and MacLean, 1968; Ohiri *et al.*, 1983). ORD and CD studies have also been performed to establish the absolute configuration (Kametani and Ihara, 1968; Ringdahl *et al.*, 1981b).

9.5 Stepholidine

Preparative TLC of the opium fraction containing minor phenolic alkaloids revealed a new alkaloid, which was purified by column chromatography. The NMR and MS spectra indicated a 2,3,9,10-substituted protoberberine skeleton. Methylation of the alkaloid with diazomethane yielded tetrahydropalmatine. The new substance proved to be identical with Stepholidine, as revealed by comparison of the respective IR and mass spectra (Brochmann-Hanssen and Richter, 1975). Several syntheses of Stepholidine have been reported (Chiang and Brochmann-Hanssen, 1977; Rajeswari *et al.*, 1977).

The stereochemistry of Stepholidine has been studied by X-ray crystallography (Wu *et al.*, 1987). Detailed PMR and MS studies have been carried out on Stepholidine (Ohiri *et al.*, 1983; Richter and Brochmann-Hanssen, 1975).

9.6 Isocorypalmine

This alkaloid has been isolated from opium (Pfeifer, 1966b) and from poppy at the stage of opium ripeness (Proksa and Cerny, 1981; Proksa *et al.*, 1979).

It has also been detected with the aid of labelled reticuline (Battersby *et al.*, 1975). Isocorypalmine can be methylated to tetrahydropalmatine with diazomethane. The NMR and MS spectra of isocorypalmine have been studied (Cashaw *et al.*, 1976; Ohiri *et al.*, 1983; Richter and Brochmann-Hanssen, 1975). The crystal structure of isocorypalmine has also been determined (Ribar *et al.*, 1992).

10 PROTOPINE ALKALOIDS
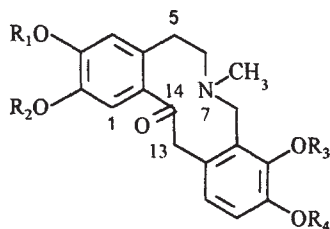
$R_1 = R_2 = CH_3$; $R_3 + R_4 = CH_2$ cryptopine
$R_1 + R_2 = CH_2$; $R_3 = R_4 = CH_3$ allocryptopine
$R_1 + R_2 = CH_2$; $R_3 + R_4 = CH_2$ protopine
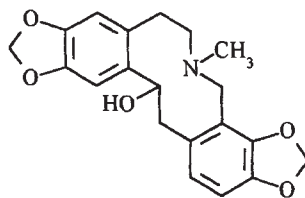
dihydroprotopine

The protopine alkaloids possess a ten-membered ring which contains a tertiary nitrogen atom and a C-14 ketonic group (Onda and Takahashi, 1988). Since these alkaloids are derived biosynthetically from tetrahydroprotoberberine precursors, they are classified among the isoquinoline alkaloids.

10.1 Cryptopine

The alkaloid cryptopine was first isolated from opium in 1867. Indian opium contains about 0.3% cryptopine. The isolation and purification of cryptopine were studied by Ramanathan and Chandra (Ramanathan, 1963; Ramanathan and Chandra, 1980, 1981). Cryptopine has also been isolated from poppy capsules (Hodková *et al.,* 1972; Szabó *et al.,* 1968).

Cryptopine has been detected in the callus tissue of the opium poppy (Furuya *et al.,* 1972; Ikuta *et al.,* 1974). The structure of cryptopine was elucidated by means of degradative (Emde, Hoffman and oxidative) reactions. It is of interest that cryptopine does not exhibit the properties of ketones. The carbonyl group yields no oxime, but it can be reduced to a secondary alcohol. Spectral studies indicate the presence of a transannular ground-state interaction between the carbonyl group and the basic nitrogen. Cryptopine and its reduction product (dihydrocryptopine) can be converted to the tetrahydroprotoberberine skeleton (e.g. isocryptopine chloride) by cyclization with acid chlorides (Dyke and Brown, 1968, 1969). Tetrahydroepiberberine has been converted to cryptopine in several steps. Oxidation with potassium chromate afforded a carbinolamine, which was reacted with methyl iodide to yield cryptopine hydroiodide. N-Demethylation of cryptopine was performed by means of cyanogen bromide. (Bentley and Murray, 1963b).

The structure of cryptopine was confirmed by spectral (NMR and MS) studies (Dolejs. *et al.,* 1964; Ma and Warnhoff, 1965; Nakashima and Maciel, 1973; Pfeifer and Thomas, 1972). X-Ray analysis was also performed in order to study the structure of cryptopine (Hall and Ahmed, 1968).

10.2 Allocryptopine

Allocryptopine exists in two allotropic modifications, the a form melting at 160°C and the ß form melting at 170°C. α-allocryptopine has been isolated from the non-phenolic fraction of opium by means of preparative TLC. It was identified via the NMR, MS and IR spectra. The content of the alkaloid in opium appears to be of the order of 0.01% (Brochmann-Hanssen and Nielsen, 1966a).

ß-Allocryptopine has been isolated from dried poppy capsules (Hodková *et al.,* 1972). Allocryptopine was detected in poppy in radioactive tracer experiments (Battersby *et al.,* 1975).

The synthesis of α-allocryptopine was reported to occur by rearrangement of the isoindolobenzazepine skeleton (Teitel *et al.,* 1973). Photochemical oxidation of canadine methiodide gave allocryptopine (Hanaoka *et al.,* 1976).

Allocryptopine can be transformed into the benzophenanthridine alkaloid chelerythine (Onda *et al.,* 1971). Treatment of allocryptopine with cyanogen bromide results in cleavage of the N-7 to N-8 bond; this reaction is in contrast with the behaviour of cryptopine (Nalliah *et al.,* 1974b).

The 13-oxo-derivative of allocryptopine has been prepared from the 13-oxoprotoberberinium methosalt (Nalliah *et al.*, 1974a).

Spectral properties (NMR and MS) of allocryptopine have been reported (Cross *et al.*, 1965; Dolejs *et al.*, 1964; Iwasa *et al.*, 1982; Nakashima and Maciel, 1973) and an X-ray crystallographic study of allocryptopine has been performed (Sakai *et al.*, 1988).

10.3 13-Oxycryptopine
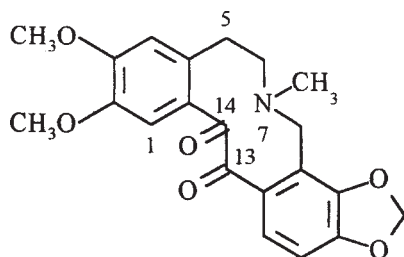
13-oxycryptopine

13-Oxycryptopine has been isolated from Indian opium. Codeine and protopine were removed from the non-phenolic fraction and the residue was subjected to preparative TLC and column chromatography (Brochmann-Hanssen *et al.*, 1970). 13-Oxycryptopine has been characterized via its spectral (UV, IR, NMR and MS) properties (Hanus *et al.*, 1967). It proved to be identical to the synthetic compound prepared from cryptopine (Leonard and Sauers, 1957).

10.4 Protopine

The separation of protopine from opium by the method of Hesse is exceedingly laborious and the alkaloid can be advantageously obtained from other plants. The presence of protopine in opium is rarely detected (Battersby *et al.*, 1975; Bessonova *et al.*, 1970; Kleinschmidt, 1959; Miram and Pfeifer, 1958; Neubauer, 1964; Neubauer and Mothes, 1961).

Protopine has been found in the callus tissue of opium poppy (Furuya *et al.*, 1972; Ikuta *et al.*, 1974).

The reactions of protopine are very similar to those of cryptopine. Protopine undergoes electrophilic aromatic substitution at C-12 on reaction with bromine and nitric acid in acetic acid (Castedo *et al.*, 1986). 13-Oxoprotopine can be obtained by the reaction of protopine with mercuric acetate (Leonard and Sauers, 1957).

Protopine has been prepared by the degradation of stylopine methiodide (Kulkarni *et al.*, 1990). The reverse process is also known, i.e. the protoberberine skeleton (coptisine, *vide supra*) can be prepared from protopine.

It is noteworthy that protopine can be transformed into the benzophenanthridine alkaloid sanguinarine (Onda *et al.*, 1968, 1969). Anhydroprotopine (prepared from isoprotopine chloride) yielded an unstable substance by photochemical reaction, which was reduced to dihydrosanguinarine (Onda *et al.*, 1971). The latter compound was oxidized to sanguinarine with DDQ. Oxidation of protopine with oxygen and a microsomal cytochrome P 450-NaDPH-dependent enzyme afforded 6-hydroxyprotopine, which underwent spontaneous cyclization to sanguinarine (Tanahashi and Zenk, 1988).

Protopine was converted to the *cis*-fused indenobenzazepine by the action of a strong base and sunlight (Blaskó *et al.*, 1981). Simpler alternative methods for the preparation of dihydrocoptisine and 13-oxystylopine from protopine have also been reported (Jeffs and Scharver, 1975).

The infrared carbonyl absorptions of protopine and α-allocryptopine in dilute CCl₄ solution and nuclear Overhauser experiments in the PMR spectra (Takahashi *et al.*, 1985) reveal that these alkaloids each interconvert between two major conformations of the ten-membered ring. The spectral characteristics of protopine (NMR and MS) have been studied in detail (Dolejs *et al.*, 1964; Nakashima and Maciel, 1973; Pfeifer and Thomas, 1972). An X-ray study of the alkaloid has also been performed (Hall and Ahmed, 1968).

10.5 Dihydroprotopine

The presence of dihydroprotopine has been reported in poppy (Stefanov *et al.*, 1972). Dihydroprotopine was detected in poppy by radioactive tracer experiments (Battersby *et al.*, 1975). It can be prepared by the reduction of protopine (Tani *et al.*, 1957).

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2. BIOSYNTHESIS OF MORPHINANE ALKALOIDS

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1 INTRODUCTION

The presence of morphinane alkaloids (thebaine, codeine and morphine), together with the secophthalidisoquinoline alkaloids (narceine, nornarceine, narceinimide), phthalidisoquinoline alkaloids (narcotine, narcotoline), benzyltetrahydroisoquinoline alkaloids (reticuline, laudanosine, codamine, tetrahydropapaverine) and aromatic benzylisoquinoline alkaloids (papaverine, pacodine) is characteristic of *Papaversomniferum* L. The carbon skeleton of these alkaloids is derived from two molecules of tyrosine (Spencer, 1968; Mothes *et al.,* 1985). The aromatic amino acids, phenylalanine, tyrosine and tryptophan are formed via the shikimate pathway. The availability of tyrosine for alkaloid biosynthetic pathways is an important determinant of the endogenous level of alkaloids.

In this chapter the enzymology and molecular biological aspects of the shikimate pathway are discussed, followed by the biosynthesis of two condensation units— dopamine and p-hydoxyphenylacetaldehyde (tyral)—from tyrosine at the interface between the shikimate and morphine biosynthetic pathways. The enzymes involved in morphine biosynthesis are covered in the last part of this chapter.

2 THE SHIKIMATE PATHWAY IN HIGHER PLANTS

Higher plants use aromatic amino acids not only as building blocks in proteosynthesis, but also, and in even greater quantities, as precursors to a large number of secondary metabolites. In addition, different plants also use the intermediates of the shikimate pathway as precursors to aromatic compounds (Figure 1). Under normal growth conditions, a significant amount of carbon fixed by plants flows through the shikimate pathway (Haslam, 1993; Connelly and Conn, 1985).

2.1 DAHP Synthase

The main trunk of the shikimate pathway, also called the prechorismate pathway, consists of seven enzymes. 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase catalyses the first reaction—the condensation of erythrose 4-phosphate and phosphoenol pyruvate (Figure 2). The product of this reaction is a seven-carbon six-membered heterocylic compound, DAHP.

Two isoenzymes of DAHP synthase have been identified in *Vigna radiata* (Rubin and Jensen, 1985), showing Mn^{2+}-stimulated and Co^{2+}-dependent activities. This pair
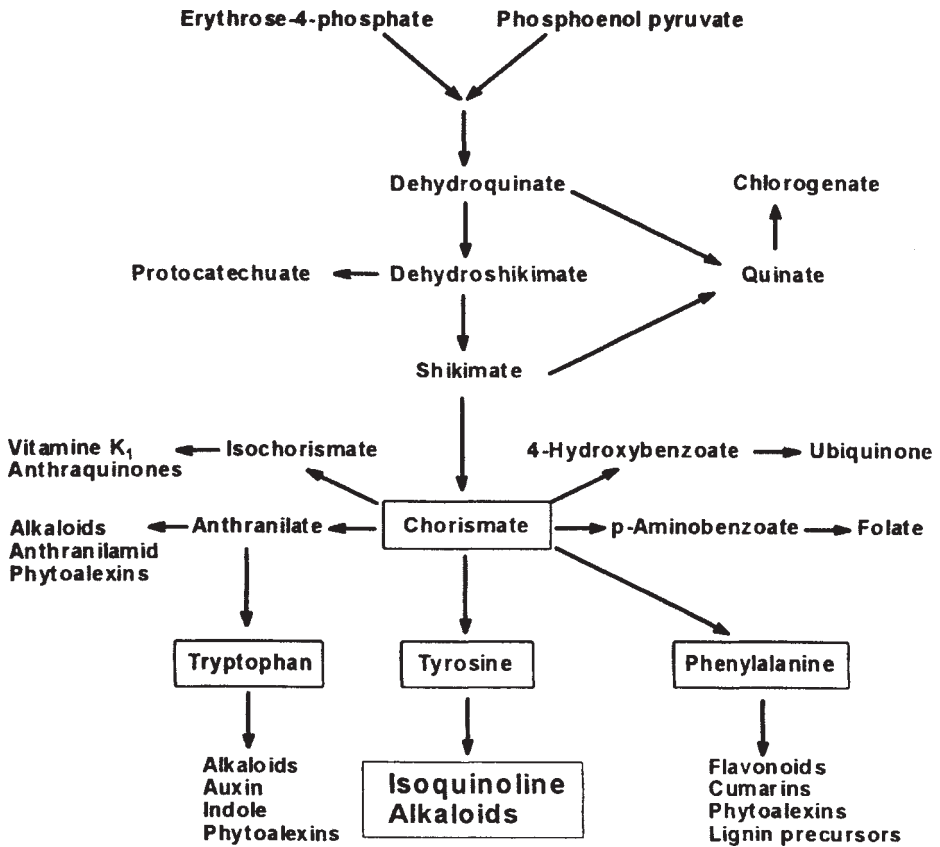
Figure 1 A schematic diagram showing different classes of secondary metabolites that arise from aromatic amino acids and from intermediates of the shikimate pathway

has been isolated from leaves and cell suspension cultures of *Nicotiana silvestris* and from various monocotyledonous and dicotyledonous plants (Ganson *et al.*, 1986). In chloroplasts prepared from tobacco and spinach leaves by sucrose gradient centrifugation only the Mn^{2+}-stimulated isoform was identified. The Co^{2+}-dependent isoform of DAHP synthase was present in a soluble fraction.

Recently the cytosolic, Co^{2+}-dependent isoform has been purified to electrophoretic homogeneity from cultured carrot cells (Suzuki *et al.*, 1996). The molecular mass of this enzyme was established to be 115kDa. The molecular masses of this isoform from other plants were reported to be around 400 kDa (Doong *et al.*, 1992). The pH optimum of the purified Co^{2+}-dependent isoform was 9.0 and the Km for erythrose-4-phosphate was 3.3 mM.

The Mn^{2+}-stimulated isoform of DAHP synthase was purified to electrophoretic homogeneity from potato tubers (Pinto *et al.*, 1986) and carrot (Suzich *et al.*, 1985).
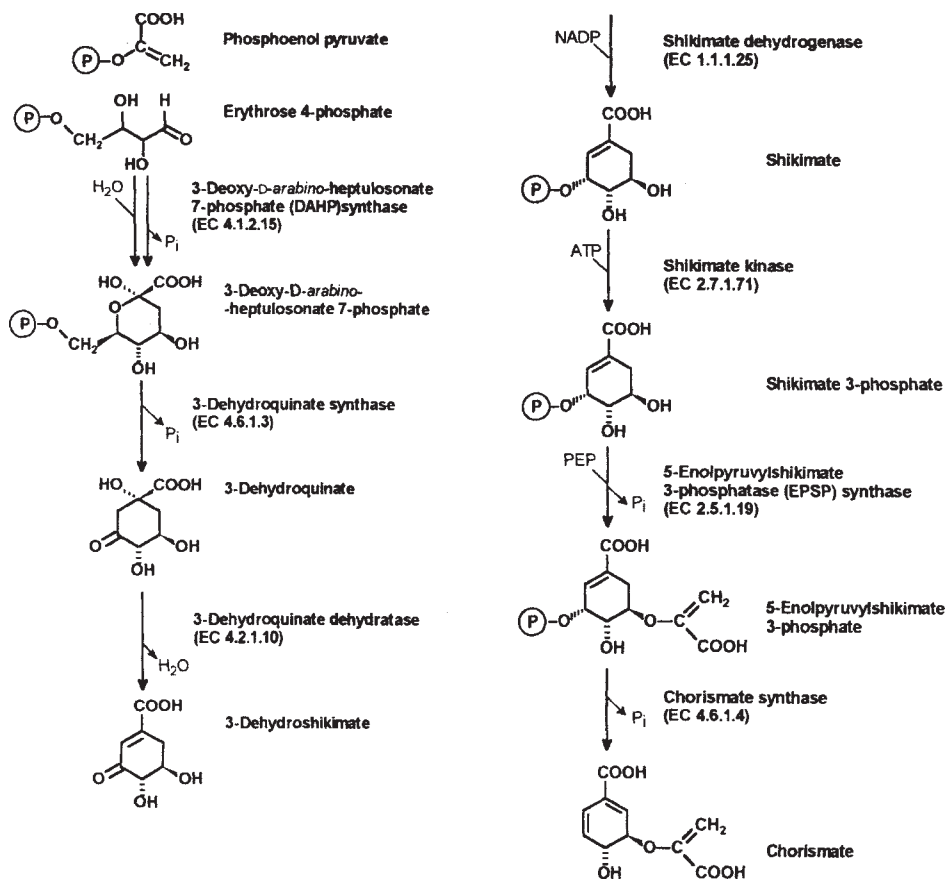
Figure 2 The shikimate pathway

To identify the first cDNA coding for plant DAHP synthase (Dyer *et al.,* 1990), a cDNA expression library from potato cell suspension cultures (Dyer *et al.,* 1989) was screened by antibodies (Pinto *et al.,* 1988) specific to the Mn^{2+}-stimulated isoform of DAHP synthase. This potato cDNA was than used to clone a second cDNA from potato (Zhao and Herrmann, 1992) and the homologues from *Arabidopsis* (Keith *et al.,* 1991), tobacco (Wang *et al.,* 1991) and tomato (Görlach *et al.,*1993a). Functional complementation of yeast (Keith *et al.,* 1991) and *E. coli* (Weaver *et al.,* 1993) both defective in DAHP synthase with cDNA from potato and *Arabidopsis* demonstrated that these cDNA encode polypeptides with DAHP synthase activity. Polypeptides obtained by translation of cDNA for DAHP synthase have N-terminal sequences resembling the transit sequences targeting the enzymes in chloroplasts (Gavel and von Heijne, 1990). Therefore, it seems likely that chloroplasts contain two Mn^{2+}-stimulated isoenzymes. Gene coding for these Mn^{2+} -stimulated isoenzymes (Shk1

and Shk2) in potato (Dyer *et al.,* 1989; Keith *et al.,* 1991) and DHS1 and DHS2 in *Arabidopsis* (Keith *et al.,* 1991) responded differently to wounding and pathogen attack. In the tomato these two genes also code for plastidic enzymes and exhibit strikingly different organ-specific expressions and sensitivity to elicitation and pathogen attack (Görlach *et al.,* 1994, 1995a,b).

2.2 3-Dehydroquinate Synthase

The second enzyme of the prechorismate pathway catalyzes the cyclization step in this pathway. For catalytic activity the enzyme requires NAD^+ and a divalent cation. By density-gradient methods 3-dehydroquinate synthase has been localized in chloroplasts from pea seedlings (Mousdale and Coggins, 1985). The purified enzyme has been obtained from the mung bean (Yamamoto, 1980) and pea (Pompliano *et al.,* 1989). Analyses of organ-specific expression of the gene encoding this enzyme in tomato plants showed that the abundance of the corresponding transcripts was relatively high in tomato flowers. A cDNA library was constructed from tomato flowers (Bischoff *et al.,* 1996) and used for complementation of *E. coli* cells defective in dehydroquinate synthase. A cDNA clone encoding dehydroquinate synthase was isolated. The N-terminal region of the deduced amino acid sequence contained a putative plastidic transit peptide, that is 3-dehydroquinate synthase appears to be a plastidic enzyme. The level of dehydroquinate synthase transcripts was highest in the roots (with an eightfold difference between roots and leaves) and lower in the flowers and stems. Strong induction of the dehydroquinate synthase transcripts was observed after elicitation of cultured tomato cells by a cell wall preparation of *Phytophthora megasperma* f. sp. *glycinea.*

2.3 3-Dehydroquinate Synthase-Shikimate Dehydrogenase

The third step of the prechorismate pathway is catalyzed by 3-dehydroquinate dehydratase. In the course of conversion of 3-dehydroquinate to dehydroshikimate the process of aromatization is initiated, i.e. the first of the three double bonds is introduced. The elements of water are released by a syn (cis) elimination (Bentley, 1990). 3-dehydroquinate dehydratase and the next enzyme of the prechorismate pathway, the shikimate NADP oxidoreductase, reside on a single polypeptide (Fiedler and Schultz, 1985; Mausdale *et al.,* 1987; Mousdale and Coggins, 1984). Within this bifunctional enzyme, the turnover of the first reaction is only one-ninth of the second reaction, that is dehydroshikimate does not accumulate during the conversion of dehydroquinate to shikimate. Pea enzymes were purified to near homogeneity (Deka *et al.,* 1994) and used to prepare antibodies against the pure bifunctional enzyme. Partial cDNA encoding the bifunctional enzymes of the pea (Deka *et al.,* 1994) and tobacco (Bonner and Jensen, 1994) have been cloned. This near full length cDNA from tobacco complemented the *aroD* and *aroE* mutants of *E. coli.*

Partially purified shikimate dehydrogenase was prepared from dark-grown three-day old poppy seedlings (Šmogrovièová *et al.,* 1981). The kinetic data of this enzyme (pH optimum 9.8; Km values for shikimate and NADP 0.59 mM and 0.018 mM respectively) are similar to those from other plants (Balinsky *et al.,* 1961; Koshiba, 1978). The activity of the enzyme was not affected by aromatic amino acids,

phenylpyruvic and chorismic acids or thebaine, codeine and morphine (up to 1 mM final concentration). Four isoforms of shikimate dehydrogenase were identified in dry poppy seeds and three isoforms were determined in individual organs of etiolated poppy seedlings.

2.4 Shikimate Kinase

Shikimate kinase catalyses an unexceptional phosphate transfer from ATP to C-3-OH group of shikimate. This enzyme has been described in different plants (Koshiba, 1979; Bowen and Kosuge, 1979; Mousdale and Coggins, 1985). A near homogeneous enzyme preparation has been obtained from spinach chloroplasts (Schmidt *et al.,* 1990) and the first cDNA encoding this enzyme has been isolated from tomato (Schmid *et al.,* 1992). Southern blot analysis showed the presence of only one gene for shikimate kinase in haploid tomato genome. When tomato cells were treated with elicitors, the abundance of shikimate kinase specific transcripts was found to increase dramatically with time (Görlach *et al.,* 1995a, b), i.e. the expression of the gene for shikimate kinase is sensitive to environmental stimuli. The pattern of organ-specific expression of the shikimate kinase gene in tomato is similar to the chorismate synthase gene and the 5-enolpyruvylshikimate 3-phosphate synthase gene (Görlach *et al.,* 1994).

2.5 5-Enoylpyruvylshikimate-3-Phosphate (ESPS) Synthase

This enzyme is the most thoroughly studied of all the enzymes in the shikimate pathway. The chemical aspects of the enzyme-catalyzed reaction of phosphoenol pyruvate and shikimate-3-phosphate to ESPS have been discussed by Bentley (1990) and Anderson and Johnson (1990). ESPS synthase has been purified to electrophoretic homogeneity from seedlings of *Pisum sativum* (Mousdale and Coggins, 1986) and *Sorghum bicolor* (Ream *et al.,* 1988). In the pea this enzyme was localized in chloroplasts and only a minor fraction was detected in the cytosolic fraction. For ESPS synthase several genes and cDNA clones have been isolated from different plants—*Petunia hybrida* (Sha *et al.,* 1986), tomato (Gasser *et al.,* 1988), *Brassica napus* (Gasser *et al.,* 1990) and *Arabidopsis thaliana* (Klee *et al.,* 1987). All these genes and cDNAs studied so far encode a plastidic enzyme. The abundance of ESPS synthase specific transcripts increased with time in elicited tomato cell cultures. Similar results were obtained when the abundance of ESPS synthase specific transcripts was analysed in infected tomato plants (Görlach *et al.,* 1995a, b). The highest level of ESPS synthase mRNA was found to be in the petals and anthers of mature petunia plants. The amount of this mRNA was very low in the leaves and pistils of petunia. In the same organs of tomato plants the amounts of ESPS synthase mRNA varied only slightly (Gasser *et al.,* 1988). The organ-specific expression of ESPS synthase gene in tomato plants is very similar to that of the shikimate kinase gene and the two chorismate synthase genes (Görlach *et al.,* 1994). In transgenic petunia and tobacco plants the tissue specific expression of reporter genes (chloramphenicol acetyltransferase or ß-glucuronidase) was analyzed under the control of different segments of the ESPS synthase gene promotor (Gasser *et al.,* 1988). ESPS synthase is the major target for inhibition by glyphosate, a broad spectrum non-selective herbicide (Bentley, 1990).

2.6 Chorismate Synthase

Chorismate synthase (CS) catalyzes a 1,4-*trans* elimination of phosphate from 5-enolpyruvylshikimate-3-phosphate (Hawkes *et al.*, 1990). In the course of this reaction the second double bond of the benzene ring is formed. CS is unusual in requiring a reduced flavine cofactor ($FMNH_2$ or $FADH_2$) for catalytic activity even though the overall reaction is redox neutral. The same is true for 3-dehydroquinate synthase requiring NAD^+ for a redox neutral reaction. The chorismate synthase from both *N. crassa* (Welch *et al.,* 1974) and *E. gracillis* (Schaller *et al.,* 1991a) is a bifunctional enzyme, containing a NADPH-driven flavine reductase activity. Chorismate synthase from higher plants is a monofunctional enzyme (Schaller *et al.*, 1990). Chorismate synthase was first described in seedlings of *Pisum sativum* (Mousdale and Coggins, 1986). A homogeneous enzyme preparation was obtained from cell cultures of *Corydalis serpervirens* (Schaller *et al.*, 1990b) and this enzyme preparation was used to obtain polyclonal antibodies. Screening of a cDNA library with polyclonal antibodies resulted in isolation of the first CS-specific cDNA from higher plants (Schaller *et al.*, 1991b). Three cDNAs (*CS1, CS2* and *CS2Δ*) corresponding to two chorismate synthase genes *(LeCS1* and *LeCS2)* have been identified in tomato plants (Görlach *et al.,* 1993b, 1995a). These cDNAs, as do those from *C. serpervirens,* encode polypeptides with N-terminal sequences resembling the peptides for chloroplast transport. Differential splicing of *LeCS2*-specific pre-mRNA results in the formation of two different transcripts *CS2* and *CS2Δ*. In tomato cell cultures exposed to elicitation the abundance of *CS1*-specific transcripts increased dramatically with time. Under these conditions the abundance of *CS2*-specific transcripts remained unchanged. Identical results were obtained with infected leaves of potato plants (Görlach *et al.*, 1995b). An analysis of the abundance of *CS1*- and *CS2*-specific transcripts in organs of tomato plants showed that the level of these transcripts is higher in the roots and flowers than the in stems, leaves and cotyledons. The abundance of transcripts of *CS1* gene is consistently higher than that of *CS2* gene (Görlach *et al.*, 1994). To characterize the biochemical properties of CS isoenzymes, cDNAs for mature isoenzymes *(CS1, CS2* and *CS2Δ)* have been expressed in *E. coli* (Braun *et al.*, 1996). The isoenzyme *CS2Δ* appeare d to be unstable. The isoenzymes *CS1* and *CS2* have been purified to near homogeneity. The only difference between the two isoenzymes were their Km values for 5-enolpyruvylshikimate-3-phosphate. The Km value of *CS2* is seven times higher than that of *CS1*.

3   BIOSYNTHESIS OF PHENYLALANINE, TYROSINE AND TRYPTOPHAN

Chorismate is a branch-point intermediate in the biosynthesis of aromatic amino acids and growth factors in bacteria, fungi and higher plants (Weiss and Edwards, 1980). At the level of chorismate (Figure 1) the shikimate pathway branches into a pathway leading to phenylalanine and tyrosine and into another branch leading to tryptophan. In some plants isomerization of chorismate to isochorismate is catalyzed by isochorismate synthase. Isochorismate is used for the biosynthesis of anthraquinones and vitamin K_1.

3.1 Biosynthesis of Tryptophan

Tryptophan biosynthesis from chorismate is catalysed by six enzymes (Figure 3).

Anthranilate synthase is a key regulated enzyme in this pathway. Genetic and biochemical aspects of plant anthranilate synthase have been actively studied over recent years. Plant enzymes catalysing the next three steps in tryptophan biosynthesis are just now beginning to be characterized in detail. Tryptophan synthase complex catalyses the last two steeps in the biosynthesis of tryptophan. Tryptophan synthase α catalyses the conversion of indol-3-glycerophosphate to indol, and tryptophan synthase ß catalyses the formation of tryptophan from indol and serine. The whole tryptophan biosynthetic pathway is localized in the chloroplasts. In the interests of brevity the tryptophan biosynthetic pathway will be not discussed here in detail. For in-depth treatments of tryptophan biosynthesis the reader is referred to several excellent reviews (Bentley, 1990; Poulsen and Verpoorte, 1991; Schmid and Amrhein, 1995; Radwanski and Last, 1995).

3.2 Biosynthesis of Phenylalanine and Tyrosine

In higher plants the biosynthesis of phenylalanine and tyrosine proceeds from chorismate via prephenate and arogenate (Figure 4).
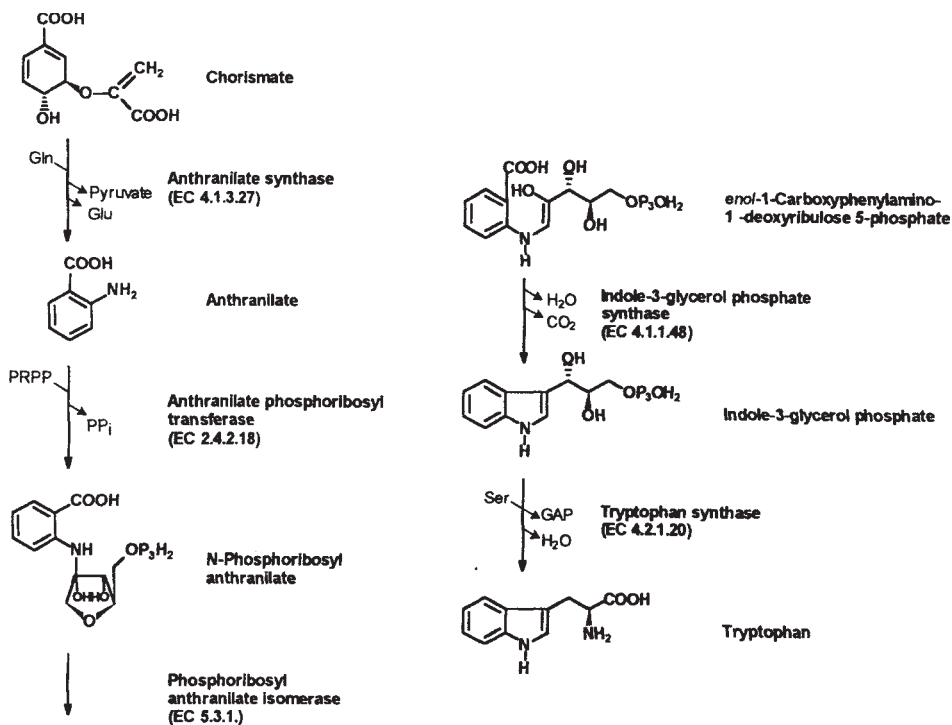
Figure 3 The tryptophan biosynthetic pathway
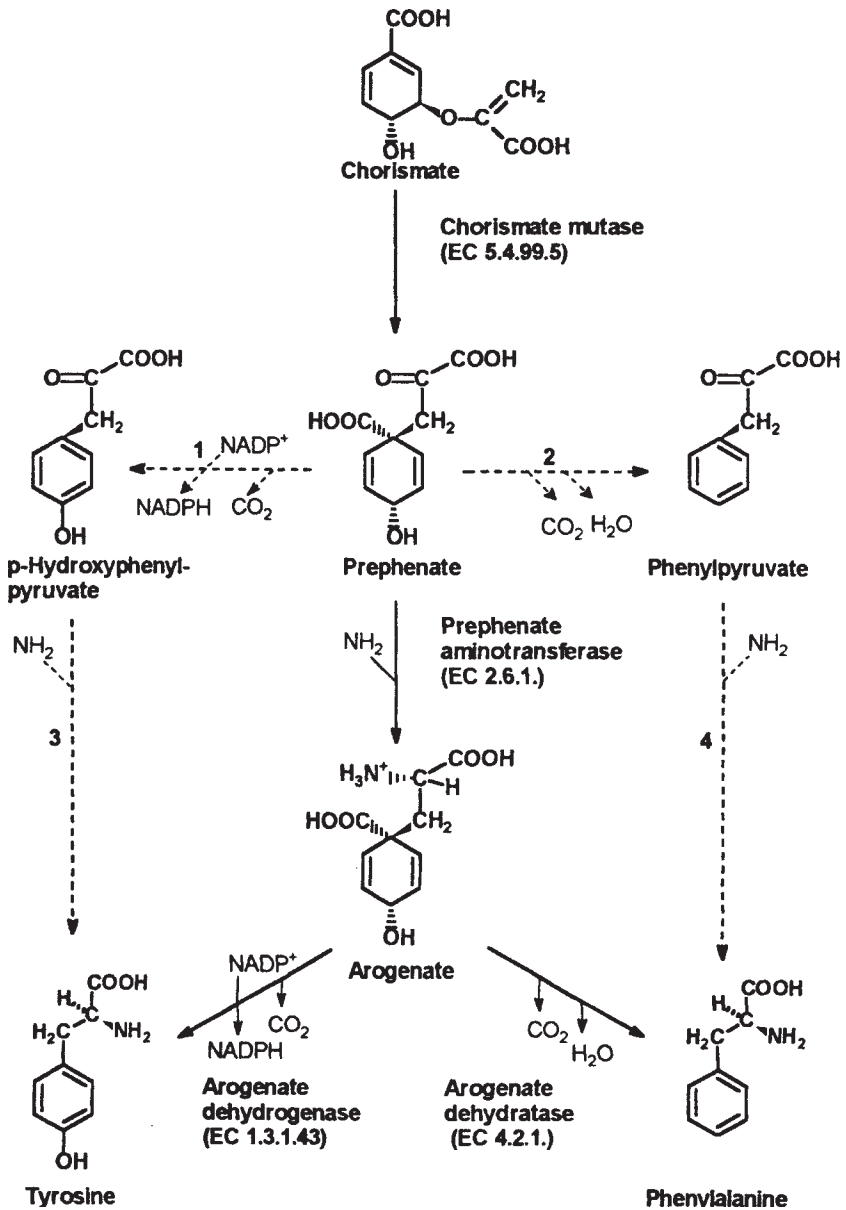
Figure 4 The pathway from chorismate to phenylalanine and tyrosine----classical pathway

3.2.1 Chorismate Mutase

Chorismate mutase (CM) catalyzes an unusual intramolecular rearrangement of chorismate to prephenate. The reaction mechanism has been discussed in detail in a recent review (Romero *et al.,* 1995). Chorismate mutase activities have been found in cell-free extracts of a variety of plants, and two isoenzymes (CM-1 and CM-2) have been frequently identified (Poulsen and Verpoorte, 1991; Romero *et al.,* 1995). The activity of CM-1 is enhanced by tryptophan and inhibited by phenylalanine and tyrosine. The activity of CM-2 is not regulated by aromatic amino acids. The isoform CM-1 was found to be located in chloroplasts while the isoform CM-2 was detected in soluble fractions (d'Amato *et al.,* 1984).

These two isoforms of chorismate mutase have been analysed in dark- and lightgrown seedlings of *Papaver somniferum* L. (Benešová and Bode, 1992). After partial purification the kinetic data and molecular masses of native chorismate mutases were determined (84kDa for CM-1 and 80kDa for CM-2). After SDS-PAGE the estimated molecular masses were determined to be 55kDa and 53kDa for CM-1 and CM-2 respectively. A chloroplast fraction prepared from light-grown seedlings contained only the isoform CM-1. The ratio of CM-1 to CM-2 activities has been found to differ between organs and during seedling growth and development.

The ratio of CM-1 to CM-2 was 2:1 in fresh potato tuber, whereas the activity ratio in green leaves was 1:4 (Conn, 1985; Kuroki and Conn, 1989). The ratio of CM-1 to CM-2 varies by a factor 20 when organismal tissue and cultured cells are compared. These results indicate that the expression of CM isoforms is organ-specific and developmentally regulated.

Two CM-specific cDNAs, corresponding to the genes *AtCM1* and *AtCM2* have been identified in *Arabidopsis thaliana* (Eberhard *et al.,* 1993, 1996b). The deduced amino acid sequence of cytosolic isoform (CM-2) is 50% identical to that of plastidic isoform (CM-1). The organ-specific expression of the two CM genes was analysed by a dot blot analysis. The highest abundance of *AtCM1*- and *AtCM2*-specific transcript was in roots, less in stems and even less in leaves. Even when the pattern of organ-specific expression of two CM genes was similar the abundance of transcripts of *AtCM1* was consistently higher than that of the *AtCM2*.

In the elicited cultured cells of *A. thaliana* only the abundance of *AtCM1*-specific transcript increased. Similar results were obtained when the abundance of *AtCM1*- and *AtCM2*-specific transcripts was analysed in infected *A. thaliana* leaves. These results show that only the expression of *AtCM1* is sensitive to environmental stimuli.

A cDNA coding for cytosolic, unregulated chorismate mutase isoform has been identified in *Licopersicon escukntum* L. (Eberhard *et al.,* 1996a). The highest abundance of the corresponding gene *(LeCM1)*-specific transcripts was in roots, lower in stems and cotyledons, and even lower in flowers and leaves. This expression pattern of *LeCM1*-specific transcripts is different from those observed in genes encoding enzymes of the prechorismate pathway. The expression of *LeCM1* appeared to be insensitive to elicitors, whereas genes coding for enzymes of the prechorismate pathway can be induced by elicitation.

It is important to realise that *AtCM2* and *LeCM1* encoded polypeptides are located in cytosol. In *A. thaliana AtCM2* encoded a chloroplast located in chorismate mutase. cDNAs and/or genes for all the enzymes of the prechorismate pathway (Schmid and Amrhein, 1995; Bischoff *et al.,* 1996) isolated so far encode polypeptides with N-terminal sequences which could target polypeptides to chloroplasts.

3.2.2 Prephenate Aminotransferase

In the classical pathway to phenylalanine and tyrosine the branch point intermediate is prephenate (Figure 4). Prephenate is first converted to phenylpyruvate (PPY) or p-hydroxyphenylpyruvate (HPP) by prephenate dehydratase or prephenate dehydrogenase (oxidative decarboxylation). These aromatic keto-acids are substrates for specific aminotransferases leading to phenylalanine and tyrosine.

In an alternative pathway first the transamination of prephenate takes place and the non-aromatic arogenate (the branch point intermediate) formed is subsequently dehydrated or dehydrogenated giving rise to phenylalanine and tyrosine, respectively (Siehl *et al.,* 1985). The two possible routes differ only in the sequence of the transamination and aromatization reactions.

Because the prephenate dehydratase activity has never been found in higher plants (Bonner and Jensen, 1987) and the prephenate dehydrogenase was reported to occur only in mung bean (Rubin and Jensen, 1979), the only possibility for biosynthesis of phenylalanine and tyrosine is via the alternative pathway. Prephenate aminotransferase and arogenate dehydrogenase were first described in the mung bean (Rubin and Jensen, 1979). Prephenate aminotransferase has been purified to near homogeneity from cell cultures of *Nicotiana silvestris* (Bonner and Jensen, 1985, 1987) and *Anchusa officinalis* (De-Eknamkul and Ellis, 1988). Prephenate aminotransferases studied so far have an unusually high substrate specificity and are highly stable to thermal treatments. Prephenate aminotransferase is located in plastid compartments (Siehl *et al.,* 1986; Jensen, 1986). For this enzyme neither a cDNA nor a gene have been isolated so far.

3.2.3 Arogenate Dehydratase

The presence of arogenate dehydratase was first described in a cultured cell population of *Nicotiana sylvestris* (Jung *et al.,* 1986). In this plant material the presence of prephenate dehydrogenase was not detected. Arogenate dehydratase was also found in spinach chloroplasts. Arogenate dehydratases from tobacco and spinach were both specific for arogenate, inhibited by phenylalanine, and activated by tyrosine. Tryptophan and caffeic acid did not affect the activity of arogenate dehydratase.

3.2.4 Arogenate Dehydrogenase

This enzyme has been observed in developing mung bean seedlings (Rubin and Jensen, 1979), in seedlings of *Zea mays* (Byng *et al.,* 1981) and in cell cultures of *Nicotiana sylvestris* (Gaines *et al.,* 1982). A high activity of arogenate dehydrogenase has been found in etiolated sorghum seedlings (Connelly and Conn, 1985). Partially purified arogenate dehydrogenase was found to be strongly inhibited by tyrosine but was unaffected by phenylalanine, prephenate and tryptophan.

So far neither a cDNA clone nor a gene coding for arogenate dehydratase and arogenate dehydrogenase have been isolated.

4 BIOSYNTHESIS OF THE PRIMARY PRECURSORS OF THE BENZYLISOQUINOLINE SKELETON

The 1-benzyltetrahydroisoquinoline (BTIQ) skeleton contains a tetrahydroisoquinoline (TIQ) segment and a benzyl-derived moiety. According to Winterstein and Trier the BTIQ nucleus is formed by Picket—Spencer condensation of dopamine with 3,4-dihydroxyphenylacetaldehyde (dopal). Tracer experiments indicated that *(S)-nor*-laudanosoline (tetrahydroxylated BTIQ) is probably the first intermediate in the biosynthesis of morphinanes (Battersby *et al.,* 1965). Tyrosine has been found to label both the TIQ and benzylic parts of morphinanes (Battersby *et al.,* 1962; Neubauer, 1965; Roberts *et al.,* 1987). When [1-^{13}C]tyramine or [2-^{14}C]tyramine were infiltrated into *Papaver somniferum* plants (Roberts *et al.,* 1987) and [2-^{13}C]tyramine into developing poppy seedlings (Loeffler *et al.,* 1987), only the TIQ portion of morphine was labelled. These findings indicate that tyramine is not a precursor of the benzylic part of the BTIQ ring system. After administration of labelled dopa and dopamine only the TIQ segment of alkaloids was labelled (Spencer, 1968). These results demonstrated that in *Papaver somniferum* tyramine is not transformed by amine oxidase into 4-hydroxyphenylacetaldehyde (tyral) as reported in *Berberis* cell cultures (Rueffer and Zenk, 1987). Highly enriched thebaine and morphine labelled specifically at position 9 were obtained after infiltration of (R, *S*)-[1-^{13}C]norcoclaurine and (R, *S*)[1-^{13}C]coclaurine into opium poppy seedlings (Loeffler *et al.,* 1987). Precursor feeding experiments to *Annona reticulata* leaves demonstrated that coclaurine and reticuline are both derived from norcoclaurine (Stadler *et al.,* 1987). These results indicated that not tetrahydroxylated norlaudanosoline but trihydroxylated norcoclaurine is the first BTIQ intermediate in the biosynthesis of opium poppy alkaloids. Feeding experiments with *(S)-*[l-^{13}C]norcoclaurine showed that norcoclaurine is specifically incorporated into protoberberine, berberine, aporphine, benzophenathridine and pavine alkaloids (Stadler *et al.,* 1987, 1989; Müller and Zenk, 1992). If norcoclaurine is the first BTIQ intermediate in the biosynthesis of 1-benzylisoquinoline alkaloids, than the carbonyl-condensation unit is 4-hydroxyphenylacetaldehyde (tyral). Tyral can arise by decarboxylation of p-hydroxyphenylpyruvate, formed by transamination from tyrosine (Rueffer and Zenk, 1987).

The proposed biochemical relationships at the interface between shikimate and norcoclaurine biosynthesis are schematically shown in Figure 5.

Transaminase, phenoloxidase, decarboxylase and oxidative deaminase activities have been reported to occur in opium poppy plants (Jindra *et al.,* 1966; Kovács and Jindra, 1965; Kovács, 1970). The alkaloids of opium poppy have been identified in the alkaloid vesicles of latex (Fairbairn *et al.,* 1974). Later on, phenoloxidase activity (Roberts, 1971, 1974) and its bound and soluble forms (Antoun and Roberts, 1975), transaminase and decarboxylase activities (Antoun and Roberts, 1975; Roberts and Antoun, 1978; Roberts *et al.,* 1983) were studied in *Papaver somniferum* latex. A specific hydroxylation of tyramine was reported by phenoloxidase from opium poppy plants (Asghar and Siddiqi, 1969).
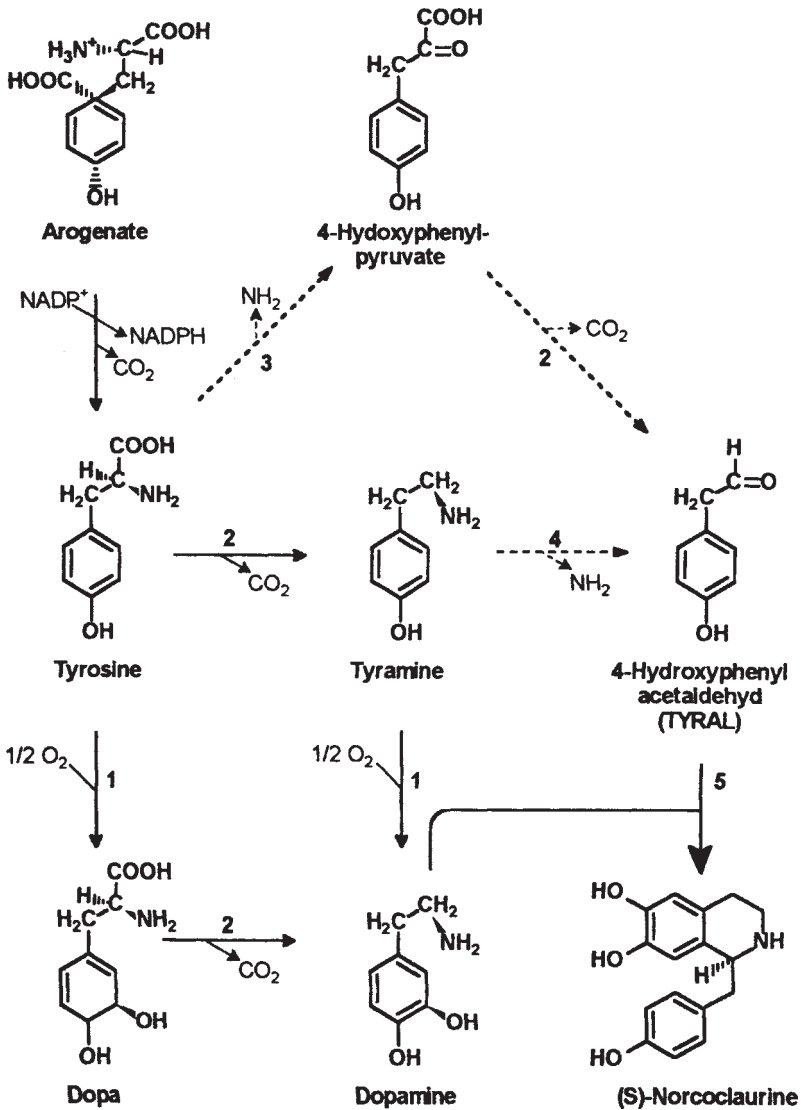
Figure 5 The enzymes in the biosynthesis of dopamine and tyral in BTIQ-alkaloid forming plants 1—phenoloxidase, 2—decarboxylase, 3—transaminase, 4—amine oxidase, 5—norcoclaurine synthase

Immobilized cells of *Papaver somniferum* retained their tyrosine/dopa decarboxylase activity for several months (Stano *et al.,* 1995).

The enzymes involved in the transformation of tyrosine to dopamine and tyral have been identified and characterized partially in opium poppy. Neither of these enzymes was purified to homogeneity and their molecular characteristics have not been investigated (Hashimoto and Yamada, 1994). Molecular analyses of these

enzymes and the corresponding genes have begun recently in order to gain a better understanding of the regulation of carbon flow between shikimate and BTIQ alkaloid biosynthetic pathways.

4.1 Tyrosine/3,4-dihydroxyphenylalanine Decarboxylase

Primers designed to two conserved domains of other aromatic amino acids decarboxylases have been used for PCR amplification of a poppy genomic DNA fragment and an insert from an authentic clone of tryptophan decarboxylase (TDC) from *C. roseus* (Facchini and De Luca, 1994). The TDC inserts and the PCR product were used to screen an opium poppy seedling cDNA library. Two different cDNAs (*cTyDC2* and *cTyDC3*) for tyrosine/dopa decarboxylase have been identified by heterologous screening by TDC cDNA as probe and a third independent *cTyDC1* was detected by the PCR fragment from opium poppy genomic DNA. Screening of a poppy genomic library by *cTyDC1* resulted in the isolation of two genomic clones (*gTyDC1* and *gTyDC4*). The nucleotide sequence of *gTyDC1* was found to be identical to *cTyDC1* and that *of gTyDC4* was 90% identical to *cTyDC1*. Members of the poppy tyrosine/dopa decarboxylase gene family have been divided into two subsets (*cTyDC1* and *gTyDC4, cTyDC2* and *cTyDC3*) according to sequence homology. Within each subset the clones exhibit about 90% identity, whereas clones between subsets share less than 75% identity. One member of each subset (*cTyDC1* and *cTyDC2*) was used to screen the poppy genomic DNA fragments obtained by digestion with four restriction endonucleases. Six to eight and four to six positive bands were found for each digestion by *cTyDC1* and *cTyDC2* respectively. These results showed that tyrosine/dopa decarboxylase is encoded in the opium poppy by a family of 10-14 genes. When *gTyDC1* and *cTyDC2* were expressed in *E. coli* as ß-galactosidase fusion proteins, these proteins exhibited high substrate specificity for dopa and tyrosine. For these enzymes tryptophan and phenylalanine have not been accepted as substrates. The Km values of the enzymes were equal for both tyrosine and dopa (Km1 mM). Both enzymes exhibited a similar broad pH optimum (pH 7.5—8.5) (Facchini and De Luca, 1995a). By *in situ* hybridization using *TyDC1* and *TyDC2* sense and anti-sense probes, an analysis of the spatial distribution of *TyDC* transcripts showed their association with vascular tissue in mature roots and stems. For analysis of organ-specific expression (roots, stems, leaves, sepals, carpels, stamens and petals) samples were collected from mature poppy plants with fully expanded flowers. RNA blot-hybridization analysis with *cTyDC1* and *cTyDC2* as probes showed that the *TyDC1*-like transcripts were expressed predominantly in roots and the *TyDC2*-like transcripts were identified mostly in stems and roots (Facchini and De Luca, 1995b) and to a much lesser extent in other organs analysed. The levels of *TyDC1*- and *TyDC2*-like transcripts were similar along the stem length. The level of both transcripts was low in the basal parts of the stem. During carpel development (from floral bud through anthesis to maturation) the levels of *TyDC1*- and *TyDC2*-like transcripts were detectable only up to anthesis. After pollination, in expanding carpels, the level of these transcripts was undetectable.

The content of thebaine and codeine was found to be constantly low in latex from expanding carpels (Zichová *et al.,* 1996). The level of morphine approximately

doubled in latex collected from expanding capsules (from pollination up to the eighth day) and remained almost constant in the next stages of capsule maturation. In latex collected from expanding carpels and during their maturation the content of tyrosine is constantly low, but the level of tyramine, and particularly that of dopamine, is high, reaching a maximum at the highest content of morphine. Whether morphine is transported into the expanding carpels or morphine is *de novo* formed in these carpels from substrates present in the latex remains to be clarified.

Another member of the *TyDC/DoDC* gene family, *TyDC5,* has been isolated from a genomic library of opium poppy (Maldonado-Mendoza *et al.,* 1996). When the coding region of *TyDC5* was expressed in *E. coli* the highest recombinant enzyme activity was with tyrosine and dopa (only 64% of that with tyrosine) and an extremely low activity was detected with phenylalanine. No activity was observed against tryptophan. The organ-specific expression of *TyDC5* has been analysed by ribonuclease protection assay. *TyDC5* has been expressed only in the roots of mature poppy plants. In other organs, including the latex and cell suspension cultures, *TyDC5* expression was almost undetectable. A high expression of this gene has been detected in developing poppy seedlings. When a promoter fraction of *TyDC5* was fused to *GUS* reporter gene, the expression of *TyDC5::GUS* gene construct expression was restricted to the roots in transgenic tobacco plants, similarly as determined by ribonuclease protection assay in roots of poppy plants. The deduced amino acid sequence and root-specific expression pattern of *TyDC5* are most similar to those of *TyDC1* (Facchini and De Luca, 1995a, b), suggesting that they are members of a common TyDC/DoDC gene family.

5 BIOSYNTHESIS OF *(S)*-RETICULINE

Reticuline is a key intermediate in the biosynthesis of a plethora of BTIQ alkaloids. The biosynthesis of reticuline from dopamine and tyral is catalyzed by a sequence of five enzyme reactions (Figure 6). The pre-reticuline pathway is common for the synthesis of different structural types of BTIQ alkaloids, and therefore various plants and cell cultures could be employed for the identification of individual enzymes of the pre-reticuline pathway.

As a result of the revision of the pre-reticuline pathway, three enzymes had to be renamed (Loeffler and Zenk, 1990): *(S)*-norlaudanosoline synthase is *(S)*-norcoclaurine synthase, (R), *(S)*-norlaudanosoline-6-O-methyltransferase is *(S)*-norcoclaurine-6-O-methyltransferase and S-adenosyl-L-methionine: norreticuline-*N*-methyltransferase is *(S)*-coclaurine-N-methyltransferase.

5.1 *(S)*-Norcoclaurine Synthase

The first enzyme in pre-reticuline pathway catalyses the stereospecific condensation of dopamine with tyral or related phenylacetaldehydes. *(S)*-norcoclaurine synthase has been isolated and partially purified from *Eschscholtzia tenuifolia* cell cultures (Rueffer *et al.,* 1981; Schumacher *et al.,* 1983). Norcoclaurine synthase is a cytosolic enzyme with a molecular mass of 16kDa. The apparent Km values for dopamine and tyral (dopal) are 1.5mM and 0.9 mM (0.7 mM) respectively. For tyral a slightly more
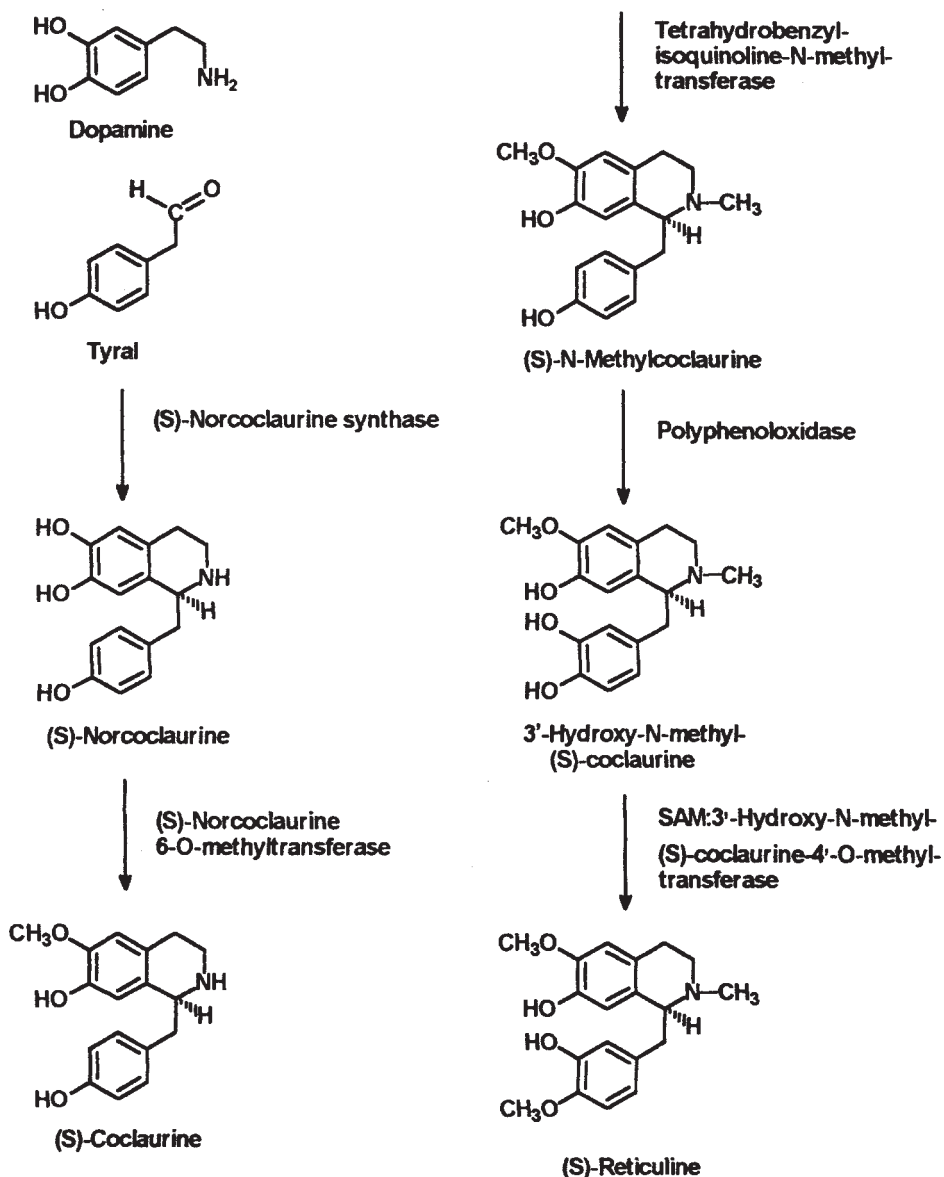
Figure 6 The biosynthetic pathway of *(S)*-reticuline

acidic pH optimum (pH-7.4) has been observed compared to that for dopal (pH-7.8). The enzyme is highly specific for phenylacetaldehydes and the corresponding phenylpyruvates have not been accepted as substrates. By disc electrophoresis and isoelectric focusing four isoenzymes have been identified in a 40-fold purified enzyme preparation from *E. tenuifolia* cell cultures.

5.2 *(S)*-Norcoclaurine-6-*O*-methyltransferase

The second enzyme of the pre-reticuline pathway catalyses formation of coclaurine (6-*O*-methylnorcoclaurine). This enzyme has been purified (80-fold) from cell suspension cultures of *Argemone platyceras* (Rueffer *et al.,* 1983). The 6-*O*-methyltransferase is a cytosolic enzyme with molecular mass of 47 kDa. An SH-group appeared to be essential for catalytic activity of 6-*O*-methyltransferase. The purified enzyme did not *N*-methylate the BTIQ alkaloids tested.

5.3 *(S)*-Coclaurine-*N*-methyltransferase

To exclude any interference by *O*-methylating enzymes tetrahydropapaverine has been used as substrate for S-adenosyl-L-methionine: coclaurine *N*-methyltransferase (Watt *et al.,* 1985). The *N*-methylating activity has been tested in a cell-free preparation from more than fifty cell cultures from plants producing BTIQ alkaloids. A partially purified (100-fold) enzyme was prepared from *Berberis vulgaris* cell cultures. The enzyme had an apparent molecular mass of 68 kDa. For this soluble enzyme only tetrahydrobenzylisoquinoline alkaloids served as substrates. The apparent Km value for (R)-tetrahydropapaverine (0.2 mM) is higher compared to that for *(S)*-tetrahydropapaverine (0.03 mM).

5.4 Phenoloxidase

The penultimate step in *(S)*-reticuline synthesis is the hydroxylation of 3'-carbon of the benzylic ring. This hydroxylation is catalysed by an ascorbate-dependent phenoloxidase (Loeffler and Zenk, 1990). A homogeneous enzyme preparation has been obtained from *Berberis stolonifera* cell cultures. The phenoloxidase appeared to be a dimer (60 kDa) composed of two identical subunits (36 kDa). This enzyme exhibited a broad substrate specificity. Purified phenoloxidase hydroxylated with similar efficiency *(R)*- and *(S)*-coclaurines, *(R, S)*-V-methylcoclaurine, tyrosine and tyramine. These results indicate that at least in *Berberis* a phenoloxidase with a dual function is present, i.e. an identical enzyme introduces an—OH group at the level of dopamine and 3'-hydroxy-N-methylcoclaurine biosynthesis. Phenoloxidases are nuclear-encoded enzymes located in or on internal membranes of plastids in both photosynthetic and non-photosynthetic tissues of angiosperms (Sherman *et al.,* 1991).

5.5 3'-Hydroxy-*N*-methyl-*(S)*-reticuline-4'-*O*-methyltransferase

The last step in *(S)*-reticuline biosynthesis is catalyzed by a 4'-O-methyltransferase (S-adenosyl-L-methionine:3'-hydroxy-*N*-methyl-*(S)*-coclaurine-4'-O-methyltransferase). This S-adenosyl-specific 4'-O-methyltransferase has been detected in cell cultures derived from thirty-six BTIQ-alkaloids containing plant species from four plant families (Frenzel and Zenk, 1990). 4'-O-Methyltransferase has been purified to near homogeneity (400-fold) from *Berberis koetineana* cell cultures. Two proteins were present in the purified enzyme preparation, one with 4'-O-methylating and another with a 6-O-methylating activity. With 3'-hydroxy-N-methylcoclaurine as substrate the kinetic data of 4'-O-methyltransferase could be determined without interference of 6-O-methyltransferase activity. 4'-O-Methyltransferase has been proved

to be an *(S)*-stereoselective and a 4'-OH regiospecific methyltransferase. The molecular mass of the 4'*O*-methyltransferase (40kDa) was determined by SDS gel electrophoresis and gel permeation HPLC. 4'-*O*-Methyltransferase is a stable enzyme when stored at—20°C. Under these conditions the 6-O-methyltransferase loses its activity after three months. 4'-*O*-methyltransferase appeared to be strongly inhibited by *(S)*-reticuline and to a lesser extent also by other alkaloids.

6 CONVERSION OF *(S)*-RETICULINE TO *(R)*-RETICULINE

Reticuline was isolated from *Annona reticulata* (Gopinath *et al.,* 1959). The stereochemistry of natural *(S)*-reticuline did not correspond to the configuration of morphinane alkaloids. Therefore both enantiomers of reticuline were infiltrated separately into poppy plants. Both enantiomers were incorporated into thebaine essentially to the same extent. Feeding experiments with reticuline labelled with ^{3}H at C-l and ^{14}C at other positions showed that incorporation into thebaine was accompanied by no loss of ^{14}C but with considerable loss of ^{3}H (Battersby *et al.,* 1965). In order to explain these results the 1,2-dehydroreticulinium ion was considered an intermediate which allows both the loss of ^{3}H and the inversion of configuration at C-l. The authentic 1,2-dehydroreticulinium chloride was incorporated efficiently into morphinane alkaloids. 1,2-dehydroreticuline was identified as a natural product in poppy plants (Borkowski *et al.,* 1978).

An enzyme able to catalyze the loss of tritium (dehydrogenation) from ^{3}H-labelled *(S)*-reticuline has been purified from *Berberis* and *Papaver somniferum* (Zenk, 1985). This enzyme was found to be absolutely specific for the substrate with *(S)*-configuration and was identical to *(S)*-tetrahydroprotoberberine oxidase.

6.1 1,2-Dehydroreticuline Reductase

The reduction of 1, 2-dehydroreticulinium ion to *(R)*-reticuline is catalyzed by an NADP-dependent 1,2-dehydroreticuline reductase (De-Eknamkul and Zenk, 1992) (Figure 7). The standard assay of 1,2-dehydroreticuline reductase is based on the solubility of (R)-reticuline and the insolubility of 1,2-dehydroreticuline in organic solvents. Reticuline reductase activity was found to be present only in plants containing
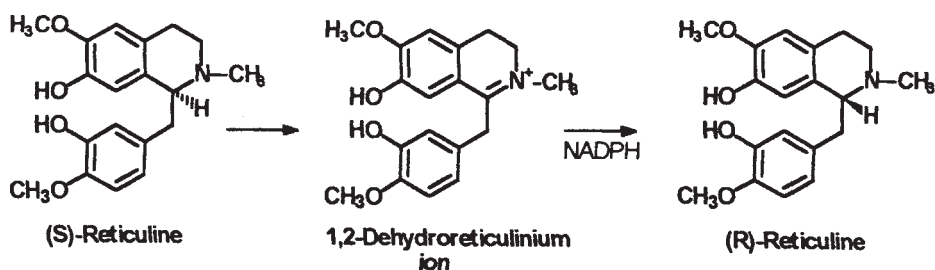
Figure 7 The conversion of *(S)*-reticuline to (R)-reticuline

morphinane alkaloids. Reticuline reductase is a soluble protein with molecular mass of 30kDa. The Km values for 1,2-reticuline and NADPH are 10μM and 7μM respectively. 1,2-dehydronorreticuline or 1,2-dehydrococlaurine have not been accepted as substrates by this enzyme.

7 BIOSYNTHESIS OF THEBAINE FROM (R)-RETICULINE

The concept of oxidative coupling of phenolic rings was introduced by Barton and Cohen (1957). From a phenolate ion, different mesomeric radicals can arise by the loss of an electron (at the oxygen atom itself or at the ring carbons *ortho* or *para* to oxygen). In reticuline each of two aryl rings is capable of forming phenoxy radicals (Figure 8). The radical pairing reactions can be carried out intermolecularly with a second molecule (e.g. for bisbenzylisoquinolines) or intramolecularly. The intramolecular oxidative coupling of a benzylisoquinoline molecule leads to a new carbon—carbon bond in the *ortho* or *para* position with respect to the free hydroxyl group in the two aryl rings. Thus, by phenolic oxidative coupling a tricyclic base (reticuline) can be transformed into a tetracylic one.

In opium poppy the aporphine alkaloids boldine and isoboldine are formed from *(S)*-reticuline by o—p' coupling (Brochmann-Hansen *et al.,* 1971). Bulbocapnine in *Corydalis cava (Papaveraceae)* is derived from *(S)*-reticuline, probably by o—o' oxidative phenolic coupling (Blaschke, 1970). Sanguinarine—a benzophenanthridine alkaloid present in opium poppy plants—is derived from *(S)*-scoulerine (Blechert *et al.,* 1995). *(S)*-scoulerine is formed from *(S)*-reticuline by the berberine bridge enzyme (Steffens *et al.,* 1984).
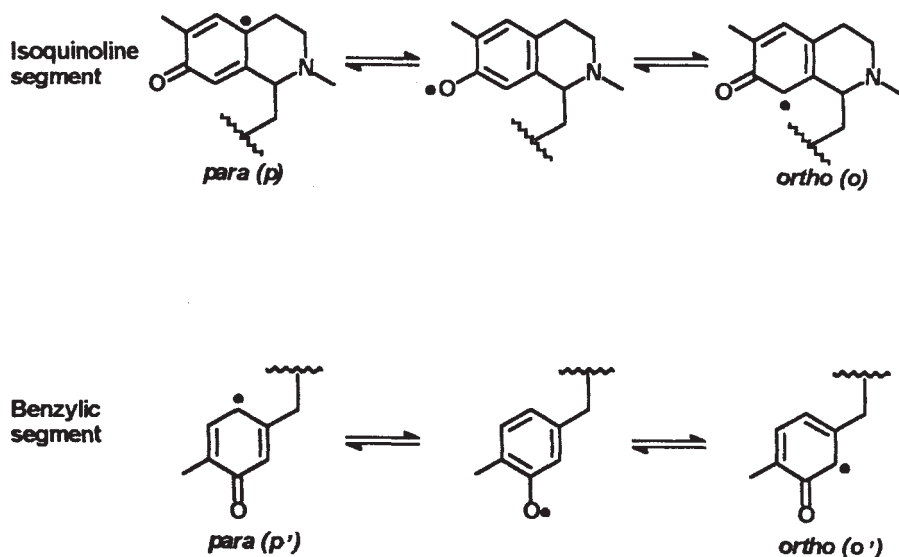
Figure 8 The mesomeric radicals of a benzyltetrahydroxylisoquinoline

(S)-reticuline is used in the opium poppy by different enzymes directing the folding of the benzylisoquinoline ring system of reticuline into the appropriate fashion (Figure 9).

(R)-reticuline is transformed into thebaine by the sequential action of three enzymes (Figure 10).

7.1 Salutaridine Synthase

The conversion of (R)-reticuline to salutaridine, i.e. the o'—p oxidative phenolic coupling of (R)-reticuline, is catalyzed by salutaridine synthase (Gerardy and Zenk, 1993a). The enzyme activity has been determined by radioimmunoassay, HPLC and radio-thin-layer chromatography using (R)-[N-^{14}CH$_3$]-reticuline as substrate. Salutaridine synthase has been found to be a microsomal protein. A microsomal fraction has been prepared from differentiated poppy plants and from a cell suspension culture of *Papaver somniferum* accumulating small amounts of thebaine. Neither (R)-norreticuline, (R)-coclaurine nor *(S)*-reticuline served as substrates. NADPH and O2 are essential for the enzyme activity. The Km values for (R)-reticuline and NADPH were found to be 17μM and 150μM respectively. Results of inhibitory studies indicated that salutaridine synthase is a cytochrome P-450 enzyme. The highest enzyme activity has been found in shoots and roots of three-month old poppy plants. Salutaridine synthase has not been identified in latex preparations from opium poppy plants and in cell cultures derived from plants accumulating significant amounts of
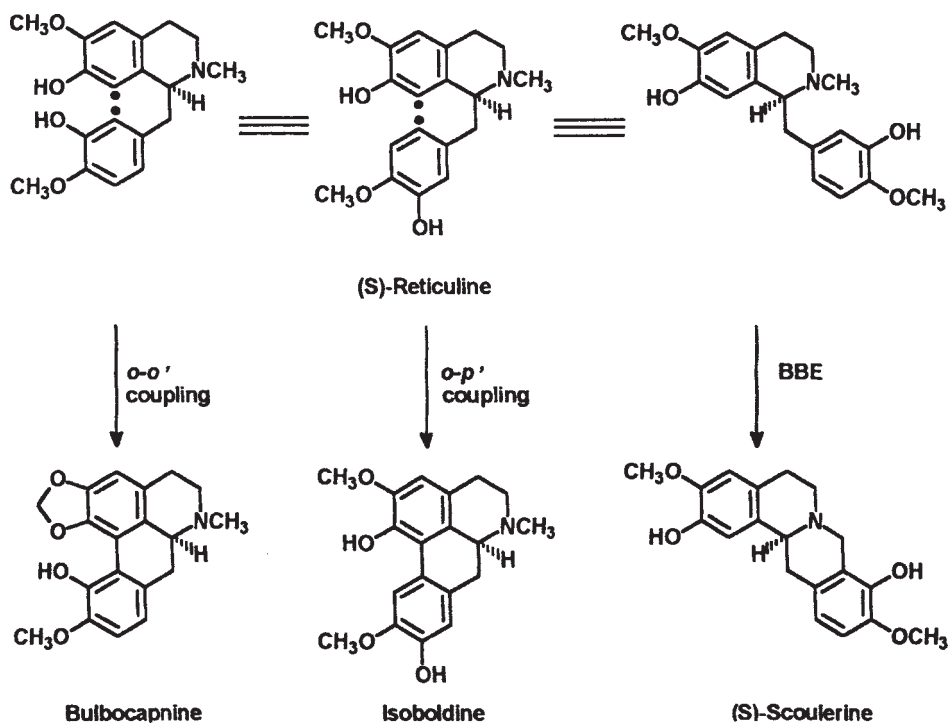
Figure 9 Biosynthesis of different benzylisoquinoline alkaloids from *(S)*-reticuline
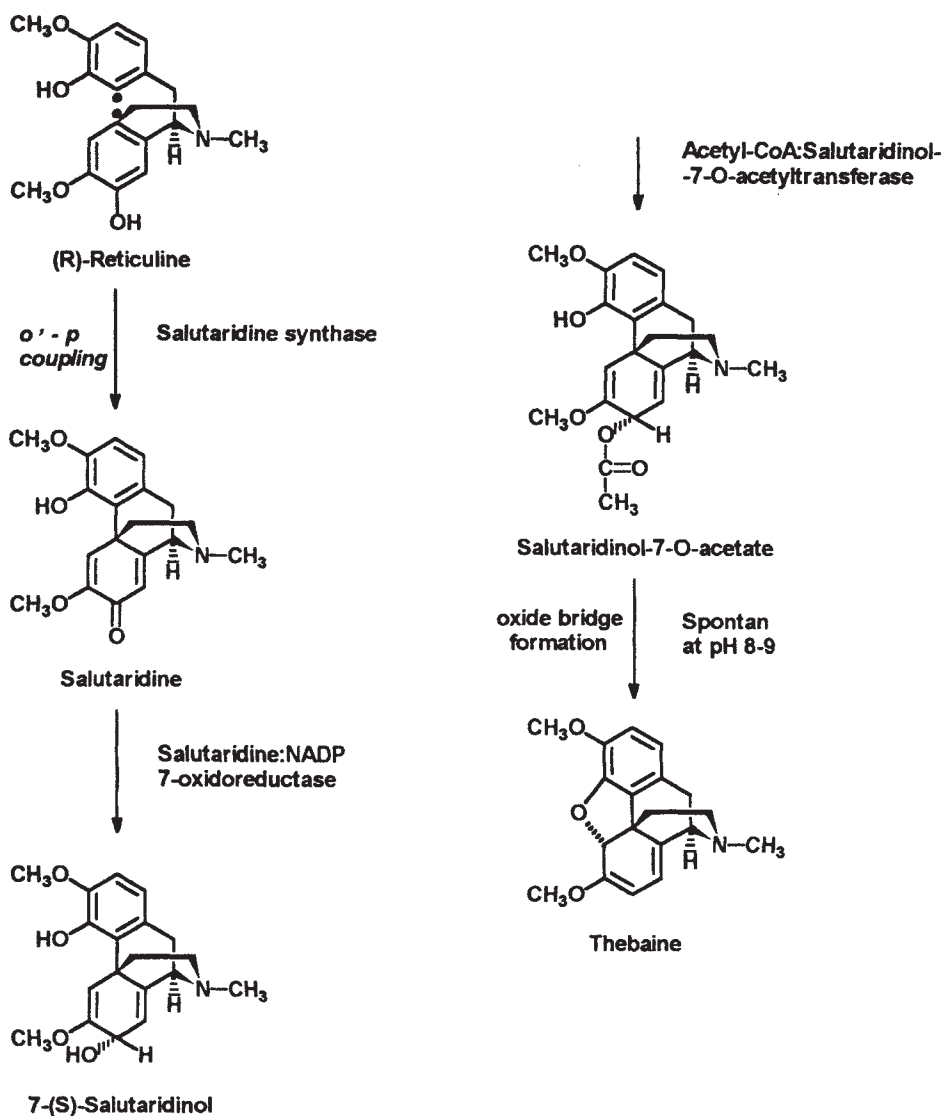
Figure 10 The pathway from (R)-reticuline to thebine

benzyltetrahydroisoquinoline alkaloids. Salutaridine synthase is NADPH-, oxygen- and cyt. P 450-dependent and a *Papaver* specific, regioand stereo-selective *o´—p* oxidative phenolic coupling catalyzing enzyme.

7.2 Salutaridine: NADPH 7-oxidoreductase

The reduction of Salutaridine to (7S)-salutaridinol is the second step in the conversion of (R)-reticuline to thebaine (Gerardy and Zenk, 1993b). Because of the solubility of

(7S)-salutaridinol and the insolubility of salutaridine in organic solvents, the enzyme activity was easily measured in the reverse direction. When [7-³H]-salutaridinol is used as a substrate for the reverse reaction, the aqueous phase can be used for counting the ³H released from the substrate. The enzyme has been purified to homogeneity from opium poppy cell cultures. Salutaridine reductase is a soluble monomeric protein (molecular mass of 52 kDa). The pH optimum of the forward reaction (pH 6.0-6.5) is different from that of the reverse reaction (pH 9.0-9.5). Salutaridine oxidoreductase is highly specific to salutaridine/ (7S)-salutaridinol. The apparent Km values for salutaridine and NADPH are 23μM and 125μM respectively. Within differentiated poppy plants, the highest specific activity was observed in developing capsules, with less activity in shoots and roots. Considerable salutaridine oxidoreductase activity has been observed in developing poppy seedlings, but the highest specific activity has been found in poppy cell cultures. Absolutely no activity could be detected in latex, developing poppy seeds and in the leaves of mature poppy plants.

7.3 Acetyl-coenzyme A: Salutaridinol-7-O-acetyltransferase

When (7S)-salutaridinol and (7R)-salutaridinol were separately infiltrated into opium poppy plants, only (7S)-salutaridinol was incorporated into morphinane alkaloids (Lotter *et al.,* 1992). Similar results were obtained with poppy cell cultures (Lenz and Zenk, 1994). Cell-free preparations from opium poppy cell cultures did not transform (7S)-salutaridinol into thebaine. When a mitochondrial fraction from opium poppy cell cultures was used as an enzyme preparation, the conversion of (7S)-salutaridinol to thebaine was detected. The presence of acetyl-coA in the reaction mixture significantly enhanced the thebaine formation. The enzyme catalyzing transition of (7S)-salutaridinol into thebaine in the presence of acetyl-coA has been purified to apparent homogeneity. This soluble protein has a molecular mass of about 50 kDa (Lenz and Zenk, 1994). 7-O-acetylsalutaridinol—the product of the acetyl-coA-salutaridinol acetyltransferase catalyzed reaction—rearranged spontaneously to thebaine at slightly alkaline pH values. It seems likely that the spontaneous transformation of 7-O-acetylsalutaridinol to thebaine is the last step in the biosynthesis of thebaine.

8 BIOSYNTHESIS OF CODEINE AND MORPHINE FROM THEBAINE

In *Papaver somniferum* and *Papaver setigerum,* thebaine is converted to morphine via codeinone and codeine (Battersby *et al.,* 1967; Blaschke *et al.,* 1967; Parker *et al.,* 1972). Neopinone has been identified in *P. somniferum* (Parker *et al.,* 1972). According to Horn *et al.* (1978), 6-O-demethylation proceeds by an oxidative mechanism with retention of the oxygen atom. Neopinone proved to be unstable and rearranges to codeinone in aqueous solutions. The isomerization of neopinone to codeinone has been analysed in detail (Gollwitzer *et al.,* 1993). At equilibrium the reaction mixture contains 52% codeinone and 48% neopinone. When a purified codeinone reductase was added to the equilibrated mixture of codeinone/neopinone a rapid decline of both ketones and an accumulation of codeine was observed. These results indisputably show that neopinone in plant cells exists in non-enzyme-catalysed equilibrium with
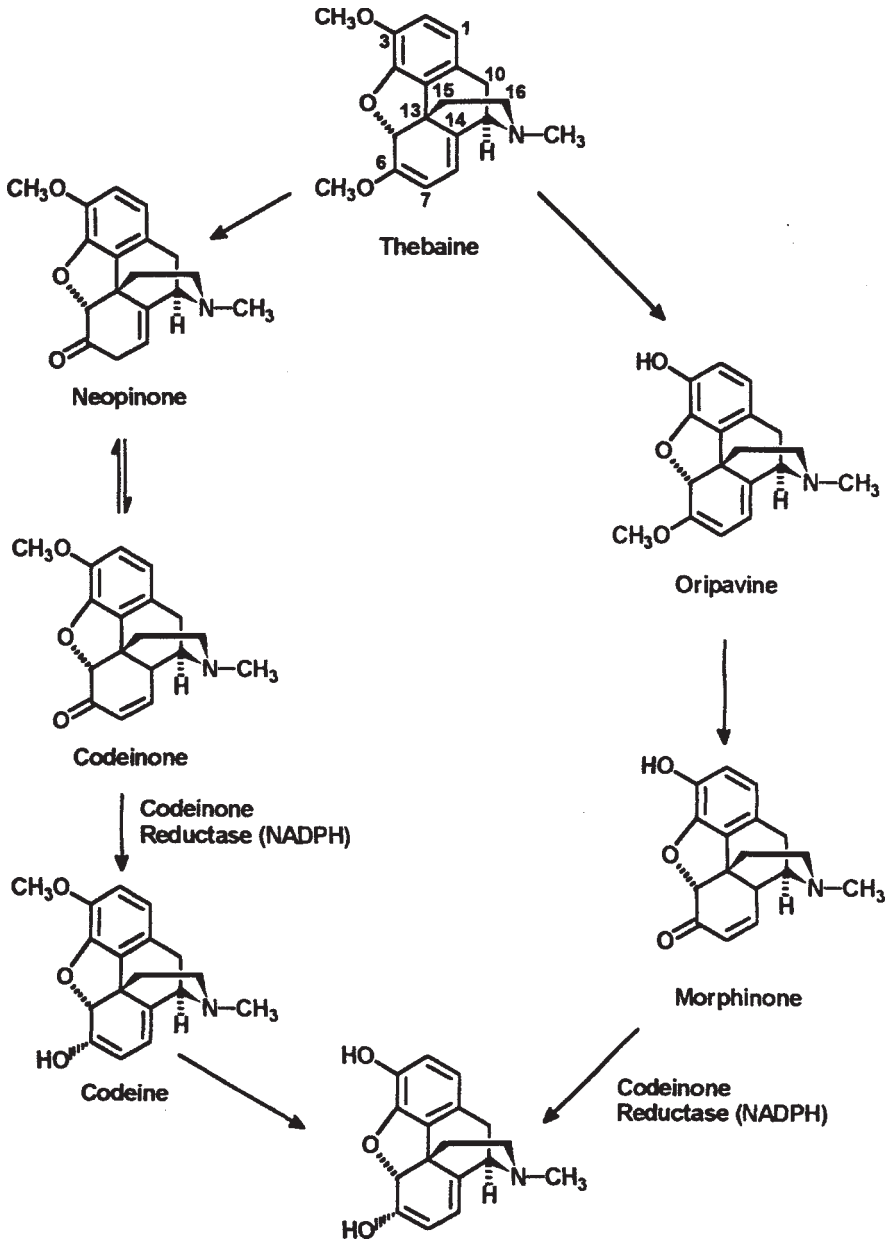
Figure 11 The pathway from thebaine to codeine and morphine

codeinone. In the presence of codeinone reductase this equilibrium is shifted quantitatively towards codeine.

In *Papaver* species except *P. somniferum* and *P. setigerum,* thebaine or oripavine are the last products of morphinane alkaloid biosynthesis. The identification of oripavine in capsules of *P. somniferum* raised a question regarding the role of oripavine in the biosynthesis of morphine (Nielsen *et al.,* 1983). The incorporation of [2-^{3}H]-oripavine into morphine and morphinone in *P. somniferum* showed that, in the opium poppy, there is a second pathway from thebaine to morphine. The intermediates of the second morphine biosynthetic pathway are oripavine and morphinone (Brochmann-Hansen, 1984) (Figure 11).

8.1 Codeinone: NADP Oxidoreductase

The *in vitro* enzymic reduction of codeinone to codeine was first observed in crude enzyme extracts from poppy cell cultures (Furuya *et al.,* 1978) and differentiated poppy plants (Hodges and Rapoport, 1980). In these studies NADH was used as a cosubstrate for the conversion of codeinone to codeine. Extracts from individual organs of poppy plants exhibited similar codeinone-reducing activities. The highest codeinone-reducing activity was detected in extracts from whole flowering plants.

A NADPH-specific codeinone oxidoreductase has been purified to homogeneity from *P. somniferum* cell cultures and from capsules of opium poppy plants (Lenz and Zenk, 1995a, b). The physiological forward reaction has been assayed by the determination of consumed NADPH after removal of codeinone and codeine by chloroform extraction. For the reverse reaction [6-^{3}H]-codeine was used as the substrate and the amount of ^{3}H released in the water phase was measured. The purified codeine oxidoreductase was found to be a soluble monomeric protein of 35kDa. In purified enzyme from opium poppy capsules two isoforms of this enzyme have been identified. Homogeneous codeine oxidoreductase was found to be a NADPH/NADP specific enzyme. In the absence of nucleotide cofactor or in the presence of NADH/ NAD, no reaction was observed. The apparent Km values for codeinone and NADPH were found to be 23µM and 168µM respectively. Codeinone oxidoreductase from opium poppy capsules was found to exhibit a higher affinity for codeinone and NADPH (Km values for codeinone 9µM and for NADPH 81µM) compared with the enzyme from poppy cell cultures. The purified codeinone oxidoreductase specifically and stereoselectively reduced codeinone to codeine and morphinone to morphine. A few synthetic analogues (hydrocodone, naloxone, naltrexone and dihydrocodeine) have also been accepted by the enzyme as substrates. These results suggest that codeinone oxidoreductase may be involved in both pathways leading from thebaine to morphine. The highest codeinone oxidoreductase activity has been detected in the capsules and shoots of opium poppy plants.

ACKNOWLEDGEMENTS

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IV.   BIOTECHNOLOGY OF POPPY

1.   *IN VITRO* BIOSYNTHESIS OF POPPY ALKALOIDS

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1 INTRODUCTION

In recent decades plant cell cultures have been favoured by plant biotechnologists for the production of prospective raw materials. Plant cell cultures have generally proved to be beneficial for biochemical and physiological studies, but progress is rather slow and difficult to predict when considering the economically important compounds (Drapeau *et al.,* 1987). A major problem is that plant secondary metabolism is regulated in an organ-, tissue- or development-specific process in the intact plant (Wink, 1989). The correct expression of the genes for biosynthesis and storage appears to be difficult to control in undifferentiated plant cells or callus cultures. Many researchers have solved these problems by employing organized tissue cultures, such as root or shoot cultures, which were facilitated by the use of transformation of plant tissue (Hamill *et al.,* 1987; Williams and Ellis, 1993; Nessler, 1994).

Alkaloids from *Papaver somniferum* callus cultures were first reported by Ranganathan *et al.* (1963) though without precise chemical identification. The numerous reports published since then on the production of morphinanes in *Papaver* tissue cultures have shown that significant amounts of these alkaloids rarely occur. Recent analyses show that concentrations of morphinanes in cell cultures do not exceed those of leaf tissues, and are much lower than the levels of alkaloid normally found in the dried latex (Constabel and Vasil, 1988).

2   EFFECT OF CULTURAL CONDITIONS AND PLANT REGENERATION ON MORPHINANE ALKALOID PRODUCTION

Plant cell suspension and tissue cultures may be regarded as a useful means of studying cell differentiation and morphogenesis by varying nutritional factors, environmental conditions and hormone regimes. The production of secondary metabolites has been associated with these factors, so the idea has been thrown up that the development of appropriate nutritional and hormone regimes would increase the production of morphinanes in the plant cell culture to levels similar to those found in the whole plant (Roberts, 1988).

Furuya and his work-team studied callus cultures of poppy and found that the origin of the explant (petiole, root, seedling, stalk and capsule) used did not effect the alkaloid patterns (Furuya *et al.,* 1972; Ikuta *et al.,* 1974).

Media after Murashige and Skoog (1962) and Gamborg *et al.* (1968) are most commonly used for *Papaver* tissue cultures and have supported successful alkaloid production. High levels of the hormone 2,4-D (2,4-dichlorophenoxyacetic acid) has often prevented alkaloid production, although Tam *et al.* (1980) successfully isolated codeine from cultures grown on a medium rich in 2,4-D.

The presence of cytokinins (kinetin and benzyladenine) seems to be favourable for morphinane alkaloid production; according to Staba *et al.* (1982) it permitted codeine formation.

Tissue cultures of *P. somniferum* have been grown in modified Murashige and Skoog (MS) media (revised tobacco medium) and B-5 medium. *Papaver* callus grown *in vitro* produced morphinane alkaloids in low concentrations. Yoshikawa and Furuya (1985) gave an account of the production of codeine and thebaine in a green callus with relatively high levels of kinetin.

There are indications that media without hormones, while promoting cell differentiation, have also supported morphinane alkaloid production. These observations suggest that alkaloid production may be closely dependent on cell differentiation. The transfer of *P. somniferum* callus to solid or liquid media without hormones led to the formation of embryoids that morphologically resembled normal seed embryos. Unlike seed embryos, however, the cell culture embryoids were found to accumulate thebaine (0.2% dry weight) (Nessler and Mahlberg, 1978). Galewsky and Nessler (1986) investigated tissue cultures of poppy (derived from seedling hypocotyls) by means of TLC and HPLC. Thebaine production was regulated by the gradual removal of auxin from the culture medium. Under these conditions neither morphine nor codeine was produced in a detectable quantity. Alkaloid synthesis in somatic embryos appears, therefore, to require a specific level of differentiation. A requirement for specific types of tissue differentiation may partially explain the conflicting reports in the literature of morphinane alkaloid biosynthesis in tissue culture.

Papaver somniferum cell and tissue cultures grown on media designed specifically to promote roots and shoots yielded significant levels of thebaine, codeine and morphine, while regenerated plantlets had alkaloid levels at least quantitatively similar to those of normally grown seedlings. The level of alkaloids, of thebaine in part, was higher than in normally produced seedlings of a similar development stage (Schuchmann and Wellmann, 1983; Yoshikawa and Furuya, 1985). Although in these experiments alkaloid levels were commensurate with levels found at the appropriate development of the intact plant, they do not compare favourably with the levels found in the natural plant (Roberts, 1988).

In the opinion of Kamo and Mahlberg (1988) most callus cells are typically parenchyma-type cells without laticifer cells. Specialized clusters of small densely cytoplasmic cells, or meristemoids, were observed to form on the surface of the callus and appear to represent a level of differentiation within the culture (Nessler and Mahlberg, 1978). These meristemoids may be similar to the albino callus first reported by Furuya *et al.* (1972). Numerous meristemoids were observed to occur randomly over the surface of a callus grown on Murashige and Skoog medium, containing various combinations and concentrations of hormones (Kamo *et al.,* 1982). Meristemoids have also been reported to contain laticifer-like cells and have been

described to give rise to green calli or to differentiate into buds and shoots (Kutchan *et al.*, 1983; Yoshikawa and Furuya, 1985).

P. somniferum callus retains its capacity to regenerate plantlets over an extended period of time. Yoshikawa and Furuya (1985) regenerated plantlets from callus meristemoids that had been in culture for nine years.

Swankar and Bohra (1989) gave account of the inhibitory effect of 2,4-D on shoot regeneration. Kinetin and IAA (indole acetic acid) are also known to induce shoots in poppy.

It is interesting, that callus cultures derived from seedling roots of poppy (Swankar and Bohra, 1989) culminated in shoot regeneration, suggesting that morphogenesis for this species is not only genotype-specific but can also be organ-specific.

Blanarik *et al.* (1989) investigated the influence of various cultivation factors on the qualitative representation of alkaloids produced by poppy cultures. The optimization of foundation, organizing and evaluation of experiments with the callus culture was studied. A sufficient number of set members and the optimum inoculum amount were statistically determined. The optimum time of the subcultivation was chosen on the basis of the results of chemical analysis of depletion of cations in a nutrient medium as well as on the results of growth dynamics and mitotic activity. The influence of the amount and the age of the inoculum on the properties of poppy tissue cultures was also studied.

Blanarik *et al.* (1992) studied the isolation and identification of alkaloids from callus cultures of *P. somniferum*. The main isolated and identified alkaloids were morphine and papaverine followed by codeine, thebaine, noscapine, and trace amounts of protopine and allocryptopine. Nadaska (1991) also investigated the production of alkaloids such as sanguinarine from tissue cultures of *P. somniferum*.

Differentiation of either shoots or roots by *P. somniferum* callus was found to occur when the callus was cultured on a variety of hormones (Kamo *et al.*, 1982). Typically the callus regenerated shoots when grown on MS basal salts medium supplemented with the growth regulators isopentenyl adenine (IPA) or kinetin (K). Roots occurred when the callus was placed on either naphthalene acetic acid (NAA) or NAA with kinetin.

Kamo *et al.* (1982) also studied the formation of morphinane alkaloids in cultured tissues and redifferentiated organs of *P. somniferum*. They examined in detail the capacity of callus tissues, meristemoids and redifferentiated roots and shoots to synthesise morphinane alkaloids when grown on media containing different combinations of natural (IAA and IPA) or synthetic (2,4-D, K and NAA) plant hormones. No amino acids were added to the media. Thus, the occurrence of alkaloids would reflect the presence in the cells or tissues of the complete biosynthetic complex for the production of morphinane alkaloids.

According to the analyses of Kamo *et al.* callus tissues contained 0.59–33.1µg total morphinane alkaloids/g tissue (dry weight). The quantity of alkaloid in the calli varied for different media. The total morphinane alkaloid content in calli grown on different combinations of IPA was relatively low. However, combinations of IPA with IAA (0.1ppm) typically resulted in a relative increase in total alkaloid in callus tissues. The highest amount of alkaloid (31.2µg) in callus occurred on media containing IPA (4ppm) and IAA (0.1ppm). Alkaloid formation on media containing different

concentrations of kinetin was relatively low; NAA alone (0.2ppm) yielded a higher total alkaloid concentration (10.3μg) than any level of kinetin employed. When kinetin was combined with NAA (0.2ppm) there was an appreciable increase in total alkaloid content on two media. The highest level of alkaloid production (33.1μg) in callus tissues was present with a combination of kinetin (4ppm) and NAA (0.2ppm). In contrast, there was a relatively low alkaloid production (1.1μg) for tissues on either 2,4-D (0.1ppm), or with a combination of 2,4-D and kinetin (0.1 ppm), although these callus tissues grew well.

Thebaine was the predominant alkaloid and was found in calli grown on all hormone concentrations used. The highest percentages of codeine compared with thebaine occurred on a medium containing a combination of kinetin (4ppm) and NAA (0.2ppm), and on kinetin (4ppm).

Callus tissue growth differed for each hormone concentration and reflected differences for alkaloid concentrations in the callus (dry weight). Growth was consistently greater for tissue grown on concentrations of IPA alone (17.7–36.9mg tissue/callus) and most kinetin concentrations (7.9–42.1mg tissue/callus) as compared with the respective concentrations of auxin and cytokinin.

Kamo *et al.* (1982) pointed out that shoots redifferentiated in greater abundance in media containing kinetin (2ppm) than on other selected hormone concentrations employed. Shoots which developed on IPA or IAA media were normal in appearance whereas they appeared abnormal when redifferentiated on calli grown on media containing 2,4-D (0.1ppm), or kinetin and 2,4-D. Shoots did not develop roots.

Shoots removed from the callus and analysed for alkaloids contained all major morphinane alkaloids (morphine, codeine and thebaine). The total morphinane alkaloid content of these shoots averaged 187μg/g dry weight—lower than that found for the shoots of intact seedlings. The percentage of each alkaloid was 40% thebaine, 12% codeine and 48% morphine. Thebaine and codeine were identified by GC/MS; morphine by HPLC.

Roots frequently redifferentiated on callus tissue, although they typically remained short. The largest number of roots developed on callus grown on media supplemented with NAA (0.2ppm), or NAA and kinetin (0.2 and 0.1ppm). Root formation at these hormone concentrations often was so profuse as to cover the callus piece completely. Roots did not commonly redifferentiate from callus grown on media supplemented with the natural hormones (IAA or IPA). Callus possessing roots, as when grown on NAA and kinetin (0.2 and 0.1ppm), had a somewhat higher concentration of thebaine (20.4μg/g dry weight tissue) than callus which lacked roots but was harvested from medium at the same time (18.9μg thebaine/g dry weight tissue).

These experiments showed that organ redifferentiation influenced alkaloid synthesis in that morphine, as well as codeine and thebaine occurred in shoot organs redifferentiated on callus. Shoots derived from the callus produced greater amounts of total alkaloids than the callus, indicating that the shoot controlled both the quality and quantity of alkaloids. The synthesis of morphine in these shoots may possibly be related to the presence of laticifers known to be present in regenerated shoots of *P. somniferum* (Nessler and Mahlberg, 1978). Redifferentiated shoots contained less alkaloid than shoots of intact seedlings. Roots on callus, and meristemoids with and without buds, produced only thebaine. Thus, thebaine and codeine can be synthesised

by callus cells as well as organs, but redifferentiated shoots seem to be necessary for the production of morphine. The only exception was that cultured meristemoid samples which contained buds and one or two small shoots possessed no detectable codeine or morphine. Although these organs were present, the relative amount of alkaloids in them was probably too low to be detected.

Kamo *et al.* (1982) observed that tissue and organ redifferentiation into shoots was a major factor that influenced alkaloid synthesis, and that morphine was synthesised only in redifferentiated shoots.

2.1 Variability of Tissue Cultures

Concerning the production of metabolites and the biochemical phenotype of cell cultures, variability is included in all research strategies for the selection of high-producing strains. This has been observed for primary metabolic processes and for secondary metabolism, such as alkaloid production.

Petiard *et al.* (1985, 1987) studied the variability of tissue cultures for alkaloid production in *P. somniferum*. Their results concerning biochemical variability expressed in plant tissue culture lead to the following three major conclusions:

— The cell-to-cell variation observed in a callus seems to be essentially due to the asynchrony of cell growth and differentiation.
— Due to the cloning process by protoplast isolation, some non-definitive variation occurs, allowing the supposition that these variations are essentially epigenetic phenomena.
— Some alkaloids of morphinane type may be accumulated in undifferentiated tissue culture in unusual forms needing specific treatment for their extraction.

From the practical point of view, the results imply the possibility that in screening tissue strains, some compounds may not be identified from the usual extraction procedures. Furthermore, these new forms of alkaloids could be of interest for the screening of new biologically active substances. Moreover, to isolate high-producing strains it seems that an additional selection phase by single cell cloning is of no interest when it is undertaken on a selected strain obtained in the classical way.

2.2 Plant Regeneration

Poppy cells grown in both liquid suspension and as calli have been regenerated to form intact plants. Cultures under both growth conditions give rise to compact masses of cells, meristemoids, which can differentiate into embryoids. Meristemoids cultured in a liquid medium have been observed to develop into 1–3mm diameter cell clusters (Nessler, 1982) and cells of meristemoids were noted to be smaller and more densely cytoplasmic than other cells of the callus (Nessler and Mahlberg, 1979; Kutchan *et al.*, 1983). The meristemoid cells were characterized by the presence of lipid droplets and vesicles with associated electron-dense deposits. The electron-dense deposits seem to be similar to the 'caps' described for vesicles of laticifer cells in intact poppies (Dickenson and Fairbairn, 1975). Some meristemoids, 2-3mm in diameter, have also been described to contain tracheids (Morris and Fowler, 1980). It appears, therefore,

that meristemoids can develop to various levels of cytodifferentiation (Kamo and Mahlberg, 1988).

Meristemoids in the cell suspension (liquid medium) were found to differentiate into torpedo-stage embryoids when subcultured on a medium lacking hormones (Nessler, 1982; Schuchmann and Wellmann, 1983). Although the torpedo-stage embryoids appeared morphologically normal, they possessed mature tracheary elements and laticifers in the position where they normally occur in association with the procambium. These embryoids germinated when cultured (12h light) on a solid medium without hormones (Nessler, 1982).

Callus cultures have formed meristemoids or albino callus (Furuya *et al.*, 1972). Yoshikawa and Furuya (1983) subcultured (16h light) callus on MS medium with kinetin (0.1-1.0mg/1) and stimulated the green buds and shoots on the callus to develop. Following root formation *in vitro* the plants grew to fertile plants in a greenhouse. It is interesting to note that whereas the intact *P. somniferum* plants contained morphine as the predominant alkaloid, the main component formed in the young plants regenerated from callus was codeine.

Yoshikawa and Furuya (1985) studied the relationship between the restoration of the ability to biosynthesise morphinane alkaloids and the appearance of some structural features, like differentiation. From cultured cells of poppy, green buds and shoots were formed at a high frequency under illumination at low temperatures (16–18°C). Differentiation was induced from meristemoids which contained large amounts of lipids. The differentiated tissues completely recovered the potential to biosynthesise morphinane alkaloids that was lost in the undifferentiated cells, i.e. calli. Moreover, it was confirmed by enzyme immunoassay (for opiates) that tissues differentiating only tracheary elements, produced morphinane alkaloids, mainly codeine. Codeine has been the principal morphinane alkaloid found in suspension cultures and in regenerated tissues from suspension cultures (Hsu, 1981; Staba *et al.,* 1982).

Although alkaloids synthesised in organs regenerated from calli may not be present in the same ratio and concentration as in intact plants, the organs regenerated from callus possessed laticifers morphologically identical to those in intact seedlings (Kutchan *et al.,* 1985; Nessler and Mahlberg, 1979; Schuchmann and Wellmann, 1983).

2.3 Morphinane Production with Laticifer Formation

Poppy latex has the unique ability of producing high yields of morphinane alkaloids. Isolated latex has been shown to undergo limited biosynthesis of poppy alkaloids, but a survey of previous work gives no evidence that the latex is the primary site of alkaloid biosynthesis, even though latex vesicles have been shown to be the major accumulation site (Roberts *et al.,* 1983; Homeyer and Roberts, 1984).

In *P. somniferum* the morphinane alkaloids accumulate in the latex, which is contained in structurally and physiologically specialized cells—the laticifers. Because morphine may constitute as much as 15-20% of the latex, the alkaloid content of these vacuoles reaches very high levels. The ability of these vacuoles to store morphinane alkaloids without significant metabolic degradation determines the high levels of alkaloids that accumulate in these plants and this suggests that, to obtain

commercially viable levels of morphinane alkaloids in tissue culture, the development of laticifer-like cells, or something equivalent, may be essential (Homeyer *et al.,* 1989).

The appearance of tracheids in cell cultures may be important in identifying differentiation that may lead to alkaloid accumulation. Details on the development of laticifers and laticifer-like cells in young seedlings and plantlet regenerants is now well documented (Nessler and Mahlberg, 1978; Roberts, 1988). Nessler and Mahlberg (1977) also dealt with the problem of concurrent cytodifferentiation and morphinane alkaloid accumulation, which appears further compounded by the detection of cells that resemble the early stages in laticifer formation, that is, cells rich in vesiculating endoplasmic reticulum that, however, do not accumulate alkaloids. An investigation by Nessler *et al.* (1985) showed the occurrence of latex-specific proteins.

Radioimmunoassay (RIA) is a useful method for analysing secondary metabolites. Its advantages over conventional chromatographic analyses are the small sample size needed, the high specificity and sensitivity, and the rapidity of the process (Weiler, 1977; Hsu *et al.,* 1983). Commercial RIA for morphinane alkaloids has been used to examine several problems associated with the production of morphinanes during the *in vitro* development and culture of *P. somniferum*. RIA has previously been used to detect alkaloids in two- and nine-week old poppy cell cultures (Hodges and Rapoport, 1982a).

Griffing *et al.* (1989) assayed poppy hypocotyls for morphinane alkaloid content using commercial radioimmunoassay (RIA) for morphine. The hypocotyls showed a sharp increase in morphinane alkaloid content on the third day after sowing, reaching a peak (9 ng mg^{-1} fresh wt) between the fourth and the fifth days. Hypocotyl explants were cultured for up to two months on solid nutrient medium containing NAA. During this time and while hypocotyls formed callus, the morphinane alkaloid content fell to a level of 0.4 ng mg^{-1} fresh weight in culture. Callus masses retained laticifer-like cells. If cell suspensions were plated with a medium lacking growth regulators, plantlets were regenerated and the level of alkaloids found for hypocotyls was re-established. If plated cell suspensions were cultured in the presence of growth regulators, organs formed, but plantlets did not regenerate and the alkaloid content remained at or near the maintenance level. Over a period of a year, callus subcultures lost their laticifer-like cells, their embryogenic potential, and their ability to maintain low-level alkaloid production. In conclusion, RIA has been used to show that there is a rise and subsequent fall of morphinane alkaloids during the growth and culture of poppy hypocotyls. With prolonged culture in suspension the cultured cells continue to harbour levels of alkaloids.

Cultures induced to become embryogenic 'maintenance' can again acquire relatively high levels of alkaloids in the absence of growth regulators. With growth regulators present, they differentiate into organs, but contain only low levels of alkaloids. The suspension cultures eventually lose the capacity to synthesise alkaloids along with their embryogenic potential (Griffing *et al.,* 1989).

3 ALKALOID PRODUCTION IN SUSPENSION CELL CULTURES

Under optimum conditions plant cells are able to grow in suspension cultures without limitation or ageing. Moreover, they are totipotent, in that one single cell or protoplast

contains all the genetic information for the differentiated plant. It can therefore be expected that under proper conditions plant cell suspension cultures are able to produce the whole range of natural products which are isolated from the differentiated plants. Plant cell cultures have several advantages over differentiated plants. For example, they are independent of geographic and climatic conditions; the price of the plant drugs could therefore be stabilized. Furthermore, they could easily be cultivated under special state control, and thus would be of great importance for the production of morphine, for example.

The production of opiates from tissue culture is dependent on the large accumulation of alkaloids in cells or the culture medium. While there have been some major successes in plant cell culture in terms of cells with high yields of isoquinolines, the most important members of this group from a commercial and pharmaceutical viewpoint—the morphinanes—have proved difficult to produce in plant cell cultures.

Most cultured *Papaver* cells, either as callus or cell suspensions, readily produce non-morphinane alkaloids such as sanguinarine, dihydrosanguinarine, norsanguinarine, and oxysanguinarine (Kuzovkina and Rabinovich, 1981; Kutchan *et al.,* 1985; Songstad *et al.,* 1989, 1990). Isolations of cryptopine (Furuya *et al.,* 1972; Anderson *et al.,* 1983), stylopine (Kamimura *et al.,* 1976), protopine (Forche and Frautz, 1981) have also been reported. Numerous reports of the production of the morphinanes, thebaine, codeine and morphine from cell cultures of *P. somniferum* occur in the literature, although yields are low compared with the high yields of plants. Tam *et al.* (1980) described the synthesis of codeine by cell suspension culture incubated in continuous light (B–5 medium with 1 mg/1 2,4-D). Tam and his work-team isolated codeine from diploid cell suspensions containing tracheids and giant cells.

P. somniferum suspension cultures have been grown in liquid media. Analysed cell cultures were found either to contain no morphinane alkaloids (Staba *et al.,* 1982; Schuchmann and Wellmann, 1983; Yoshikawa and Furuya, 1985; Shamina *et al.,* 1988) or to contain morphinane alkaloids (Tam *et al.,* 1980; Kutchan *et al.,* 1983; Heinstein, 1985). The suspension cells were described as consisting of meristemoids or light and dark coloured cell aggregates rather than being a suspension of single cells. Meristemoids formed in suspension cultures have the capacity to be embryogenic and can differentiate to form torpedo-stage embryoids in liquid media (Nessler, 1982).

Three groups have demonstrated that the differentiation of suspension cells into somatic embryoids or roots was correlated with thebaine and/or codeine production (Staba *et al.,* 1982; Schuchmann and Wellmann, 1983). The results were consistent with alkaloid biosynthesis occurring in callus possessing the presence of differentiated tracheidlike or laticifer-like cells. The *P. somniferum* suspension cells grown by Tam *et al.* (1980) synthesised codeine, and the cell aggregates of this suspension were described as having differentiated.

Several factors in addition to cell and tissue differentiation may affect morphinane alkaloid biosynthesis, e.g. age of cultured cells, explant used, *Papaver* variety, medium or hormones used, and alkaloid analysis techniques. Cells grown for one to two months in liquid suspension contained more thebaine than five-month old cultures which possessed only traces of thebaine. From the available literature, all researchers reported the use of callus from seedling explants to initiate suspension cells, except Morris and

Fowler (1980), who used callus from the stems of intact plants. Plant growth regulators have a remarkable influence on the production of benzylisoquinoline alkaloids in some cultures. The *P. somniferum* variety of cells used varied for each researcher. Suspension cells were grown either in Murashige and Skoog (1962) or Gamborg (1968) B5 medium containing kinetin, NAA or 2,4-D. There seems to be no explanation for the differences in alkaloid biosynthesis of the various suspension cell cultures based on the plant variety, explant used or culture medium, although the data are incomplete.

Suspension cell cultures have proved useful for transformation studies. Besides the possibility of producing alkaloids *in situ* the synthesising capacity of the plant cells can be used for biotransformation. However, few experiments have been reported so far. The codeine-producing suspension cells described by Tam *et al.* (1982) were capable of converting the precursor codeinone to codeine. Another essential factor that might induce the production of benzylisoquinolines could be the addition of biosynthetic precursors to the medium.

Cell cultures which did not produce morphinane alkaloids (Furuya *et al.,* 1978) were also found to be capable of reducing the precursor codeinone to codeine through biotransformation. The cell cultures were unable to metabolize thebaine, codeine or morphine. When these cells were fed /RS/-reticuline, /—/—/R/-reticuline—a precursor of morphinane alkaloids in plants (Borkowski *et al.,* 1978)—was not metabolized which verified the absence of morphinane alkaloid biosynthesis by these cell cultures.

Radioactive tyrosine is another precursor that has been given to suspension cells (Hsu, 1981), because it has been shown that tyrosine serves in the synthesis of morphinane alkaloids in intact plants (Fairbairn *et al.,* 1968, 1981). The suspension cell cultures converted the tyrosine to thebaine, codeine and morphine with codeine as the main component.

The biological pathway from the primary metabolite L-tyrosine to /S/-reticuline, the universal precursor to a large variety of isoquinoline alkaloids in intact plants, has been established in detail (Zenk, 1995) at the enzyme level. The conversion of /S/-reticuline to its /R/ epimer opens the morphine pathway, which is restricted in plants of the genus *Papaver*. While the precursor molecule /R/-reticuline only contains three rings with one asymmetric centre, the morphine skeleton consists of five rings with five asymmetric centres.

It has been suggested that the crucial ^{12}C—^{13}C bond of morphine alkaloids that generates the fourth ring may be envisaged as being formed by intramolecular phenolic coupling of /R/-reticuline. Indeed, experimental proof at the precursor feeding level to whole plants has been obtained with /R/-reticuline being transformed to salutaridine by regioselective para-ortho oxidative coupling.

Lenz and Zenk (1995b) have studied the mechanism by which the plant enzyme is capable of closing the oxide bridge during the transition of salutaridinol to thebaine acetyl coenzyme A: salutaridinol-7-O-acetyltransferase has been purified from *P. somniferum* cell cultures. The salutaridinol-7-O-acetate thus formed subsequently spontaneously closes the oxide bridge at a cellular pH 8–9 by allilic elimination of acetate to furnish the morphine precursor thebaine.

The effect of stress on alkaloid biosynthesis has been examined using *Papaver* suspension cultures. Laughlin and Munro (1983) observed a 75% increase in morphine concentration of leaves and stems subsequent to infection of plants with *Sclerotinia*

sclerotiorum. These observations have prompted further investigations with cell cultures. Elicitors derived from pathogenic micro-organisms, that is, autoclaved broadspectrum wilt fungi conidia and homogenates, have been used with *P. somniferum* to increase yields of alkaloids. Heinstein (1985) inoculated *P. somniferum* suspension cultures with autoclaved conidia from either *Verticillium dahlia* or *Fusarium monoliforme*. The result was a substantial, minimum tenfold increase in yield of both morphine and codeine. Several other phytoalexin elicitors have been inoculated into *P. somniferum* suspension cell cultures, but there were no detectable morphinane alkaloids (Eilert *et al.,* 1985). Suspension cultures inoculated with *Botrytis* turned brown and were structurally like parenchyma-type cells (Eilert and Constabel, 1985). The extent to which alkaloid production in cell culture results from stress factors deserves further investigation, since such a reaction may throw light upon the factors that initiate enzyme formation and activation, and subsequently, alkaloid biosynthesis.

Tyler *et al.,* (1988, 1989) elicited *P. somniferum* cells with a *Botrytis* sp. homogenate and cultured them by a semi-continuous process. Elicitation induced synthesis of sanguinarine and dihydrosanguinarine. Sanguinarine, a benzophenanthridine alkaloid, has considerable market potential in oral hygiene products. Williams *et al.* (1992) investigated the effect of polymeric adsorbents on the production of sanguinarine in *P. somniferum* cell cultures.

The sensitivity of *P. somniferum* cell line PBI=2009 to elicitation results in the accumulation of the benzophenanthridine alkaloids sanguinarine and dihydrosanguinarine at levels of 3% or more of cell dry weight, i.e. levels of interest from a commercial point of view. However, a great many obstacles must be cleared in developing a quantitative, controllable process from laboratory-scale, largely qualitative observations. Before answering questions such as 'what type of process lends itself best to large-scale production?' and taking the economics of production into consideration, fundamental aspects must be thoroughly examined. These aspects are: cell line stability, managing variability in the response to elicitation, and establishing culture conditions and protocols for maximizing product yield (Tyler *et al.,* 1989). Hopefully, the answers to such questions derived from future elicitation studies on *P. somniferum* can be related to other plant cell-elicitor systems for the benefit of plant cell culture technology as a whole.

4 BIOTRANSFORMATIONS OF ALKALOIDS

Poppy cultures with low production levels of morphinane alkaloids may be ideal for investigating alkaloid biotransformation. The production of morphinane alkaloids absolutely requires /R/-reticuline. Although in whole plants, /S/-reticuline is formed from /S/-norlaudanosoline, it is readily converted to the /R/ isomer in *P. somniferum* (Zenk, 1985). In plant cell cultures /R,S/-reticuline was stereospecifically converted into /S/-scoulerine and /S/-cheilanthifoline, but no apparent utilization was made of the /R/-reticuline (Furuya *et al.,* 1978). This group also presented evidence for the conversion of /-/-codeinone to /-/-codeine and showed that their cell cultures would not further metabolize thebaine, codeine or morphine. Tam *et al.* (1982) studied cell cultures of *P. somniferum* and found them able to convert thebaine to neopine (3%) and codeinone (1.5%) while unable to metabolize codeine, neopine or DL-norlaudanosoline.

Some cultures have a limited capacity to biotransform thebaine to codeine (Grutzmann and Schroter, 1966) or codeinone to codeine (Furuya *et al.,* 1978), while the cultures themselves do not produce any alkaloids.

Further progress in this field has been made with the immobilization of *P. somniferum* cells. Immobilized cell systems have been rapidly developed over recent years. These systems have practically been applied to the industrial production of useful compounds, such as foodstuff additives and drugs. Immobilized cells were first used as a non-viable catalyst for single enzyme reactions. The application of immobilized living cells to the production of useful compounds utilizing multi-enzyme reactions has been the subject of many papers (Furuya *et al.,* 1984).

4.1 Bio transformation of Codeinone to Codeine

Besides the possibility of producing alkaloids *in situ* the synthesising capacity of plant cells can be used for biotransformation. Investigations of biotransformations with cell cultures thus highlight some of the problems in the biosynthesis of morphinanes and at the same time show that operative enzymes under ideal conditions can produce potentially commercially useful levels of a given product.

Lenz and Zenk (1995a) described the biosynthetic transformation of codeine and morphine from the biological precursors: codeinone and morphinone.

Neopinone, which is derived from thebaine by enol ether cleavage (Horn *et al.*, 1978), and codeinone exist in a thermodynamic, non-enzyme catalysed equilibrium as reported previously (Gollwitzer *et al.,* 1993). The subsequent stereospecific reduction of codeinone to codeine is an important reaction in morphine biosynthesis, because the catalytic activity of the enzyme involved (previously named codeine: NADP$^+$ oxidoreductase) regulates the sequence from neopinone via codeinone to codeine (Lenz and Zenk, 1995a).

Codeinone-reducing activity was first detected in crude enzyme extracts from *P. somniferum* suspension cells, as well as in capsule tissue from differentiated poppy plants, but only when NADP$^+$ was used as the co-substrate. This indicates that the enzyme was dependent on NADPH/NADP$^+$ and not on NADH/NAD$^+$ as it was previously postulated (Furuya *et al.,* 1978; Hodges and Rapoport, 1980).

Since Brochmann-Hanssen (1984) clearly showed that a second alternative pathway from thebaine to morphine via oripavine and morphinone was operative in poppy plants, the codeinone-reducing enzyme has also become an interesting research area. Codeinone and morphinone vary only in the presence of a methyl group at position 3 in the former compound. Therefore this reductive enzyme could possibly be involved in both pathways, leading from codeinone to codeine as well as from morphinone to morphine. In order to investigate its properties and regulatory function in morphine biosynthesis, purification of this important enzyme was necessary. Lenz and Zenk (1995a) described the isolation and characteristics of the codeinone-reducing enzyme, codeinone reductase (NADPH), from *P. somniferum* cell cultures.xxx

Suspension cell cultures are useful in transformation research. The codeine-producing suspension cultures of Tam *et al.* (1982) were capable of converting the precursor codeinone to codeine, whereas other precursors—codeine, neopine,

papaverine—were not metabolized. Thebaine was converted to neopine, which differed from the thebaine pathway noted in intact plants (Parker *et al.*, 1972). These results support the interpretation that cell cultures do not demethylate thebaine or codeine.

Suspension cultures which did not produce morphinane alkaloids (Furuya *et al.*, 1978) were found to have the capacity to reduce the precursor codeinone to codeine with a biotransformation ratio. The cell cultures were unable to metabolize thebaine, codeine and morphine. Furuya *et al.* (1978) tried to produce morphinane alkaloids by the addition of reticuline, known to be an important intermediate in their biosynthesis. /S/-reticuline was converted to /S/-scoulerine (14.7%) and further on to the protoberberine cheilanthifoline (0.5%). /R/-reticuline, the isomer needed for the morphinane pathway, remained unchanged, probably indicating that the enzymes catalysing the steps to salutaridine and further on to codeine and morphine were missing. The only reaction that could be performed in this pathway was the reduction reaction from codeinone to codeine (67%), but no further metabolism to morphine could be observed.

To provide a basis for isolating the enzymes responsible for alkaloid biosynthesis, Hodges and Rapoport (1980) utilized cell-free extracts from entire poppy plants to demonstrate the specific *in vitro* conversion of codeinone to codeine. The method of quantifying this conversion at the nmol level was developed to allow the possibility of preparing active enzyme extracts.

In studies on immobilized plant cells, various reactions concerning alkaloids have been attempted for the production and biotransformation of secondary metabolites. Furuya *et al.* (1984) concentrated on the reduction of codeinone to codeine by immobilizing living poppy cells entrapped in calcium alginate beads. Various matrices had been employed, but calcium alginate or carrageenans seemed to be favourable as the entrapment because a high retention of cell viability was noted with these media. Of these two matrices, calcium alginate has been especially used in the immobilization of plant cells.

Furuya *et al.* (1984) described the biotransformation of codeinone to codeine by the cell suspension culture and the cell-free system. This reduction required NADH as a co-factor in the enzyme system. In the immobilized cell system, however, this reaction proceeded without NADH. Using a column bioreactor packed with the immobilized living cells, Furuya *et al.* (1984) investigated the effects of various conditions, such as temperature and aeration, on the conversion of codeinone to codeine and the cell viability.

4.1.1 Immobilisation of Cell Cultures

Intact cells of *P. somniferum* were entrapped in calcium alginate beads under sterile conditions. The immobilized cells slightly increased in the beads when cultured in a flask supplemented with a growing medium in a rotary shaker. Poppy cells never leaked from the beads into the medium. It can be presumed from microscopic observations that this phenomenon (non-leakage from the beads) is due to the very large size of the *P. somniferum* cultured cells. Moreover, respiratory activity confirmed (Furuya *et al.,* 1984) that the cells continued to live for over six months on subculturing at one-month intervals. Furuya *et al.* (1984) used the immobilized living cells after a one-week culture in a shake flask and studied them under various reaction conditions.

4.1.2. Biotransformation in a Shake Flask

/-/-Codeinone (5mg) was administered to the immobilized cells after one week in culture. After three days of shaking in a rotary shaker, the cultures were harvested. The immobilized cells and the medium were separately extracted with chloroform—isopropanol (3:1) at pH 8.5, and codeine (3.10mg in the immobilized cells and 0.42 mg in the medium) was identified by TLC and determined by a SIM (selected ion monitoring) method (m/z 299 M$^+$) in GC/MS. The total conversion ratio was 70.4% to the codeine administered. This ratio was higher than that of the cell suspension culture (60.8% after three days). This excretion (or release) is of considerable importance for the utilization of immobilized plant cells for the production of secondary metabolites and this reaction can therefore be described as the focus for an investigation into the applications for production by a bioreactor.

4.1.3 Biotransformation by a Column Bioreactor

The bioreactor used in the experiments of Furuya *et al.* (1984) was designed by revising the column. An air supply to the entrapped cells was achieved by direct upward aeration, i.e. airlift mode. After reacting for an appropriate time, samples were taken from the base of the column by stopping the air pump. Fresh medium previously reserved in the tank was supplied from the top of the column by a pump. The substrate (codeinone) was injected from the upper inlet through a millipore filter. Continuous utilization of the immobilized *P. somniferum* cells was investigated by following the biotransformation of codeinone to codeine using the bioreactor under three different temperatures and three rates of aeration.

Furuya and his team (1984) first studied the intensity of aeration. The column bioreactor was functional for 30 days under optimal conditions (20°C, 3.75 vvm in aeration) and had a conversion ratio of 41.9%. The biotransformation rate in the bioreactor was lower than in the flask (70.4%) and in the free cells (60.8%). This was obviously caused by the inhibitory effect of the permeability in the alginate beads, because the immobilized cells entrapped in the beads require five times the amount of oxygen compared to that in the free cells. The cells gradually lost their viability due to the lack of oxygen and at the same time lost their biotransformation potential. Therefore, in order to utilize immobilized cells for long periods, a modification of the column bioreactor or the other matrices must be devised.

The biotransformation of codeinone to codeine proved to be possible in the immobilized cells. The biotransformation of thebaine via codeinone and codeine to morphine is now being investigated with other strains of *P. somniferum* cell cultures.

4.2 Conversion of Codeine to Morphine

P. somniferum plants were employed by Hsu *et al.* (1985) to demonstrate the biotransformation of morphinane alkaloids. In the biosynthetic pathway Hodges and Rapoport (1980) demonstrated the conversion of codeinone to codeine by cell-free extracts of the entire plant of *P. somniferum*. With the same cell extract Hodges and Rapoport (1982b) reported a high yield conversion of /^{3}H/reticuline to /^{3}H/

salutaridine. Neither cell-free extracts nor latex had previously been used to study the conversion of codeine to morphine, until Hsu *et al.* (1985) investigated this important step in biotransformation, using isolated capsules of poppy. The aim of their investigation was to determine the role of enzyme co-factors and other factors in controlling the conversion of codeine to morphine.

One week after petal fall, young isolated capsules of poppy were excised and incubated with /^{14}C/codeine in MS medium. The uptake of radioactivity into the capsules increased with increasing incubation time. Uptake reached a near maximum level at 10 h incubation; at that time the amount of radioactivity in the capsules was only 15% of the /^{14}C/codeine initially added to the medium. This isolated capsule system offers an ideal means for investigating the biotransformation of morphinane alkaloids. The advantages of the system are that it is close to the *in vivo* plant system, it does not denature the biosynthetic enzyme system, and it provides uniform precursor uptake.

The biotransformation of codeine to morphine is not a simple one-substrate to one-product reaction. Several possible reactions have to be considered. In a study by Hsu *et al.* (1985), /^{14}C/codeine and /^{14}C/morphine were converted into their radioactive N-oxides. Latex or whole poppy plants have the ability to convert morphine and codeine into their N-oxides. Demethylation of morphine has been established as an active meta bolic process. Normorphine has been found in raw opium (Hsu *et al.,* 1985). The morphine degradation pathway involves an initial demethylation to normorphine, which is subsequently degraded to non-alkaloid metabolites. Morphinane alkaloids may play an active metabolic role, perhaps as specific methylating agents. In the presence of H_2O_2, horseradish peroxidase transformed alkaloids into N-oxides and morphine to pseudomorphine (Vágújfalvi and Petz-Stifter, 1982). The crude poppy enzyme fraction showed the same activity. The rate of reaction was influenced by phenolic compounds and their reaction was influenced by the concentration of H_2O_2 and the presence of ascorbic acid (Vágújfalvi and Petz-Stifter, 1982). Hsu *et al.* (1985) showed that the oxidation reaction was the major degradation process. Norcodeine and normorphine, the demethylation products of codeine and morphine, were detected only in trace amounts, which prevented any further structural analysis. The dimerization product of morphine, pseudo-morphine, was not found in the isolated capsule system. Ascorbic acid and several other phenolic compounds, such as DOPA and p-cumaric acid, did not significantly interfere in the conversion of codeine to morphine. The reducing agents seemed to be important factors in controlling the degradation process of morphine and codeine. Reducing agents such as dithiothreitol, glutathione and ß-mercaptoethanol strongly promoted codeine and morphine degradation, while morphine formation remained at a constant level. The presence of hydrogen peroxide (concentration>0.25mM) resulted in the conversion of codeine and morphine to N-oxides by non-enzymatic oxidation.

Hsu and his work-team (1985) found that co-factors such as nicotinamide adenine dinucleotide, adenosine-5'-triphosphate, S-acetyl coenzyme A and pyridoxal phosphate were not required in the conversion of codeine to morphine. Isolated capsules of *P. somniferum* offer a method of studying the biotransformation of codeine to morphine.

Hsu and Pack (1989) also studied the metabolism of /^{14}C/codeine in cell cultures of *P. somniferum*. These studies indicate that cultured poppy cells have the ability

to synthesise morphinane alkaloids at a stage closely related to the differentiation of some tissues and organs. However, the concentration of morphinane alkaloids formed in these cells was not as high as that in intact capsules. A large yield of morphinane alkaloid could be produced if the cultured cells regenerated into plants. Based on the inherent problems of producing sufficient amounts of morphinane alkaloids in poppy cultures, emphasis was placed on factors that could affect the biotransformation of codeine to morphine. In this report the ability of codeine to convert to morphine in cultured cells was investigated by a radio-isotope technique. The results suggest that enzymes involved in the oxidative degradation of codeine dominate the biotrans formation of codeine to morphine in *P. somniferum* cultured cells.

5 SUMMARY

In summary, the future potential of producing benzylisoquinoline alkaloids using pharmaceutical biotechnology looks promising. The fact that successful production of morphinanes has not been achieved so far is disappointing. Nevertheless, this problem might be overcome with proper selection of the methods employed and with an intensified search for an optimum medium. Gene transfer technology offers new opportunities to modify the synthesis of plant secondary products through metabolic engineering. These examples suggest that future applications of plant biotechnology are a real possibility.

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2. *IN VITRO* CULTURE TECHNOLOGIES

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1 INTRODUCTION

The opium poppy, like many other important crop species, has provided a number of technical challenges for the application of modern molecular approaches. These difficulties include, but are not limited to, problems in protein and nucleic acid purification, as well as the transformation and regeneration of fertile plants. Over the past decade most of these barriers have been overcome so that the opium poppy is poised to become a model system for the study of latex cell gene regulation as well as a target for metabolic engineering of important pharmaceuticals.

This section will review the features which make the opium poppy recalcitrant to standard molecular procedures and how protocols have been successfully adapted in order to work with poppies. In addition, the cloning and characterization of several poppy genes will be described. Their potential application in the metabolic engineering of transgenic poppy plants for altered alkaloid synthesis is also discussed.

2 CHARACTERIZATION OF MAJOR LATEX PROTEINS

Laticifers are internal secretory systems which occur in both primitive and advanced flowering plants. Although they are present in only fifteen families, latex-bearing plants may be quite valuable due to their ability to accumulate useful products such as alkaloids, hydrocarbons, or enzymes.

Opium is the air-dried cytoplasm of the opium poppy laticifer which remains metabolically active at maturity. Isolated latex is able to convert radioactive precursors into morphinane alkaloids (Bohm *et al.,* 1972; Fairbairn and Wassel, 1964a,b; Fairbairn *et al.,* 1968; Fairbairn and Djote, 1970; Kleinschmidt and Mothes, 1959). Enzymes of general cellular metabolism have been detected in isolated poppy latex (Antoun and Roberts, 1975a, b), as have enzymes which may be involved in alkaloid biosynthesis (Antoun and Roberts, 1975a; Roberts and Antoun, 1978; Roberts *et al.*, 1983).

Biochemical investigations of poppy alkaloid metabolism have been complicated by the presence of robust polyphenol oxidase (PPO) activity which can interfere with many enzyme assays when latex is exposed to air (Roberts, 1971). Opium poppy has one of the most active PPO enzymes in the plant kingdom. Most plant PPOs have a rather low substrate affinity with a K_m of about 1 mM (Flurkey, 1985), however, the opium poppy enzyme has a K_m of only 1µM (Ashgar and Sidiqi, 1970).

Studies of fractionated latex have shown that PPO activity sediments with a 1000g vesicle pellet in centrifuged latex and is present in both aqueous and detergent-soluble forms (Roberts, 1971, 1974). These two forms of PPO have different substrate affinities and are distributed in separate cytoplasmic compartments: a lighter organelle containing the soluble PPO and a heavier organelle with the bound form (Roberts *et al.,* 1983). In *Papaver bracteatum* (Nessler and Mahlberg, 1978) PPO activity has been localized to largemembrane-bound inclusions within laticifer plastids which are also present in opium poppy laticifers (Nessler and Mahlberg, 1976). In view of what is now known about PPOs in the plastids of other species, it seems likely that the membrane-bound form of PPO in opium poppy latex described by Roberts (1971) is the plastid form.

It is not possible to directly separate the proteins in unfractionated opium poppy latex by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) because they precipitate in the presence of morphinane alkaloids, even when boiled (Nessler *et al.,* 1985). Although many latex proteins remain soluble when whole latex is mixed 1:1 with a sample buffer containing a non-ionic detergent such as NP-40 (Nessler *et al.,* 1985), the most efficient way to isolate latex proteins is to separate them from the latex alkaloids before mixing with SDS sample buffer (Nessler, 1988).

Morphinane alkaloids and their precursors are stored in membrane-bound alkaloidal vesicles within the latex (Fairbairn and Djote, 1970). Alkaloidal vesicles and their contents can be easily separated from the latex serum by gently mixing freshly collected latex with 0.05 M phosphate buffer (pH 7.0) containing 0.5M mannitol and subjecting the mixture to a 10 second spin in a microcentrifuge. The mannitol acts as an osmoticum which prevents lysis of the alkaloidal vesicle pellet leaving a clear supernatant serum fraction which can be drawn off and analysed by SDS-PAGE (Nessler, 1988).

Electrophoretic analysis of poppy latex has revealed a distinct group of abundant, low molecular weight polypeptides which we have termed the major latex proteins or MLPs (Nessler *et al.,* 1985). Protein gel blots and immunocytochemical investigations show that MLPs are constitutively expressed in latex (Nessler *et al.,* 1985; Griffing and Nessler, 1989) and thus represent good markers for laticifer development.

Two dimensional electrophoresis of poppy latex separates the MLPs into a series of discrete polypeptides with pIs ranging from 6.0 to 3.5 (Nessler *et al.,* 1985). Molecular analysis suggest that each of these MLPs is encoded by a separate member of a small gene family (Nessler, 1994).

Isolation of clonable nucleic acids from opium poppy tissues is difficult because of its abundant PPO activity (see above) and the high concentration of dopamine in the latex which is quickly converted by PPO into highly reactive quinones in the presence of oxygen. Poppy PPO is so active that it will even use the phenol at the interface of phenol—chloroform extraction buffer as a substrate. Thus, protocols involving a phenol extraction step yield dark brown RNAs that cannot be copied by reverse transcriptase and DNA molecules that do not digest with restriction enzymes. The CTAB protocol for isolation of plant nucleic acids developed by Taylor and Powell (1982) does not use phenol and thus works well with poppy (Nessler and Vender Haar, 1990). Additionally, because dopamine and other potential PPO substrates are confined to the alkaloidal vesicles, it is also possible to isolate latex RNA directly from the serum by removing the vesicular fraction as outlined above.

MLPs are encoded by a family of nine genes which can be divided into two distinct subfamilies based on DNA gel blot analysis (Nessler, 1994). Genomic clones and cDNAs have been obtained for four members of this family: MLP15 (Nessler and Vonder Haar, 1990; Nessler *et al.*, 1990), MLP22 (Nessler and Burnett, 1992) and MLP146/MLP149 which are physically linked and separated by approximately 5.5kb (Nessler, 1994). The organization of the MLP family is consistent with the triploidhybrid origin of the opium poppy as proposed by Kadereit (1986).

The biochemical function of the opium poppy MLPs has not been determined, however, homologous genes have been identified in several other plant species, all of which lack laticifers (see Pozueta-Romero *et al.*, 1995). It has been proposed that these proteins participate in early disease resistance responses (Breiteneder *et al.*, 1989) which is consistent with the concept that laticifers are 'pre-wounded' cells that constitutively accumulate defence compounds that can be immediately released when the plant is injured. It will be interesting to see if the molecular regulation of laticifer-specific gene expression is controlled by signal transduction paradigms similar to those of woundinduced genes in non-laticiferous plants.

3 PRODUCTION OF TRANSGENIC PLANTS

In order to produce transgenic plants one must be able to: (1) stably integrate foreign DNA into its genome; and (2) regenerate fertile plants from transformed tissues. It is now possible to do both of these in opium poppy.

Long-term callus and suspension cultures of opium poppy have been maintained on defined media in several laboratories (reviewed elsewhere in this volume). Both roots and shoots have been regenerated from callus (Nessler and Mahlberg, 1979), however, a much simpler method for regenerating poppy suspensions through somatic embryogenesis has been developed (Nessler, 1982). Opium poppy somatic embryogenesis parallels the development of zygotic embryos, with both tissues passing from globular, to heart and finally torpedo stages. One unusual feature of somatic embryos is the presence of laticifers among elements of the procambium, in contrast to the zygotic embryos which develop laticifers only *after* germination. Somatic embryo cultures have been shown to synthesise complex morphinane alkaloids that are not produced by normal seed or undifferentiated callus (Galewsky and Nessler, 1986).

Large numbers of plants have been regenerated from suspension cultures via our somatic embryogenesis procedure and grown to maturity. Normal R_1 plants have been also obtained by selfing these regenerated plants (Nessler, 1990). The ability to induce regeneration easily from opium poppy tissue cultures through somatic embryogenesis has greatly facilitated the development of transformation/regeneration protocols for this species.

3.1 Biolistic Transformation of Poppy Somatic Embryos

Globular somatic embryos can be maintained in suspension cultures for many months in Murashige and Skoog's medium (MS) (Murashige and Skoog, 1962) in the presence

of 0.25mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and retain their ability to regenerate whole plants (Galewsky and Nessler, 1986). These cultures are ideal 'targets' for particle bombardment because they continually produce new embryoids by periclinal divisions of the protoderm (Nessler, 1982). Thus the coated particles do not have to penetrate deeply into the embryoid to deliver their DNA to cells with morphogenic potential.

For Holistic transformation, a PDS-100 helium gene gun (DuPont) was used to introduce 1.0μm gold particles coated with the pBI221 plasmid into cultured embryoids. The pBI221 plasmid encodes a bacterial ß-glucuronidase (GUS) reporter gene under the control of the cauliflower mosaic virus 35S promoter and is conveniently assayed by histochemistry or fluorometry (Jefferson *et al.*, 1987).

A series of transient assay experiments were conducted to optimizee parameters for biolistic transformation of opium poppy somatic embryos. The best results were obtained when stationary cultures, harvested 24 days after transfer to fresh media, were bombarded from a distance of 20cm at 1550 psi. A DNA concentration of 10μg/50μl of gold particle suspension was found to produce the greatest number of GUS-positive spots.

For stable transformation, pBI221 was mixed 1:1 with the pHYG plasmid which contains a 35S::hygromycin resistance gene construct as a selectable marker. Following bombardment under the optimized conditions the embryoids were placed onto 1% agar solidified media (MS+0.25mg/l 2,4-D) for a 48 h recovery period and then transferred to the same media containing 25 mg/1 hygromycin for selection.

Within two weeks the embryoids not expressing hygromycin resistance turned dark brown and died. The remaining embryoids remained white and continued to grow on the hygromycin-supplemented solid media when subcultured at two-week intervals. After three rounds of selection, individual embryoid colonies were transferred to liquid media (Nessler, 1982) without hygromycin and taken through the standard regeneration protocol (Nessler, 1990).

Fertile, GUS-positive poppy plants were recovered from several embryoid lines transformed by particle bombardment. However, since direct DNA transfer often leads to a high copy number and rearrangements of the inserted DNA (Buchholz *et al., 1996*), biolistics may not be the best transformation approach for an annual species, particularly if the transgenic material is going to be incorporated into an ongoing breeding programme.

3.2 *Agrobacterium*-Mediated Transformation and Regeneration of Poppy

Agrobacterium-mediated transformation, unlike direct DNA transfer methods, generally results in the integration of a low copy number (1–5 copies) of the T-DNA insert (Simpson *et al.*, 1986). It is therefore the method of choice for those species that are susceptible to *Agrobacterium* infection. Although there is at least one report in the literature that opium poppy does not infect with wild-type *A. tumefaciens* (De Cleene and De Lay, 1976), the primary reference in this review is old (1924) and apparently only one bacterial strain was tested. It is not surprising, however, that poppy might not form tumours with wild-type *A. tumefaciens* since the auxin:

cytokinin ratio needed for optimal callus formation in poppies is different from that of tobacco or tumour forming species.

More recently a new strain of *Agrobacterium rhizogenes* (MAFF 03–01724) has been shown to transform opium poppy (Yoshimatsu and Shimomura, 1992). Unfortunately, since this wild-type strain of *A. rhizogenes* was not disarmed, the morphology and alkaloid content of the recovered plants were altered by the phytohormone genes transferred with the T-DNA.

Our laboratory has developed an *A. tumefaciens* based method for transforming poppy tissues from which fertile plants can be recovered via our somatic embryogenesis procedure. Because we use disarmed vectors, callus growth and embryogenesis are strictly functions of the hormonal composition of the culture medium.

The best transformation efficiencies were obtained using the *A. tumefaciens* strain EHA105 which is derived from A281 containing the supervirulent pTiBO542 plasmid (Hood *et al.,* 1984). The EHA105 strain was transformed with the hygromycin resistance binary vector pBIG (Becker, 1990) into which the 35S::GUS intron (Vancanneyt *et al.,* 1990) reporter gene construct was cloned.

Hypocotyls were excised from 7–10 day-old sterile opium poppy seedlings and incubated for 10 min in a stationary-phase *Agrobacterium* culture. The hypocotyls were then blotted dry and placed in Petri dishes containing H medium (Nessler, 1990). After two days without selection the tissues were transferred to fresh media supplemented with 25mg/l hygromycin and 200mg/l cefotaxime. Within 4–6 weeks hygromycinresistant calli formed at one or both ends of approximately 10% of the treated hypocotyls. Resistant calli were subcultured every four weeks for two more passages and then transferred to antibiotic-free media. After twelve weeks of selection all of the surviving calli were GUS-positive and no untransformed 'escapes' were produced.

Transformed calli were subcultured to fresh media at four-week intervals and the embryoids which sporadically developed on their surface were transferred to liquid media (MS+0.25mg/l 2,4-D) and taken through the standard regeneration protocol (Nessler, 1990). Transgenic plants were recovered within six to eight months, transferred to soil, and grown to maturity.

4 CLONING OF ALKALOID PATHWAY GENES

All opium poppy alkaloids are derived from two molecules of tyrosine which are enzymatically modified to form dopamine and 4-hydroxyphenylacetaldehyde (Stadler *et al.,* 1988). Condensation of dopamine and 4-hydroxyphenylacetaldehyde produces norcoclaurine, the key intermediate in the synthesis of protoberberine, benzophenanthridine and morphinandienone alkaloids (Stadler *et al.,* 1987).

Although the biochemistry of morphinane alkaloid synthesis has received considerable attention, the early steps in the pathway have not been fully characterized. For example, although dopamine is clearly abundant in poppy latex (Roberts *et al.,* 1983), it is not known if dopamine is derived from the decarboxylation of 3,4-dihydroxyphenylalanine (DOPA), the hydroxylation of tyramine—the decarboxylation product of tyrosine—or by both.

Molecular cloning of pathway genes has provided a means to resolve these steps through the expression of individual genes and analysis of their enzyme's substrate specificities without contamination from other isozymes with different activities. Two distinct cDNAs and two related genomic clones encoding aromatic amino acid decarboxylases have recently been isolated from opium poppy (Facchini and De Luca, 1994). When expressed in bacteria as fusion proteins the enzymes encoded by both cDNAs showed their highest activities against DOPA, but also accepted tyrosine as a substrate thus identifying them as tyrosine/DOPA decarboxylases (Facchini and De Luca, 1994, 1995a).

Our laboratory has recently isolated a new member of the T*y*DC/DODC gene family, designated *TyDC5,* which shares extensive identity with the other opium poppy genes (84%), but is more active against tyrosine than DOPA when expressed in *Escherichia coli* (Maldonado-Mendoza *et al.,* 1996). *TyDC5,* like *TyDC1* (Facchini and De Luca, 1995b) is expressed primarily in the roots of mature poppy plants. A peak of *TyDC5* expression was also seen during germination corresponding to the time when the radicle penetrated the seed coat.

We observed similar expression patterns in tobacco transformed with a *TyDC5* promoter fragment translationally fused to the GUS reporter gene. In *TyDC5::GUS* tobacco, GUS activity transiently appeared in all parts of the seedling during germination, but was limited to tissue roots in older plants. GUS staining patterns in tissues of the root were not consistent. Sometimes staining was localized to the epidermis and cortex but was also occasionally concentrated in the vascular tissue. Similar patterns of T*y*DC gene expression have been reported in opium poppy by *in situ* hybridization, with higher levels of expression confined to the vasculature in mature tissues (Facchini and De Luca, 1995b). These results suggest that the differential expression of TyDC/ DODC gene family is transcriptionally regulated and that promoters from these genes may be useful for the metabolic engineering of different alkaloid pathways within poppy.

Unlike the morphinane alkaloids, benzo(c)phenantridine alkaloids are not limited to genus *Papaver,* but are found in many other species throughout the *Papaveraceae.* One of these species, *Eschscholzia californica* (California poppy), has been used as a model by Zenk's group to elucidate each of the enzymatic steps in the entire benzo(c)phenantridine pathway (Kutchan and Zenk, 1993). Of particular note is the cloning of the berberine bridge enzyme (Dittrich and Kutchan, 1991) which is the key branch point enzyme that converts the N-methyl group of (S)-reticuline into the berberine bridge carbon, C-8, of (S).scoulerine. Using the *E. californica* clone it should be possible to isolate the homologous gene from *P. somniferum* for use in the metabolic engineering of poppy alkaloids.

5   FUTURE PROSPECTS: METABOLIC ENGINEERING OF ALKALOID PATHWAYS

The availability of reliable transformation/regeneration systems for opium poppy and the cloning of alkaloid pathway genes means that it should be possible to apply metabolic engineering to alter the quantity and quality of alkaloids in this species in the very near future.

Experiments are currently underway in our laboratory to overexpress tyrosine decarboxylase in transgenic poppies to determine which subclass of alkaloid might increase with a rise in the tyramine and dopamine pool sizes. Anti-sense and co-suppression approaches are also being explored to see which subclasses of alkaloids can be lowered by decreasing precursor availability. If sufficient suppression of the TyDC/DODC gene family is achieved it may even be possible to recover plants which produce very low alkaloid levels or no alkaloids at all. Such plants would undoubtedly be very useful for examining the ecophysiological roles of alkaloids in nature.

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V. RAW MATERIAL PRODUCTION

1. CULTIVATION OF POPPY IN THE TEMPERATE ZONE

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1 INTRODUCTION

Poppy is cultivated in several countries of the temperate zone. It is cultivated for two purposes: for straw, which is an important pharmaceutical raw; and for seeds and fatty oils which are used in both alimentary and industrial production processes. In certain cases and countries the seeds are marketed as the only product of local poppy cultivation (e.g. in Germany and Austria). However, both the straw and the seed have significant market value and use of them both obviously increases the profitability of the crop.

Poppy plants also have a small but increasing importance as an ornamental species and the dried capsules are used in dried flower compositions. Special cultivars have been developed and produced for this purpose.

Considering the production areas and quantities of harvested poppy straw and seed, the main European countries involved are Hungary, Spain, France, Romania, Slovakia, the Czech Republic and Austria. Although very low production capacity in the 1990s has been recorded in Poland, Bulgaria and Yugoslavia (INCB, 1995), these countries are traditional poppy producers and after the recent economic crisis they may become important players in the market again. Turkey is reported to cultivate several thousand hectares of poppies for purposes other than opium production.

The production of opium has been significant in Turkey, Iran, some parts of the former Soviet Union (e.g. Kirghizia, Kazakhstan, Ukraine) and the former Yugoslavia in the past. However, no licit opium production has been registered in these countries since the 1980s, so opium production in countries of the temperate zone is not considered here.

2 CULTIVATION OF STRAW AND SEED

This work describes cultivation methods for both seed and capsules (straw), combining the main elements of numerous European cultivation practices, but essentially based on Central European methods.

Two main forms of poppy cultivation are found in the temperate zone—the so-called 'winter' (autumn-sown seed) and 'spring' (spring-sown seed) technologies. Spring poppy cultivation is more widespread in the temperate zone, because

overwintering of the plants cannot be ensured in all regions (Bernáth and Tétényi, 1982a, b). Apart from low temperatures, winter precipitation may also cause significant damage to autumn-sown crops. However, in some countries autumn sowing is relatively common, especially in Bulgaria, where it is considered the optimal method (Ilieva, 1967). Autumn sowing is almost never carried out in northern parts of the temperate zone (e.g. in Poland (Ruminska, 1973)) but can be practised in southern parts of Hungary and Austria with the occasional risk of damage to the crop taken into account.

It is already known that 'spring' and 'winter' poppy cultivation methods do not differ in agro-technology only. The two types of cultivation are also distinguished by the use of special poppy ecotypes characterized by differing levels of frost resistance (Bernáth and Tétényi, 1982a, b).

There are also differences between agro-technologies applied in large-scale production and in smaller private gardens and farms. Figure 1 shows an example of large-scale spring poppy cultivation in Hungary. The biological considerations and some agrotechnological aspects are the same in both cases, but the required seed and straw production can be achieved in different ways depending on the technology and tools available, finance, market aims, etc. In the next sections large-scale technology and some alternatives are presented. An overview of large- and small-scale technologies is given in Table 1.

Figure 1 Large-scale 'spring-sown' cultivation of poppy in Hungary

Table 1 The main elements of highly mechanized and small-scale technologies of 'spring' poppy production in Europe

Time period	Mechanized cultivation	Small-scale cultivation
October–November	• ploughing	• digging (or ploughing)
November	• fertilization	• raking
	• smoothing, harrowing	
February–March	• soil sterilization	• soil sterilization
	• fine smoothing, rollering	• fine smoothing
	• sowing by machine with treated, irradiated or granulated seeds	• sowing by hand, seeds mixed with other small grains
	• herbicide treatment	• hoeing
April–May	• herbicide treatment	• hoeing
May	• herbicide treatment	• hoeing
May–June	• spraying twice minimum	• spraying 2–3 times
June–July	• spraying in case of epidemic (once or twice)	• spraying
July–August	• harvesting by combine or special harvester and threshing	• cutting by hand, separation by hand
	• cleaning, separation and storage	• pre-cleaning, cleaning
		• packing and storage

3 LAND AND CLIMATIC REQUIREMENTS

Poppies can be successfully grown under various ecological conditions. The required temperature is 20–22°C which is ensured in the majority of European areas. Using suitable cultivars, the amount of daylight may not be a limiting factor. However, the amount and distribution of precipitation limits the range of cultivation areas. For the poppy the greatest demand for water occurs at the times of shoot elongation and capsule development and in some cases artificial irrigation becomes necessary. On the other hand, too much rain can be unfavourable during flowering and capsule ripening, decreasing both dry matter yields and alkaloid content In humid regions of the temperate zone damage from plant pathogen fungi can also be a problem. Wind can also cause damage to poppy crops. In the very early developmental stage, soon after germination, the small plants may be driven out while fully developed plants may be broken or blown down before ripening.

The poppy also has special demands on the soil type, preferring a neutral or slightly alkaline pH, and clay—loam soils of high fertility. The most suitable soil types are: chernozem, forest and washland soils of neutral character (Földesi, 1994).

In the temperate zone, poppies can be successfully grown when planted after several other different crops. The most important characteristics of the preceding crop are that it should leave the soil weed-free and with sufficient nutrients. It should be an early harvest crop which provides enough time for soil preparation before sowing the poppy seed. Papilionaceous annual and manured, hoed species are considered to be the best preceding crops. However, under European conditions poppy is most frequently cultivated between two cereal crops (Mórász, 1979).

The new problem of herbicide sensitivity has been described recently (Hörömpöli, 1995): triasine and carbamide residues and other compounds can accumulate in the soil and harm the crop.

4 PREPARATION OF SOIL AND FERTILIZATION

Because of the more widespread cultivation of spring varieties, the agro-technology used for this type is detailed, while differences concerning the growth of winter poppies are briefly mentioned as they occur.

As poppy seeds are very small, careful soil preparation is required. After harvesting preceding crops, continuous cultivation of the soil is necessary to keep it free of weeds. Deep ploughing—maximum 22–29cm (Mórász, 1979) or minimum 15–18cm (Popov *et al.*, 1971)—is carried out until the beginning of October, which is followed by smoothing until a properly compacted soil is attained. In this way, by early spring no further deep soil preparation is required and sowing can be carried out at the right time after preparing well pulverized seed beds with a light roller or harrow. This is one of the main prerequisites for optimum plant stand. Although there are references in the literature to the application of spring harrowing (Shulgjin, 1969) it is generally thought that too much soil preparation in the spring is unfavourable.

In small-scale cultivation the same soil conditions are obtained by the using methods and means available locally. In the case of the winter poppy all these operations are again required, but the time period for soil preparation is much shorter because the pulverized seed bed must be ready for sowing at the beginning of the autumn.

Fertilization requirements depend on the soil type, preceding crop, etc. Because of the short vegetation period of poppy, easily absorbed nutrients are used. Farmyard manure is generally avoided because of its favourable effect on weed growth and because it generally lowers the efficacy of herbicides in weed control. Manure is more suitable for use on the preceding crop.

Many factors have to be considered in the application of fertilizers and the optimum dose varies from site to site and is thus characterized over broad intervals. The main macro elements are added in the following dosages, calculated in terms of active agents: nitrogen, 140–160kg/ha; phosphorous 70–110kg/ha; potassium, 80–100kg/ha. Nitrogen is the most effective nutrient. It has been noted, however, that in certain cases high doses of nitrogen may harm the plant and application rates above 60–80kg/ha are not recommended in these cases (Ruminska, 1973). Nevertheless, higher nitrogen rates are required to obtain economical yields under intensive cultivation methods and under irrigated conditions increases in nitrogen may not be effective until 200–240kg/ha doses are applied (Földesi, 1994). The contradictory observations concerning the effectiveness of nitrogen might be a result of differences in both soil type and nitrogen reserves existing in the soil (Dachler, 1990). According to Földesi (1992), a crop producing 3500kg/ha vegetative mass and 1200kg/ha capsules with seeds had been supplied with 102.5kg/ha nitrogen, 192kg/ha phosphorous and 11.4kg/ha potassium. Recently, in open field experiments the effect of phosphorous was shown to be very important: its increased supply resulted in higher straw and alkaloid yields

(three-to fourfold), while nitrogen and potassium had only a moderate effect (20–40% increases) (Földesi *et al.*, 1987). In some regions granulated superphosphate is applied simultaneously during sowing to accelerate the growth and development of seedlings (Shulgjin, 1969). The effect of potassium has hardly been studied in open field conditions and, according to Bulgarian practice its application may even be omitted (Popov *et al.*, 1971).

Boron should also be mentioned as one of the most important micro elements. Generally, an amount of 25–30 kg/ha ensures the proper development of poppy plants. According to Bulgarian results the application of boron with Zn, Mn and Cu had a more beneficial effect, increasing yields by 28% (Popov *et al.*, 1971).

As spring poppy is sown as early as possible, it is recommended that the total fertilizer dose is given during the autumn plough to ensure permeation throughout the soil. However, on very light soils, where water passes through easily, the nitrogen dose may be split and one half or one third of it can be given in the spring.

In the case of the winter poppy, the addition of phosphorous is recommended before sowing at the time of soil preparation, while nitrogen is mostly used as a top-dressing with one third of the dose given in March and two thirds in April.

5 SOWING

One of the conditions for successful poppy production is an optimal time of sowing. In Hungary sowing should be carried out from February until the end of March, generally depending on the weather and soil conditions. Further to the North, for example in Poland and Germany, sowing is postponed to the second half of March until the middle of April. In southern parts of Europe both autumn and spring sowing is carried out. In general, poppy should be sown as early as possible in the spring, not later than the early grain crops of the given region.

When the surface of the soil is dry and its condition is suitable for preparing a very fine pulverized seed bed, poppy seed can be sown. Spring poppy is not sensitive to moderate frosts.

Winter poppy is sown in the second half of September, so the seedlings should reach a four-six leaf rosette stage before the onset of the low temperatures of winter. If the plants are in an earlier development stage at that time, their chances of overwintering become more uncertain. In Bulgaria the optimal sowing time is considered to be between 20 September and 1 October, immediately before sowing winter cereals. From the data of Popov *et al.* (1971), this method could produce three to five times higher yields than those of spring-sown seeds in Bulgaria.

In the former Soviet Union sowing was carried out in the autumn with the aim of getting the seeds into the soil but without them germinating before winter frosts. In such cold regions poppy plants are not able to overwinter, but this method of sowing may accelerate development in spring. In this case sowing takes place in the second half of October to the beginning of November (Hotin *et al.*, 1967).

High-quality propagation material consists of 99% poppy seed with the amount of

seeds of other species being less than 100 pieces/kg. The germination capacity then reaches 90% and the water content does not exceed 9% (Földesi, 1994).

The quantities of seed used vary over a rather wide range and depend mainly on the soil preparation and cultivation technology. On average, 300000–400000 individual plants are required per hectare for optimal development and production (Földesi, 1992). As a consequence of naturally occurring limiting factors, this number of plants can be ensured if about twice as many seeds are sown (800000–1 000000). According to the cultivation technology, this plant density can be achieved by using different row and plant spacings. A row separation of 25–40cm has generally been proved to be the optimum. However, on soils infested with a large amount of weeds and where the application of herbicides is limited, an increased row separation of 45 cm—to ensure that mechanical weed control can be undertaken—is generally advised.

There is some contradiction between the optimum number of plants required per unit area and the amount of seed that has to be sown. Because of the small size of poppy seeds, only relatively great doses, 2.0–3.5kg/ha—depending on purity and germination capacity—are able to provide a satisfactory plant stand. The germination power of single seeds is also quite poor so a higher seed density is necessitated. In this way the number of plants becomes much higher than is considered optimal for shoot formation, flowering and capsule formation and as a result of this contradiction thinning has become one of the basic elements of poppy cultivation.

In small-scale cultivation thinning does not usually present an enormous problem. The labour of thinning has been reduced by the use of special sowing methods, i.e. mixing the seeds with small grains of inert material, e.g. semolina, bran, sand. In experiments carried out in the 1950s and 1960s, some seeds were killed by heating (Mórász, 1979) and these sterile poppy seeds were used for mixing. However, because the treated grains did not germinate at all, the sprouting of the viable seeds also became uncertain. As a result of the poor germination rates the number of individual plants became much less than the optimum, and the whole stand had to be frequently ploughed off.

For reducing the labour requirements of this procedure and making cultivation more profitable on a large scale, special methods have been developed with the same goals in mind—ensuring good germination without sowing a large quantity of seed which necessitates thinning afterwards.

A method using an irradiated poppy seed mixture was described by Földesi (1972) and this became one of the most widespread technologies applied in large-scale cultivation (Mórász, 1979). According to this method the propagation material consists of 80% irradiated seeds and 20% non-irradiated (viable) seeds. A well defined radiation dose is used which damages the embryos but does not kill the seeds. The irradiated seeds do not lose their germination capacity totally, rather they are an aid to the germination of the viable seeds and contribute to form a complete stand (Figure 2). The irradiated individuals do not form leaves, they die prematurely and thus thinning is unnecessary. Perfectly homogenous mixing of the irradiated and viable seeds is very important and can be ensured by using standardized seed material for preparation of both components of the mixture. The seeds used for mixing must be from the same cultivar to avoid problems which may arise if some of the irradiated seeds remain viable. The seed rate used with this

Figure 2 The use of an irradiated seed mixture for sowing is one of the most widespread technologies applied in large-scale poppy cultivation in Hungary. The irradiated seeds do not lose their germination capacity totally; they aid the germination of the healthy ones but die prematurely making thinning unnecessary

method is about 3.0kg/ha but on soils which crack easily, a 15–20% increase in this rate is suggested; winter poppy seed should also be sown at a slightly increased dose. In Bulgaria, up to 40–50% more seeds are advised for autumn sowing (Popov *et al., 1971*).

Seed dragees, as used in the propagation of several crops, especially vegetables, can also be used for poppy cultivation (Földesi, 1994). The dragees consist of two or three seeds, because of the weak germination power of single seeds. They can be sown by precision machines at a rate of 7–10kg/ha. About 50–60% of seeds will germinate to give an optimal plant density. Application of this method requires high water saturation in the soil at the time of germination.

In practice, the sowing depth can be between 0.1 and 1.5cm, but with optimal soil conditions a depth of 0.5–1.0cm gives the best results. In very loose soils seeds are sown at a depth of 2–3cm; this is common practice mainly in south eastern parts of Europe (Shulgjin, 1969).

Rolling is an important operation which can be performed both before and after sowing. In some cases, for instance when the soil is frozen under the surface, rolling

seems not to be necessary before sowing. The type of the roller is chosen according to the soil quality.

6 PLANT CARE

Poppy germinates and comes up within 10–14 days of sowing. During this period cracking of the soil surface may severely hamper or even stop this process and thus soils liable to cracking have to be loosened regularly. In early spring in the case of the winter poppy, rolling with a light roller is necessary if the soil structure of the plantation is frost damaged.

If the plantation is set up using traditional methods, the poppy stand should be thinned when the plants are at the 4–6 leaf stage. On smaller fields or in high-density stands thinning has to be repeated twice (at the 2–3 leaf and 5–6 leaf phases), but this procedure requires a lot of labour and costs can reach a third of the whole production expenses (Ruminska, 1973; Földesi, 1992).

6.1 Plant Density and Thinning

The optimum plant density should be reached in order to get optimal yields. Plants at a distance of 3–4cm apart are capable of developing one capsule per plant, but those spaced 10–20cm apart may produce 3–4 capsules; higher capsule numbers per plant are, however, dependent on many other climatic and agro-technical factors. Muchova *et al.* (1993) proved that a significant positive correlation exists to a certain extent between seed yield and the number of plants per unit area. The optimal plant separation can be determined according to the cultivar characteristics and the harvesting method. In cases where combine harvesters are used, a uniform plant height is required; this can be achieved by using a higher plant density but this also results in lower capsule numbers per individual.

Special machines to mechanize thinning have been proposed but their use is thought unlikely to become widespread because their effectiveness is uncertain in the diverse ecological and biological circumstances of poppy cultivation in the temperate zone (Mórász, 1979).

In the 1950s and 1960s thinning was attempted by harrowing the rows in cross directions and experiments using specially designed machines were also carried out to reach this aim. According to Bulgarian practice, the optimal width of the strips proved to be 15–16cm (Popov *et al.,* 1971). However, this technology was not able to produce optimal plant densities and yields hardly exceeded those obtained without thinning. Another method, applied in Hungary, was thinning the poppy stand by spraying with a herbicidal preparation (Mórász, 1979). Plants were killed off in 15cm wide strips. According to further experiments the two above mentioned methods could be applied in parallel (Budzynski, 1986). However, by using these thinning methods small bunches of plants still remained, the utilization of space by the plants was not optimal and some manual work was required afterwards to manage the proper plant standing.

Winter poppy crops are thinned later on in their development stage than the spring types. They are thinned at the 6–8 leaf stage which is only reached in spring, depending

on geographical region, e.g. in Hungary it is during the second half of March and in Bulgaria it is February or the beginning of March.

6.2 Irrigation

Irrigation may be necessary on very loose soils or regions where precipitation levels are extremely low during sprouting, at development of the leaf rosette and at budding. According to Shulgjin (1969) the usual quantity of water used is 800m^3/ha, which should be applied when the soil moisture content falls below 70%. In southern European conditions, e.g. in Bulgaria, spring-sown poppy is irrigated regularly— three or four irrigations are recommended in the phases of leaf rosette and stem development and in the budding stage; however the amount of water used per irrigation is much lower than that mentioned above (300–400m^3/ha). The usual method in these areas is furrow irrigation. However, the majority of poppy cultivation in the temperate zone is managed without irrigation or it is only applied in extremely dry years because of economic considerations.

Under irrigated conditions top-dressings of about 20kg/ha nitrogen (ammonium-nitrate) in the phases of rosette formation and budding and 20kg/ha magnesium chlorate at the start of flowering have been proven to be beneficial (Shulgjin, 1969)

6.3 Weed Control

The growth of poppy is very slow in the early stages and the small plants are unable to compete with weeds. After germination, when rows become visible the weeds should be removed by hoeing. By using only mechanical methods, weeding may be needed three to five times during the vegetation period or even until the budding— flowering period.

In large-scale cultivation effective weed control is one of the most important elements of cultivation technology. Weed control in these conditions is based on herbicides, although poppy is rather sensitive to these chemicals. Experience has shown that effective weed control cannot be based solely on preparations which have long-term effectiveness—complex technology is necessitated, which depends on the development of the poppy plants, the spectrum and density of weeds and weather conditions.

Herbicide control begins within three days of sowing. For pre-emergent treatment until the last stages of development nitrofene (8–101/ha) was the most widely used agent, but its effect only lasts for a few weeks and is strongly dependent on the weather. Recently, chlortoluron (1.0–1.5kg/ha) can be mentioned as the most frequently used herbicide at this stage of poppy growth, but several other materials have also appeared, e.g imidasolin (0.3–0.41/ha), nitrofen, buminafos (Pank *et al.*, 1987). This treatment should ensure a clean stand for 4–6 weeks.

If strong or resistant weeds develop during this period, diquat (2.5–31/ha) spraying may be necessary and this destroys the weeds by singeing the leaves. The application of diquat is possible when the poppy has at least 2–4 leaves until the 8–10 leaves has formed. The aim of this treatment is to keep the plantation weed-free from the first pre-emergent herbicide application until the time of the second post-emergent treatment.

The second post-emergent herbicide application may be carried out after the poppy plants have developed at least 8–10 leaves when several different compounds are applicable. The aim of the second post-emergent herbicide treatment is to kill existing weeds and prevent the further germination of both monocotyledon and dicotyledon species. The application of chlortoluron is very commonly used to reach this goal in 2 kg/ha doses. The utilization of many other compounds have been reported: pyridate (3–4 kg/ha), fluroxipir (1–1.21/ha) or fluazitop-butyl against monocotyledonous weed species (Zemanek and Mikulka, 1986); and diclotop-methyl, flamprop-isopropyl (Pank and Buhr, 1987). Combinations of chlortoluron+asulum (8–101/ha) and chlortoluron+metolachlor (1.5–2kg/ha) preparations are also applicable according to the weed spectrum, and can give a better result than single chemicals (Földesi, 1982). The poppy stand can be treated with appropriate herbicides during the whole vegetation period, even in the budding and flowering stages, when the tolerance of the poppy increases more quickly than that of some dangerous weed species, e.g. *Chenopodium, Amaranthus* (Nagy and Földesi, 1989). However, such a late treatment seems to be necessary only if the large amount of weeds endangers the harvesting process.

In the case of the winter poppy a post-emergent treatment is generally necessary in the spring. This can be repeated once, but the fallen weed leaves usually cover the soil quickly, preventing further weeding by acting as a mulch (Figure 3). The same chemicals as those applied to spring-sown crops can be used.

Figure 3 Differences in the development of 'winter' and 'spring' poppy stands in the second half of May under Hungarian cultivation conditions; the poppies sown in the autumn are in full blossom, while the spring stand has just finished rosette formation

At the application of herbicides, the slight singeing seen on poppy plants does not usually cause a decrease in the yield. Damage to plants can be minimized by lowering the drop size of the spray to 250–300µm and increasing the volume used to 300–600 1/ha. The occurrence of singeing is more frequent in rainy conditions which tend to thin the waxy layer of the leaves. The waxy layer begins to thicken 3–4 days after rain, so further herbicide application is beneficial.

In herbicide technology, pre-emergent treatments are the most important, while post-emergent applications are often substituted by manual work.

Herbicides may not be combined with insecticides or fungicides, which have a different action. If mechanical weed control is also undertaken, the condition of the plants must be considered carefully because damaged leaves are more susceptible to harm from herbicides.

Weed control is thus a crucial component of cultivation technology. If it is not performed correctly the crop may be spoiled and harvest may have to occur at a non-optimal time and in a non-optimal manner; infection by fungi may also increase and seeds and capsules cannot be harvested and cleaned afterwards.

7 PLANT PROTECTION

Unfortunately, poppy may be attacked by a number of parasites and pests during the vegetation period. The most dangerous insect in poppy plantations are weevils (*Ceutorrhynchus macula-alba* or *C. denticulatus* in southern regions) which chew the leaves and other plant parts and lay eggs in the capsules. The larvae leave the capsule by chewing through the wall. Latex emanating from and drying on the capsule shows the presence of weevil. The most effective way of protection is early sowing, so that the poppy plants can reach flowering before the multiplication period of the weevil. In some cases hexachlorane preparations can be introduced into the soil to kill the weevil larvae (Shulgjin, 1969). The most usual method in Central Europe is preventive protection with insecticides at the time of stem emergence with aminophos-methyl, furathiocarb, dimethoat, diasinon or malathion containing insecticides. Efficient protection against the imago can be maintained up to flowering by applying, for example, metileparathion powder. Treatment is applied around the poppy field in 10–20cm wide stripes to prevent the migration of the insect. Maximal damage occurs during flowering, but at this time only bee-safe and less efficient preparations (e.g. dioxacarb, endosulphane, pyrethroids, bensultap) may be applied; this can be done by spraying from an aeroplane in the case of large-scale cultivation.

Poppy fly *(Dasyneura papaveris)* and poppy gnat *(Perrisia papaveris)* are parasites which use the holes made by weevils for laying eggs in the capsule. In certain years plant lice *(e.g. Aphis fabae)* can also create significant damage. Protection against them is via the use of universal agents. Under certain conditions soil parasites can cause severe harm and protection against such pests necessitates soil sterilization, e.g with diasinon, before sowing.

Diseases of the poppy are increasingly damaging in rainy weather. Treatment of the seed before sowing is useful against some fungi (*Fusarium* spp., *Ervinia*

spp., *Helminthosporium* spp.) and is also part of the protection against peronospore *(Peronospora arborescens),* which is the most harmful disease to poppy. The first symptoms are light spots on the surface of the leaves which then begin to grow and form a mould layer. In severe cases this disease may deform flowers and capsules, lower yields or even kill the plant. Apart from seed treatment, spraying with copper-containing preparations may be necessary after the appearance of the symptoms and the treatment should be repeated until the danger of further infection exists. Powdery mildew *(Erysiphe communis)* damages firstly the stem, then attacks the leaves which begin to blacken. The damage is more severe when both precipitation levels and temperatures are high. Later sown plantations are mainly affected. Sulphur-containing preparations are the most useful for protection and should be applied at the time of the first appearance of the disease. As a secondary pest, smut-mould (*Apiosporium* spp. or *Microsphaerella tulasnei*) can often appear in the form of a black layer on damaged capsules. It occurs in connection with lice, so protection against them, as well as spraying with copper-containing preparations or mancoceb and an optimal harvesting time can moderate the injury.

In certain cases some other parasites and pests which necessitate action, for example *Ceutorrynchus denticulatus, Stenocarus fuliginosus, Clinodiplosis papaveris, Timaspis papaveris, Opatrum sabulosum, Ervinia carotovora, Entyloma fuscum,* can occur in poppy crops.

Because of the waxy layer on the poppy leaves, the adhesion of insecticides and fungicides may be strengthened by moisturising agents.

Winter poppy is almost never affected by weevils, because of its earlier flowering and capsule development. This also means that secondary parasites cannot cause significant damage. However, protection against fungi may be necessary and should be applied in similar ways as the above described methods for spring poppy.

The methods used for the protection of poppy against diseases and parasites in large-scale cultivation are summarized in Table 2 (Földesi, 1994).

8 HARVESTING AND POST-HARVEST TREATMENT

In the temperate zone poppy is usually cultivated for both straw and seed and it therefore has to be harvested when the seeds are completely ripe and the capsules are 'straw-yellow' or greenish yellow and crack when pressed (Figure 4). Harvesting usually takes place in the second half of July to the beginning of August in the case of the spring poppy and about a month before for the winter poppy. A delay in harvesting can cause problems, e.g. increased likelihood of leaning, seed and capsule breakage, weed infection, alkaloid loss and mould growth as a result of rain. The optimum conditions for harvesting are dry weather, after dew.

The simplest method, which also ensures the best quality harvest, is to cut the heads by hand. They are cut with an 8–10cm long stem. This is also the method universally used in small-scale cultivation. In certain cases whole plants are pulled out, bound into sheaves and allowed to ripen fully and dry. Thereafter all the material is threshed or the capsules are cut and threshed.

Table 2 Plant protection methods used in European spring poppy culture

Phenophase	Time period	Pest or parasite	Active agents used	When applied
6–10 leaf stage	beginning of May	plant lice	dimetoate, parathion, pyrimicarb	at first appearance
Green bud stage	2nd half of May	weevil	dioxacarb, phosmet, methylparathion	1–2 insects/m^2
	beginning of June	*Peronospora, Helminthosporium*	mankoceb, propineb, cuprochelate	epidemic
Flowering	2nd half of June	weevil	dioxacarb	1–2 insects/m^2
		poppy fly	endosulphane	
		plant lice	deltametrine	
		Peronospora, Helminthosporium	propineb	epidemic
Capsule growth	July	weevil	dioxacarb	1–2 insects/m^2
		poppy fly	deltametrine	
		Helminthosporium	mankoceb	epidemic

Figure 4 In the temperate zone poppy is harvested when the seeds are completely ripe and the capsules are 'straw-yellow' or greenish yellow and crack when pressed

Two methods of mechanized harvesting are also practised.

(i)   A special harvesting adapter was developed in Hungary, which can be assembled on universal mowers and cuts the capsules with a 10–20 cm long stem. After cutting, the separation of capsules and seeds is carried out at the farm centre, where the crop can be stored in heaps until the time of processing.

(ii) A common method is to harvest and thresh using a cereal combine harvester. Poppy heads with 10–20cm long stems are cut by combine harvesters which are modified in certain parts so as not to damage the seeds. A clean, weed-free poppy stand is required for this method. After harvesting the capsule parts, green weed fragments and other impurities have to be immediately separated from the seed. A better efficacy of harvest can be reached by singeing the plantation with herbicide 6–8 days before harvesting and this is particularly effective in rainy and cooler regions or years. With the use of this singeing method harvesting can take place 5–7 days earlier and the moisture content of the harvested capsules is considerably lower (Földesi, 1992).

There are advantages of combine harvesting—labour and costs are reduced—but several disadvantages must also be mentioned, such as a higher proportion of damaged seeds and the loss of seed and capsules during the harvesting process.

Separation of the seeds from the capsules and other plant parts is carried out in different ways. After hand cutting, the seeds are also taken from the capsules by hand. The seeds can also be separated from the capsules by threshing machines and it is possible to do this by using machines originally built for other purposes, but which can be adapted to ensure an optimal quality.

The threshed material is separated further and the seeds are cleaned using different grades of sieves. When separation and cleaning is not carried out properly, capsule residues—in the form of very fine dust—create severe problems. Such contamination may result in measurable morphine traces on the seeds which, in Hungary for example, may not exceed a level of 20 mg/kg (Földesi, 1994).

After harvesting and separation both the seeds and the capsule straw have to be stored properly. Capsules are at first stored in 5 cm thick layers and turned over every day. In general, artificial dryers are not necessary, but natural air ventilation is advised in wet weather conditions. After some days the product is packed in into sacks. The required water content of the capsules at this point is 10–12%—it cannot be stored if this value is over 14%. A precondition for long-term storage is a water content of the seeds of about 9% (Hörömpöli, 1995).

Yields vary quite considerably and are between 0.3 and 1.2t/ha for seeds and 0.5–1.0t/ha for capsules. The winter poppy has slightly higher yields than the spring types.

The quality requirements of seeds and capsules are regulated by national and industrial standards.

9 CHARACTERISTIC CULTIVARS

In general, the capsules of the oil poppy have much thinner skins and less developed latex vessels than those of the opium poppy. In each country the main characteristics of the cultivars reflect the ecological conditions and product orientation of the area. The aims of breeding special types are therefore varied: high or very low alkaloid content according to the main product; special alkaloid composition in the case of pharmaceutical applications; high seed and oil yields for alimentary uses; resistance against frosts or pests; winter or spring ecotype characteristics, etc.

The more frequently used cultivars in poppy farming under temperate conditions and some of their main characteristics are as follows.

'Kompolti M'. Hungarian cultivar, strong growth, height 11–140cm, 2–3 capsules which are elongated. Vegetation period is 120–130 days. The flowers are white with a violet base. The seeds are light blue. Morphine content: 0.6–0.8%.

'Kék Duna'. Hungarian cultivar, medium height of 110–120 cm. Capsule number reaches 3–4. Vegetation period and flowers are similar to variety described above. Capsules have an elongated pea form and are ribbed. It is also a morphine-producing variety, but its resistance to pests and drought is better. Morphine content: 0.6–0.7%.

'Kék Gemona'. Hungarian cultivar producing narcotine (and morphine), 90–100cm in height, middle-early ripeness. The flowers are white with a violet bottom. The seeds are blue. Morphine and narcotine content: 0.8–1.4%.

'Monaco'. Hungarian codeine (morphine) chemovariety. Vegetation period is 110–120 days. Stem has 2–4 offshoots. The seeds are greyish dark blue. Morphine content: 0.8–0.8%; codeine content 0.5–0.7%.

'Novinka 198'. Ukrainian variety, middle ripening, drought resistant. Morphine content: 0.6–0.8%.

'Kozmosz'. Hungarian winter poppy. Characterized by intensive growth. Its height reaches 130–160cm and has 3–4 capsules. Flowers are light-violet, with a dark spot at the bottom. Capsules are big, ribbed, semi-cone or cone form. Seeds are greenish blue. Morphine content 0.4–0.5%.

'Edelweiss', 'Edelrot'. Austrian varieties, developed from **Zwettler Graumohn;** differences exist only in the colour of petals (white and red). They are special cultivars for seed; oil content reaches 42.5%. The seeds are big and the capsules have an anthocyanin colouring.

'Marianne'. Dutch variety with white flowers; the capsules (2–3 per plant) are small and closed; seed yield is high.

'Dubnik'. Slovakian hybrid variety (**KM 52–2598XMarianne).** Vegetation length is 135 days, height 127cm, white flowers, high capsule yield potential (>1.6t/ha).

'Gerlach'. Slovakian hybrid variety (**CH-47XMarianne).** The flowers are white with a violet bottom. Thousand seed weight reaches 0.6g, yield up to 2.0t/ha. It is relatively resistant to pests.

'Albin'. Slovakian variety, height 130cm, pink flowers, seeds are white and big (thousand seed weight 0.7g), yields are high (1.4t/ha).

'Amarin'. Czech variety, morphine content: 0.69%.

'R1', 'R8'. French varieties, morphine content: 0.68–0.74%.

'Extra 2'. Rumanian variety, morphine content: 0.5%.

'Niebieski KM', 'Modry'. Polish varieties.

'Rosemarie'. Dutch variety.

'Flora', 'Soma'. Swedish varieties.

'Reading'. British variety.

'Sanofi'. French variety.

'Mahndorfer', 'Nenga', 'Pilot'. German varieties.

'Parmo', 'Luna', 'Lori', 'Löfti'. Danish varieties.

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2. CULTIVATION OF POPPY UNDER TROPICAL CONDITIONS

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1 INTRODUCTION

Although the gene centre of the opium poppy is identified as the Near East and Middle Asia, the plant can be successfully grown in such diverse areas as most parts of Europe, Asia, North and East Africa, South America, Australia, etc. In tropical countries the plant is mainly cultivated for its opium (Singh, 1982). The seed, which is considered to be a by-product, is also utilized in cooking and is considered to be a good source of oil and protein.

The main product, however, is opium, which is the sun-dried latex of the unripe capsule. It is obtained by lancing the capsule and is formulated afterwards by a simple technological procedure. From the practical point of view five alkaloids of main importance can be distinguished in opium—morphine, codeine, thebaine, narcotine and papaverine. Opium on a dry basis contains 9–14% morphine, 0.7–2.5% codeine, 0.3–1.5% thebaine, 5.5–11.0% narcotine and up to 1% papaverine (Madyastha and Bhatnagar, 1982). The ratio of alkaloid content may vary from country to country. For instance, Indian opium is richer in codeine as compared to the codeine content of opium produced in more temperate climates. There are also significant differences in composition of opium produced at the same location depending on the actual climatic conditions experienced in a certain year. From the data of Kaicker *et al.* (1978) the morphine content of opium can even reach 18–20% in the Delhi region due to extended ranges of temperature, as well as a higher relative humidity. Bernáth *et al.* (1988) also noted the importance of the origin of the plant material in the composition of opium.

2 LICIT PRODUCTION OF OPIUM IN INDIA

2.1 Main Production Areas

From the data of Bryant (1988), 800–1000 tonnes of opium are produced in India yearly. This amount of opium produces about half of the world's annual morphine demand. There is no real competition to Indian opium producers, because India remains the only country in which the cultivation of poppies for opium is still legal. In previous decades other countries were active in the production of opium, such as Turkey, Iran, Yugoslavia, Romania, Bulgaria and southern regions of Russia. In these

countries no legal opium production is carried out nowadays, however, the production of poppy seed and straw is legal in some cases.

The first records on the cultivation of the opium poppy in India date back to the 15th century (Kohli, 1966). At first it was cultivated along the sea coast and penetrated into the peninsula afterwards. During the Moghul Empire the production of opium became of great importance and was a valuable means of trade with China and other countries. In the second half of the 18th century the East India Company took the rights for controlling opium production, especially in Bengal and Bihar, which afterwards went into the hands of the British Governor. From that time until India's independence, the British authorities controlled all the fields of production, processing, distribution and sale of opium.

Since 1st April 1950 the Indian Government took control of both poppy cultivation and opium production businesses. A central organization—The Narcotics Commission—was established to unify and rationalize the control system throughout the country. Some important aspects of this policy were to restrict the production of opium to the quantity actually required for export for medicinal purposes and to concentrate poppy cultivation areas to the regions where traditional producers were based. These regions were the States of Uttar Pradesh, Madhya Pradesh and Rajasthan where the yield is maximal and proper control over production could be exercised.

2.2 Method of Cultivation

The production of opium is a legal procedure in India, where its strict control is governed by The Narcotics Commission. Annual licences are issued to the growers, based on the market possibilities of the actual year whilst avoiding illegal production and trade. The cultivation is thus restricted to eleven districts of the above mentioned states. The cultivation area was 63 885ha in 1977–78, which decreased to about 40 000ha in 1984. The present cultivation area is not larger than 24 500ha (Gupta, 1984; Bryant, 1988).

2.2.1 Selection of the Land

From practical and scientific experience the opium poppy needs deep, clay—loam soil of high fertility. The soil should be rich in organic matter and nutrients. At the same time a good water supply is required which can be obtained using irrigation facilities. The optimum pH value is around neutral, between 6.0 and 7.0. To obtain the required soil conditions, adequate manuring and fertilization is needed, especially on sandy-loam lateric soils. The climate of the land has to be moderately cold (20°C), with adequate sunshine in the vegetative growth phase. However, in the reproductive phase the optimum temperature is much higher (30–38°C) and adequate sunshine is more crucial. Cold, cloudy, windy weather in this phenophase could have an adverse effect on the crop, reducing the flow of latex after lancing. Heavy rainfall in this phenophase can ruin the crop, leaving only a little or even no latex for collection. The opium poppy cannot endure extreme cold. Frost, which sometimes even occurs in the Indian climate during the time of the vegetation cycle, can be disastrous to the crop. Dull, cloudy or rainy weather reduces not only the quantity, but also the quality of opium (Singh, 1982).

The yield of the opium poppy crop can be increased by choosing a suitable preceding crop (Jain and Solanki, 1993). The latex yield was found to be 9.12 and 10.14% higher when poppy was grown after legume crops *(Phaseolus mungo, Arachis hypogea)* respectively in Rajasthan. Growth of a poppy crop after maize *(Zea mays)* resulted in a much lower yield. Based on local experience, Singh (1982) states that crop rotation, though necessary, is seldom practised in India. Opium poppy crops are grown in the same field year after year, carrying over pathogens and resulting in diseases of the crop.

2.2.2 Plant Material

The plant material which is cultivated in India is commonly known as opium poppy. It is a plant of about 120cm in height, with brightly coloured flowers. The form of the capsule is oblong to globose and is filled with white flat seeds. The length of the vegetation cycle usually varies from 140 to 160 days. However, many different types of poppy are known to be grown in India. Early investigations (Singh, 1982) attempted to describe three main varieties as follows.

(i) *P. somniferum var. nigrum* D.C. is a semi-naturalized form with purple-red flowers and roundish oblong open capsules. The colour of the seeds is greenish-black and they are a good source for oil production.
(ii) *P. somniferum var. album* D.C. has open white flowers. The capsule is round-ovate and has no open pores after ripening. It is widely used for opium production.
(iii) *P. somniferum var. normale is* characterized by small flowers, which are streaked with green and red; the petals are crumpled and never expand fully. The capsule is oblong-roundish with open pores when ripe. It is an ornamental type.

Poppy populations cultivated for opium can be distinguished on the basis of colour and texture of the capsules and are usually known by their local names. There are about 20–25 different forms which are known as cultivated types in India (Singh, 1982). The characteristic features of some of the cultivated populations of great importance and those of new selected races are as follows.

'**Telia**' is an early flowering type with a short vegetation cycle (140 days). The flower is a shade of pink and the form of the capsule is oblong-ovate.

'**Kutila**' possesses bristles on the flower stalk and the form of the capsule is oblongovate, having reddish-coloured latex in the capsule at the time of lancing.

'**Kaladanthi**' possesses an oblong-ovate glaucous capsule with a bluish black flower stalk. The latex is slightly reddish at the time of lancing. Opium production from this race is high.

'**Galaria**' is about 120cm high with white flowers coloured by dark pink borders. The capsule is round-oblong, 7.5cm×6.3cm size. The latex is light brown at lancing.

'**Ranjhatak**' is a cultivar of medium height with a moderate vegetation period. It has white and light pink flowers. The capsule is elongated and slightly flattened on the top and is mature for lancing 125–130 days after sowing.

'**Dhola Chota Gothia**' is a cultivar that can be characterized by restricted growth and short vegetation period. The plants are 85–90cm in height and form pure white flowers. The shape of capsule is oblong-ovate and is ready for lancing 105–115 after sowing.

'**MOP 3**', '**MOP 16**', '**IARI 1**', '**IARI 2**', '**NBG 1**', have been selected for increased latex and seed yields and better ecological adaptability (Sethi *et al.,* 1990).

2.2.3 Preparation of the Soil and fertilization

The cultivation of the poppy starts after the rainy season in India. After the harvesting of previous crops, e.g. maize, chilli, peanut, etc. the field has to be ploughed and planked. The land is separated afterwards into smaller fields to provide suitable conditions for watering during the vegetation cycle.

Manures and fertilizers are added at the time of soil preparation. The farmers prefer to apply 10–20 tonnes/ha of farmyard manure which helps to maintain the soil in good physical condition and accelerates the initial and vegetative growth of the plant. Organic manures are applied by broadcasting over the field. Application of half the quantity of the manure across the field and the second half alongside, helps ensure a uniform distribution of manure. The effectiveness of nutrients has been proved by both farmers and nutritional experiments (Sharma, 1978; Jain, 1990a, b) and the application of such nutrients varies depending on the soil conditions, cultivation area and the preceding crops (Jain and Solanki, 1993). On average 30–50kg of P_2O_5, and 25–30kg K_2O dosages are put into the rows in 5–6cm deep, before sowing. The application of nitrogen is quite different. The amount used varies between 60 and 90kg/ha and the dosage is divided into three equal parts. The first dose is given at sowing, and subsequent doses are given 30–40 days and 60–65 days after sowing. However the actual dose depends on the circumstances of cultivation which are affected by climatic conditions (Atal and Kapur, 1984). In the region of New Delhi, both morphine and opium yields doubled with the application of 50kg N/ha (Turkhede *et al.,* 1981b). A similar effect from the addition of P_2O_2 was registered up to a 21.5kg/ha dose, however the increase in morphine yield was not significant. From the results of Turkhede *et al.* (1981a) optimum nitrogen fertilization protects the plants against frost damage which occurs occasionally in February. Such frosts can have an adverse effect on the young and succulent capsule, decreasing its latex flow and ultimately the yield of opium.

2.2.4 Sowing

The seed is sown in a very well pulverized seed bed. A rate of 8kg/ha is suggested, but a 5–6kg/ha dose is sufficient, using rows 30 cm apart (or 25cm in light soils). When sowing is done by broadcasting, the seed is mixed with ash to give a uniform spread of seed in the beds. The seed is covered afterwards with a thin soil layer to ensure the right conditions for good germination. Germination normally takes 5–10 days depending upon the moisture content of the soil (Gupta, 1974).

The optimum time for sowing varies according to the cultivation region. As examples, seed sown in the second week of November gives a good crop in the Neemuch—Chittorgarh belt, while in the Eastern Uttar Pradesh region a late October sowing seems to be the optimum. In Himachal Pradesh a field experiment was

conducted by Kharwara *et al.* (1988) to determine the optimum sowing time. From their results it was concluded that sowing poppy seed in the second half of November significantly increased opium and seed yields and also increased morphine and oil contents in the seeds, compared with later sowings. Sowing after December 1st reduces the crop productivity significantly.

2.2.5 Plant Care and Irrigation

The plant grows very slowly in the first phase of its development. The right time for weeding and hoeing is about 3–4 weeks after sowing. This can be followed by irrigation. A second weeding should be carried out 50–60 days after sowing and used, combined with thinning, to get a 10cm distance between plants in the rows. The best time for thinning is when the plants are 5–6cm high with 3–4 leaves. The optimum spacing for the opium poppy is reported to be 30cm (row)×10cm (between plants) which provides a plant population of about 333 thousand plants per hectare. This procedure is followed by more weeding and irrigation depending on the state of culture.

Usually the crop need as much as 7–12 irrigations during the cultivation period. It has been established by experiments that there are some critical phases for lack of moisture. In particular, a shortage of water from the rosette stage until the appearance of capsules causes enormous losses in latex and seed yields. The data of Turkhede *et al.* (1981a) revealed a second critical phase for seed yield: stress at the time of flowering, due to the parallel translocation of photosynthetates into latex and seeds, may result in a decrease in both products. Under other ecological circumstances in Bangalore (Tomar *et al.*, 1990), seed and husk yields were found to be significantly reduced from water stress at all the physiological stages, with the exception of water stress at flowering which had little effect on seed yield. The greatest reductions in seed and husk yields (29.4% and 32.5% respectively) were obtained with water stress imposed at bud and early capsule stages. Irrigation is not used during the period of lancing and latex collection.

Herbicides can be used to save labour if necessary, however hand weeding has many advantages. From the experience of growers in India, a pre-emergent application of Asulox (61/ha) and Dicuran (1.5kg/ha) is effective in light soils, while chlortoluron (1.5kg/ha) is suitable for heavy ones.

2.2.6 Plant Protection

For good germination the land and the seed should also be treated with insecticides and fungicides before sowing. To protect plants from termites and shoot-cutting caterpillars, some insecticides can be applied to the soil at the time of land preparation. A presowing treatment of the seed by fungicides may be effective against the action of fungal infections.

Under Indian conditions the crop can be damaged by many diseases and insects. The most important of these are as follows.

(i) Downy mildew *(Peronospora arborescens)* may harm the plants in all vegetation phases. In the early form the infected plants develop curling, chlorotic abnormal leaves. The secondary infection starts when the plants are about one-month old.

Irregularly shaped brown spots appear on the leaves. At maturity the whole plant is spotted and the majority of leaves become dry. With the use of fungicides the disease can be checked, but the damage of infection can hardly be eliminated. Crop rotation and sowing healthy seeds can be an effective preventative method.

(ii) Powdery mildew *(Erysiphe polygoni)* usually appears 90–100 days after sowing. Blackish spots appear on the leaves but the infection is more severe on the stems than on the leaves and capsules. The crop can be protected by using systemic or contact fungicides. At the time of flowering the treatment has to be repeated several times in 15-day intervals to prevent spreading of the disease. Plants infected at and early stage of the vegetation period should be removed and burnt.

(iii) Root rot *(Rhizoctina bataticola)* infects the plants resulting in withering and drying of the leaves. If the capsule is infected it becomes dry prematurely, giving little or no latex yield. The infected plants have to be pulled out and burnt.

(iv) Curly leaves (resulting from viral infection) are frequently observed in cultivation. The disease is spread by *Jassids* and *Aphis* species. Virus-affected plants should be burnt.

(v) Soil nematodes, together with the action of fungi and bacteria, have an adverse effect on latex production and seed yield and cause a special disease named plant leprosy (Ramanathan, 1983). Poppy plants affected by root-lesion nematodes (*Pratylenchus* spp.) and root-knot nematodes (*Meloidogyne* spp.) show wrinkled and distorted leaves with black spots with the terminal leaves being yellow, thick and brittle. The tap roots of plants become dark and the plant dies soon afterwards. The application of nematocides before sowing may result in a satisfactory prevention.

2.2.7 Harvesting

The optimum time for lancing is a cultivar-dependent phenomenon. The Indian cultivars begin flowering 90–115 days after sowing and are in blossom for 5–6 days. To arrive at the maturity stage for lancing ('industrial maturity') about 20–25 days more are required. At lancing the capsule has reached its full size, but it is green and immature. A special knife (naka) is used for lancing, which has 3–4 sharp-edged blades fitted at a distance of 1.5–2mm from each other. A number of longitudinal incisions of 1–2mm depth are made on the immature capsule. The length of the incision should be one-third or less than the full length of capsule. Based on experience, bright sunny days are the most beneficial for a good latex yield, especially during the early afternoon hours. The latex flows out from the cut end and is deposited on the capsule surface. Four lancings are usually made on each capsule at two-day intervals. Ramanathan (1982) and Turkhede and Singh (1981) determined that opium from the first lancing has the highest morphine content and the morphine content decreases progressively in opium obtained from subsequent lancings. Lancing must be performed very carefully as a deep incision may result in exudation of latex inside the capsule, spoiling the quality of seeds. The latex which exudes through these lancings becomes thick during the cold night. Latex collection is carried out early in the morning with a blunt-edged iron scoop called a Charpala. Collection should occur before 8–10a.m., otherwise the viscosity of the latex decreases in the heat of the day and collection

becomes more difficult. The latex is usually collected in plastic containers. Using good varieties 50–60kg of opium yield is expected from one hectare. However, the average yield in India is only about half this value.

The lanced crop is left for 20–25 days on the land while the capsules reach full maturity. The capsules are then picked and spread over an open yard for drying. The seeds are separated afterwards by beating the capsules with a wooden rod. The average seed yield is about 500–600kg/ha in India.

2.3 Processing of the Opium

After collection, the raw opium is stirred and churned every day to make it homogeneous while its water content is decanted off. Farmers bring the raw opium to weighing centres, where officers also make a preliminary quality control by touch and observation. Material of acceptable quality is then transferred into bags of uniform volume.

In the processing centres, the bags are opened and a sample is taken from each bag to determine the morphine content and the water content. The bags are then emptied into large vats of 3–30 tonnes capacity. Equal smaller amounts of opium (10–35kg) are then dried in wooden trays. During the drying process the opium is stirred manually or by using mechanical equipment. The dried opium is then formed into cakes, the size and weight of which depend on market requirements. After wrapping the cakes in paper or synthetic material, they are placed in wooden chests for transportation. Four different types of opium product are well established in India.

(i)   Export opium is a product of high quality made by manual drying using solar energy as well as steam. Its consistency is about 90% pure and each lot is tested for purity and morphine content and packed into 1, 2 or 10kg packages as required by the buyer.
(ii) Medicinal opium is used as an ingredient in many pharmaceutical preparations in India, manufactured and sold as Opium IP and Opium Powder IP. The first contains not less than 9.5% anhydrous morphine and the powder contains 9.5–10.5% anhydrous morphine. Medicinal opium is sold to pharmaceutical firms holding valid licences.
(iii) Excise opium is made from raw materials and contains a lower percentage of morphine. Excise opium is sold to registered medical patients in India.
(iv) Opium alkaloids, including morphine, codeine, thebaine, their semi-synthetic derivatives, as well as their salts are manufactured in India. The products of Government Opium and Alkaloid Works Undertaking at Ghazipur (U.P.) and Neemuch (M.P.) are sold in wide ranges in India.

3   ILLICIT OPIUM PRODUCTION BY ETHNIC GROUPS (EAST, SOUTH-EAST ASIA)

3.1 Main Production Areas

Referring to the data of Bryant (1988) around 40000 tonnes of opium is produced world-wide and only 5% of it is used officially as a raw material of industrial

production. Large amounts of opium are produced and used by the inhabitants of poppy growing areas for curing, opium addiction, religious ceremonies, etc. Based on World Health Organisation (WHO) estimates, in many countries where the opium poppy is grown, substantial segments of the rural populations are dependent on opium (Smart and Archibald, 1980). The level of addiction amongst adults in these countries, including large areas of the Golden Triangle as well as areas of India, Iran and Pakistan, varies between 3% and 10%. However, poppy is cultivated in the above mentioned countries not solely because of the drug requirements of the locals themselves, but also because it is one of the few cash crops available. This situation was surveyed very carefully at the request of the Government of Thailand in 1965/1966. A United Nations (UN) team (Philips *et al.*, 1968) concluded that poppy is grown in this region primarily as a source of cash income. Some of this income is needed to buy rice, since the average family in these regions usually produces less than its annual rice needs. The remainder of the cash income is used to acquire necessary goods, clothing and 'luxuries' such as torches and watches. Opium income is also used as a form of savings. In a year of very good production the income may be enough to allow purchases beyond the ordinary. In a poor harvest, goods bought in more productive years are re-sold for cash to buy necessities. Income from opium varies greatly according to the fertility of the various areas, the climate of an actual year and many other factors.

3.2 Method of Cultivation

The majority of published information has concerned opium production by ethnic groups in the regions around the Golden Triangle, typified by the example of the hill tribal peoples of Thailand. Analyses of the situation have been carried out by several research groups supported by WHO, United Nations Division of Narcotics and other UN authorities (see e.g. Suwanwela *et al.,* 1976, 1978; Chindarsi, 1976; Geddes, 1971; Saihoo, 1963; Crooker, 1988; Crooker and Martin, 1992).

3.2.1 Selection of the Land

The economy of ethnic groups living in poppy cultivating areas is mainly based on agriculture. The main crops in this region are rice, maize, bean, gourd, melon, potato, chilli and other different kinds of vegetables.

The poppy is cultivated in the surroundings of the settlements of ethnic groups. However the actual place of cultivation is dependent on many factors. UN experts (Anonymous, 1967) determined that climatic conditions, especially the altitude of the land, are of great importance in this respect. In some tropical regions cultivation is restricted to median altitudes, about 1200m. The second criterion for selection is the terrain—it must not be too severe and must be free of erosion processes. The third criterion is that the land should be relatively fertile, which can be achieved with clearance of the original vegetation. The high alkalinity of the soil must be also be considered. The fields are abandoned when the fertility of the soil decreases after a cultivation period of three or four years. This field may then be utilized again after a resting period of 10–15 years.

The size of the cultivation area is dependent on many factors such as the structure

of the farms, the spectrum of the species cultivated, the sizes of the farming families and the amount of labour available. The size of poppy fields vary over a large interval but are generally reported to be between 1.2 and 8ha.

3.2.2 Growing Procedure

Poppy seed is usually sown at the end of August or in September. In some districts different cultivars ('Taw', 'Phi', 'Clang') are used in order to produce a longer harvestin season. Using these cultivars the planting time may continue up to October or November. The ground is prepared meticulously; thoroughly broken up and cleared of all grass and weed. Before sowing the seeds are mixed with lettuce, parsley or other small seeds. The mixture is sown broadcast and lightly covered with soil which is then pressed down using fingers. After germination the poppy field needs constant weeding. Thinning is the next step in the cultivation process, combined with the removal of other plants which have germinated from the original seed mixture.

The poppy fields generally need no irrigation in this growing area. The overall water content of the soil in forest regions, the relatively temperate climate and heavy dew at nights provide satisfactory conditions for poppy growth and development. However, the constant weeding that is required to keep the fields clean at all the time demands a lot of labour. Insects sometimes cause damage to the field, but this can be protected by watering with an infusion of tobacco leaves.

Four months after sowing flowers appear, followed by the quick development of capsules. The optimum green capsule stage for tapping starts in November and lasts till February or March in the case of late cultivars. The plant height in this phase changes between 40 and 100cm depending on the soil conditions and other ecological factors. A good crop is characterized by the number of capsules which form on individual plants. Under poor conditions only one capsule is formed, while on good fields six or more capsules per plant may be found.

3.2.3 Harvesting

In the early stages of development, young leaves of the plants are consumed similarly to the leaves of the other edible vegetables, but the consumption of older leaves has to be avoided because of their toxicity. The main product is, however, opium which is taken from the capsule by a process known as tapping. Each capsule is usually tapped only once, but in a very good areas it can be done a second time. This is a very laborious and time-consuming job, involving all the members of the families. Tapping has to be started after flowering and its effectiveness depends on many factors. Work usually begins early in the morning and continues until sunset. Bright sunny days are considered to be the most beneficial for a good resin yield. In contrast, rainfall—which is very rare during this period—can ruin the whole crop.

Special iron knives, whose form and construction vary from one region to another, are used to remove the resin from the capsule. The tapper makes two or three incisions on the capsule from which the milk-white resin slowly flows out. The resin is left on the capsule to dry for a day and is scraped off the next day into broad moon-shaped

brass or iron plates. The yields obtained in these regions are highly variable, from 10kg/ha up to 30kg/ha.

3.3 Processing of the Opium

The semi-dry and sticky resin is collected in bamboo or iron containers. In the course of drying the resin turns from a white colour into dark brown. Processing of the opium is limited to the small amount that is consumed locally. The raw opium is broken into small pieces and boiled in water. The mixture is then passed through a fine cloth strainer and the mass left in the strainer is boiled again. This process is repeated several times until the liquid which passes through the strainer is colourless. This is the sign that all the opium has been extracted. All the strained liquid is collected during this procedure. It is then boiled again to evaporate off all the water. The solid residuum is then ready for use.

4 OPIUM PRODUCTION IN THE GOLDEN CRESCENT

4.1 Main Production Areas

The main producers in this region are Afghanistan and Pakistan. Only a little information is available on production in Iran and Lebanon. From INCSR estimates (Gordon, 1994) the total cultivation area of poppies which are planted for opium in this region is thought to be about 30 000ha.

4.2 Method of Cultivation

4.2.1 Growing Procedure

The poppy is cultivated in this region of the world mostly as an autumn-sown crop, with sowing taking place between October and December. The exact sowing period is dependent on the ecological conditions of the countryside and starts earlier in cooler climates, in some cases in September. Irrigation possibilities may also affect the determination of the sowing date.

According to the data available, the amount of seed used for plantation varies in a wide range between 5–10kg/ha.

In many areas of this region, especially in Afghanistan and the dry areas of Pakistan, the precondition for success in cultivation is proper irrigation. Irrigation is usually started in the spring and is repeated 8–14 times until ripening of the capsules is underway, according to the weather conditions. No irrigation is allowed during the lancing period.

4.2.2 Harvesting

Lancing of the capsules begins after 15–20 days of flowering, when the maximum yields of latex and morphine are expected. Lancing in Afghanistan is performed six

times in a year and repeated at intervals of two or three days in each session. In the majority of cases it is carried out during May and June, but it has also been reported that lancing can take place as early as April in the extremely warm regions, or as late as August if the weather is cool. In Pakistan, the plant development is accelerated due to the warm conditions and the harvest can be started even earlier, in March.

4.3 Processing of the Opium

In this region clandestine laboratories are reported to be very active in processing opium to obtain the commercial products of morphine and heroin. According to Government data of Lebanon (INCB, 1995), 2.5–3 tonnes of morphine base are converted into heroin annually in that country.

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3. POPPY CULTIVATION IN AUSTRALIA

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1 INTRODUCTION

Poppies (*Papaver somniferum* L.) were grown in Australia on a very small scale throughout the 19th century by some medical practitioners for the production of opium to be used in their individual practices. This was in the form of tinctures of opium *(laudanum),* a common item of medical practice in this period (Beeching, 1975). More comprehensive plans to establish a poppy industry based on opium production were considered in the state of New South Wales (Turner, 1891), however planned production was never brought to fruition at that time.

World War II was the event which gave a strong motivation for the commencement of poppy production based, not on opium, but on dry poppy 'straw' (the capsules and a small quantity of stem). Morphine and related derivatives which were normally imported from Northern Hemisphere sources were in very short supply at that time and an experimental programme on the agronomy of *P. somniferum* was initiated by the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO). Experimental plots were set out in the Australian Capital Territory at Canberra (Loftus Hills, 1945) and in collaboration with State Departments of Agriculture in Tasmania (Walker and Raphael, 1975), Victoria and South Australia. Small areas of semi-commercial production of poppies followed the trials in these Australian states during the war years and a small amount of morphine was extracted.

Poppy production in Australia was not continued in the early post-war period, with supplies of medicinal morphine again being imported from Northern Hemisphere countries. However in the early 1960s an experimental programme of poppy production was commenced in Tasmania by the English pharmaceutical company McFarlane Smith, a subsidiary of Glaxo. The motivation for this resurgence of interest was due to the fact that the major pharmaceutical companies in the Northern Hemisphere drew their supplies mainly from India and Turkey with small amounts from Eastern Europe. Supplies of poppy straw and opium from these traditional areas of production were subject to fluctuation because of the vagaries of weather and production problems. Because of this, a strategy was developed to draw supplies of morphine and related alkaloids derived from dry poppy straw from Australia

Figure 1 A commemorative plaque relating to the poppy industry in front of the Department of Primary Industry and Fisheries Laboratories at Stoney Rise, Devonport, Tasmania
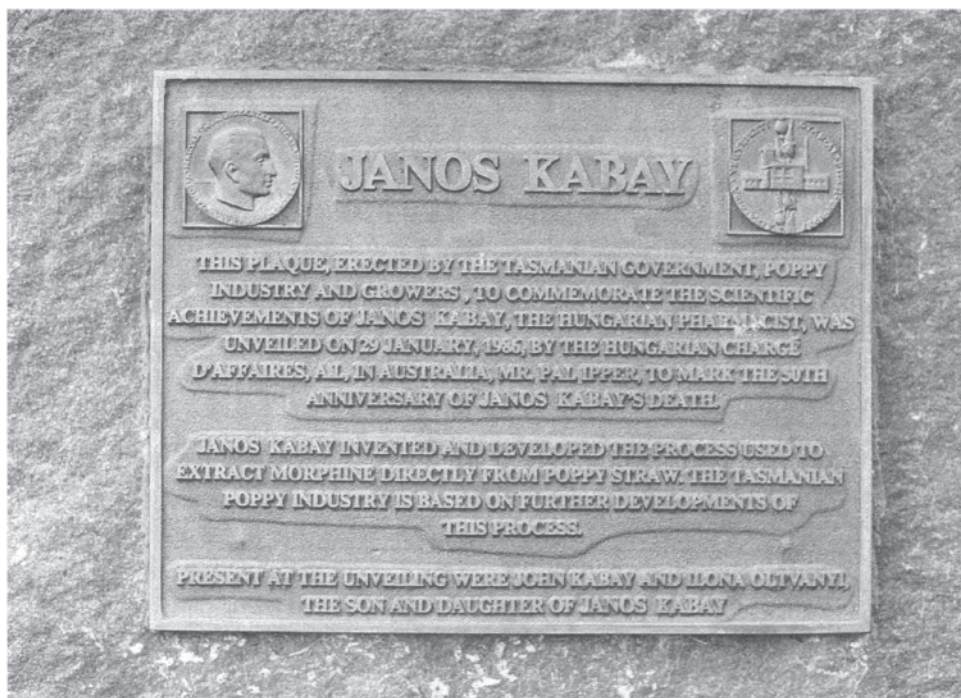
Figure 2 A close view of the plaque which commemorates the 50th anniversary of the death of the Hungarian Pharmacist Janos Kabay and the influence of his work on the Tasmanian poppy industry

which is politically stable, has modern agricultural expertise and infrastructure and reliable climatic conditions. In the extraction of morphine from dry poppy straw a modified Kabay process (Kabay, 1990) was used. Figures 1 and 2 show photographs of commemorative plaques to Kabay, indicating the importance of his work to this industry. In addition to a reliable supply of poppy alkaloids, the out-of-season nature of production in the Southern Hemisphere spread the supply of poppy straw and derived pharmaceuticals to complement supplies drawn from the Northern Hemisphere.

In the late 1960s, commercial production of poppies began in the island state of Tasmania with farmers being contracted by Glaxo Australia Pty Ltd (now Glaxo Wellcome). In the early 1970s a second pharmaceutical company, Abbot International, entered the industry in Tasmania using the name of Tasmanian Alkaloids. This latter company is now owned by the large American pharmaceutical company Johnson and Johnson. In 1971, due to a formal agreement between all of the six Australian States, the production of poppies was exclusively restricted to Tasmania. The main reason for this was the isolation of the island state of Tasmania which gave added security against any illegal use of poppy crops.

Poppy production on private farms in Tasmania is administered by a system of licenses issued under the direction of the State controlled Poppy Advisory and Control Board (PACB). Licenses are only issued after farmers have measured up to a stringent range of criteria. Inspectors from the PACB monitor all crops throughout the growing season for any evidence of illegal use and to ensure that crop straw is properly disposed of after harvest to minimize any re-growth problems. Another important function of the PACB is to ensure that production of poppy alkaloids is carried out strictly in accordance with Australia's international obligation to the 1961 Single Convention on Narcotic Drugs (as amended by the 1972 Protocol). The objective of this Convention is to ensure that the production of poppy alkaloids is restricted to recognized medicinal and scientific needs. This is accomplished by limiting production to expected market demand.

Contracts for the production of poppy crops in Tasmania are made with individual farmers by the two commercial companies Glaxo Wellcome and Tasmanian Alkaloids who supply the seed, organize the drilling, supply cultural advice and harvest the crops. Poppy seed forms a valuable by-product of these operations and is used primarily for culinary purposes. In the 1997–1998 season about 14 000 hectares of poppies were cultivated in Tasmania and the total area which has been used since 1970 has ranged from about 500 to 14 000 ha. The isolation of Tasmania and the stringent security measures have ensured that any illegal use of crops has been minimal. Useful abbreviated general outlines of poppy and alkaloid production in Tasmania have been recorded by Allen and Frappell (1970), Walker (1977), Davies (1985), White (1985), Bremner (1989), Wallis (1994) and Allwright (1995).

2 CLIMATE AND ENVIRONMENT

Poppy growing in Tasmania is carried out in a cool temperate maritime environment. The crop is cultivated from latitude 41°S to 42°30'S with the largest area located in the North West region of the state. Over most of the poppy growing areas of Tasmania, the physical environment is characterized by gently rolling or hilly terrain (Figure 3) with a limited contribution from flat areas. Figure 4 illustrates the range of locations and Table 1 shows the mean monthly meteorological data for day length, temperature, rainfall, sunlight and wind at Forthside (Lat. 41°12'S; Long. 146°E, altitude 150m) in the North West region.

3 SITE SELECTION

Poppies do not grow well on acid soils in Tasmania (Temple-Smith *et al.*, 1983) and in selecting sites for commercial crops, efforts are made to obtain locations with a pH of at least 5.7 (see Section 5.5, Nutrition). The terrain on the coastal strip in the North West region is generally flatter and more amenable to easier cultivation and harvesting. In addition to the physical environment being more hilly back from the coastal strip, rainfall increases at these higher altitudes and the soil pH generally decreases. Moreover, the incidences of frosts increase and although poppies are generally tolerant to frost at the early stages of growth some cultivars do tend to be

Figure 3 A field of poppies in bloom (December) in North West Tasmania showing the typically undulating topography of the region. (By courtesy of *The Advocate* Newspaper, Tasmania.)

susceptible during the stem elongation phase. For all of these reasons the less hilly areas closer to the coast are preferred; in addition is the fact that the logistics of transport of harvested crops to processing and extraction plants is easier. Similarly, the areas of river flats of the Derwent and Coal Rivers in the South of Tasmania have also been chosen for poppy production.

4 GERMPLASM AND SEED SELECTION

The poppy ecotypes cultivated in Tasmania are long-day plants which have been specially developed for Tasmanian conditions. These plants have been bred for the basic fundamentals of high capsule yield and high concentration of poppy alkaloids, morphine and codeine in particular. In addition to alkaloid content, factors such as straw length, standing ability and resistance to disease have also been incorporated into the various breeding lines.
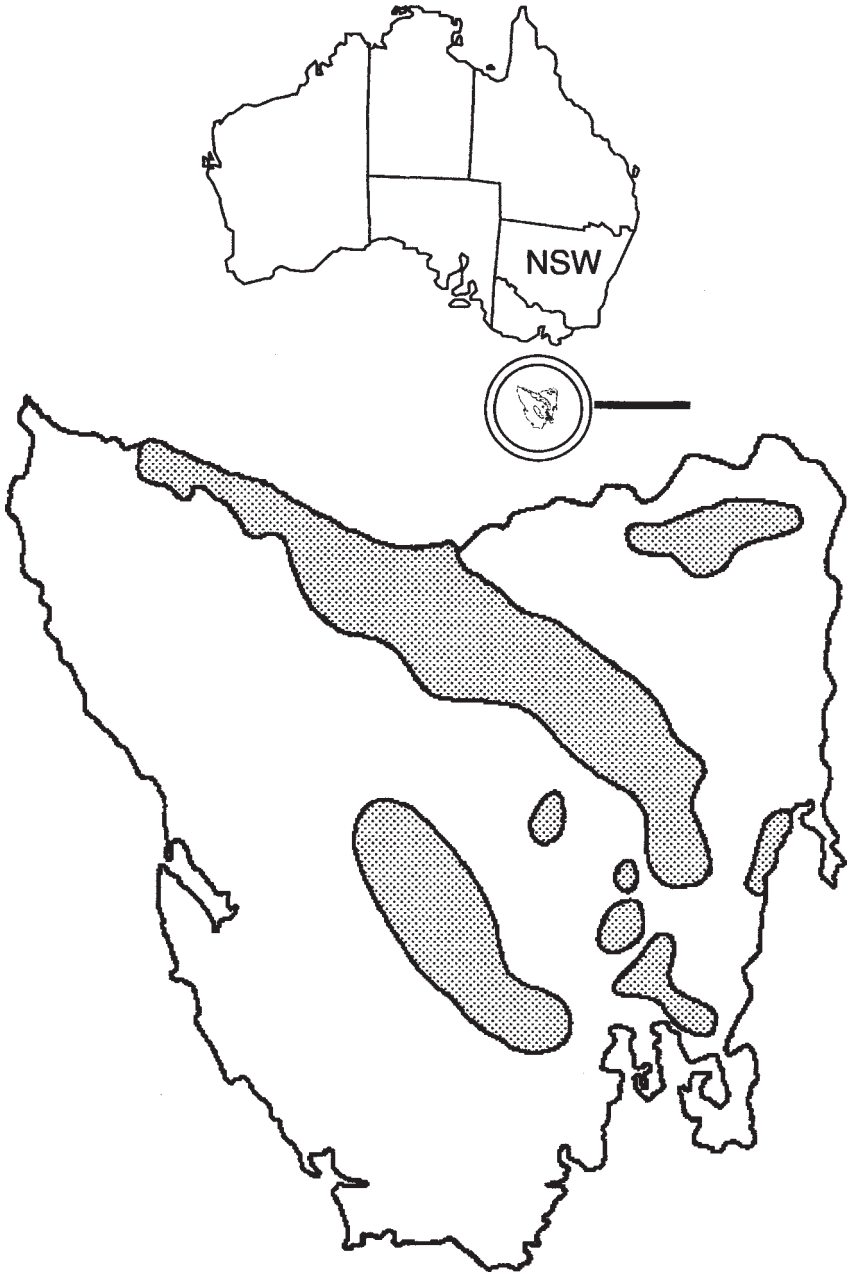
Figure 4 The regional locations of poppy production in Tasmania

Table 1 The long-term average monthly meteorological data for Forthside Research Station, Tasmania

	Temperature (°C)		Rain (mm)	Wind (km)	Sun (h)	Day length	
	Min	Max				(h)	(min)
Jan	11.0	20.6	46.1	6411	253.1	14	49
Feb	11.6	21.2	50.2	5390	223.1	13	41
Mar	10.4	19.7	54.4	5391	197.9	12	25
Apr	6.2	14.8	80.3	6248	198.2	11	02
May	6.5	14.0	102.8	4970	121.6	9	51
Jun	4.4	12.1	100.3	4775	107.0	9	13
Jul	3.5	11.1	122.8	5311	115.2	9	28
Aug	4.0	11.7	114.2	5592	127.5	10	28
Sep	5.0	14.1	90.3	6161	161.5	11	47
Oct	6.3	15.3	84.2	6515	227.9	13	07
Nov	8.5	17.2	74.1	6241	225.7	14	25
Dec	9.8	18.9	76.0	6648	244.1	15	08

A greenhouse experiment was carried out in Tasmania on the effect of seed size on establishment in response to abnormal seedling growth in autumn-sown crops (Beattie, 1977). A commercial line of seed with a mean 1000 seed weight of 465mg was divided into three categories of 'small' (423mg), 'medium' (440mg) and 'large' (476mg). The 1000 seed weight of the 'small' seed was only 11% less than the 'large' seed but after 14 days its percentage emergence was 53% less than the 'large' seed. The conclusion to this experiment was that size grading of poppy seed was an important contributor to uniform establishment.

The germplasm utilized and the seed selections developed in Tasmania have been the exclusive and independent prerogative of the two contracting pharmaceutical companies, Glaxo Wellcome and Tasmanian Alkaloids. The combination of germplasm and seed selection, climatic environment, soil type and farming techniques have resulted in the alkaloid yields per hectare of Tasmanian poppies being the highest in the world.

5 PLANT CULTURAL TECHNIQUES

5.1 Crop Rotation

In Tasmania poppies are typically grown on mixed farming enterprises which may include any or all of a very wide range of vegetables, pasture, cereal or other essential oil, herb or insecticidal crops. These crops may include green peas *(Pisum sativum)*, potatoes *(Solanum tuberosum)*, onions *(Allium cepa)*, brassicas (*Brassica* ssp.), mint *(Mentha piperita)*, fennel *(Foeniculum vulgare)*, pyrethrum *(Tanacetum cinerariifolium)* and others. There is a generally accepted policy on the part of both poppy contracting companies that there should be a three to four year rotation between poppy crops because of the potential for disease with shorter rotations. However there is no general agreement on the specific crops which should precede poppies.

Good poppy yields have been recorded when a range of the above vegetable, pasture, or cereal crops have preceded poppies.

5.2 Time of Establishment

Early spring (August—September) is the preferred sowing time for most poppy crops in Tasmania with flowering occurring in December and a dry mature harvest (12% moisture in capsules) during February/March. Trials have shown that spring sowing later than September resulted in lower capsule dry matter yields and lower morphine concentration (Frappell, unpublished work). Experience with autumn sowing in the drier southern areas of the State with mean annual rainfalls of 500–600mm compared to 900–1000mm in the North West region has generally shown lower capsule yields with lower alkaloid levels. Currently all poppy crops in Tasmania are spring sown.

5.3 Soil Selection and Cultivation

The soil types which are utilized in Tasmania to grow poppies range from the red basaltic krasnozem soils of the North West and North East regions to the sandy soils of the drier South East. Poppies are also grown on a range of intermediate clay loam soils in the Central North areas of the state. Approximately 70% of poppy crops are grown on the red basaltic krasnozem soils in Tasmania. This soil type is preferred for not only poppy production but also for most of the high-value vegetable crops because of its excellent texture and free draining structure. This free draining characteristic allows for cultivation or the passage of harvesting equipment very quickly (one to two days) even after heavy rain. Where a cycle of cultivation, drilling and harvesting operations must be planned for a large number of crops this soil's structural characteristic is a very significant advantage.

   Although krasnozems are the preferred soil type, good yields can be achieved on the other soil types which are used to cultivate poppies in Tasmania. Cultivation techniques are reasonably uniform throughout and consist of an initial ploughing with a mould board plough followed by cultivation with a disc implement and a number of passages with tyned harrows. Rolling may be used to consolidate the soil depending on circumstances.

5.4 Seed Drilling

Seed is supplied by each of the companies contracting the crop. Two main techniques are used to drill the seed, namely either (i) mixed with a 50:50 combination of lime superphosphate or (ii) mixed with an inert substance and drilled at a rate of 0.75–1.0kg of seed/ha. The choice of drilling technique may be controlled by soil fertility (see Section 5.5).

5.5 Nutrition

A range of NPK (nitrogen, phosphorus, potassium) nutrients and sometimes micro nutrients are applied to poppy crops. In addition, liming is a common practice.

5.5.1 Soil pH and Liming

Poppies do not grow well on acid soils. On soils with a marginal pH poppy seed is often drilled intimately mixed with a 50:50 lime superphosphate combination (see seed drilling). Generally sites for potential poppy crops are preferred to be at least pH 5.7 and preferably pH 6.0. In two field experiments on krasnozems in Tasmania, ground limestone was applied at 0, 2.5, 5, 10 and 20t/ha to sites of pH 5.6 (Forthside) and 5.1 (Elliott) in the top 150mm of soil (Temple-Smith *et al.*, 1983). The soil pH increased to a maximum of 6.1 at Forthside and 6.0 at Elliott with 20t/ha of limestone. The yield of both capsules and morphine also increased to a maximum at 20t/ha of limestone with a two- to threefold increase above zero lime at both sites. Poppy yield responses to liming at these sites were attributable mainly to an alleviation of aluminium toxicity. In some situations toxic levels of manganese may also be a problem for poppies on krasnozem soils of low pH.

5.5.2 Methods and Rates of Fertilizer Application

The krasnozem soils of Tasmania have excellent structural characteristics and their red colouration is attributable to a high content of free ferric oxide. This iron oxide has the ability to combine with the phosphorus component of applied fertilizer and convert it into a relatively insoluble and unavailable form. This capacity for phosphorus 'fixation' dictates that phosphorus (P) fertilizer is always band placed on the Tasmanian krasnozem soils and most commonly this method is also used on other soil types which produce poppies in Tasmania. Potassium (K) and nitrogen (N) fertilizers are also commonly banded in a basal application. The rate of application of a 14:16:11 NPK mixture varies from 150 to 300kg/ha depending on soil type, soil analysis and management practices.

The specific depth of fertilizer band application varies from approximately 50mm directly below the seed to 50mm below the seed and 25–50mm to the side. If specialized seed and fertilizer placement drills are not available then 'pre-drilling' of the cropping area is carried out. In this approximation to precise band placement the NPK fertilizer is banded in narrow rows (150–200mm) and seed is then drilled in a separate operation approximately 50mm above the level of the fertilizer. In this technique the location of the fertilizer band will be displaced at random from directly below to a maximum of 75 or 100mm to the side of the seed. In experiments on the Tasmanian krasnozem comparing broadcasting and uniform mixing, precise band placement and pre-drilling the two banded methods gave similar capsule and morphine yields and both methods gave about 50% higher yields than broadcasting and uniformly mixing (Frappell, unpublished data).

In a greenhouse experiment, using a Tasmanian krasnozem soil, radioactive ^{32}P labelled superphosphate bands were placed 40 and 75mm directly below poppy seeds. When harvested 50 days after drilling the plant tops from the 40mm bands contained 42% more phosphorus derived from the fertilizer than the tops from plants with fertilizer 75mm below the seed (Laughlin, 1978). This result confirmed that banded fertilizer should not be placed as deep as 75mm below the seed.

5.5.3 Nitrogen

In contrast to phosphorus and potassium, nitrogen is very susceptible to leaching by rain and irrigation with movement through the soil profile and out of the root range of poppies. Because of this, poppies often respond to supplementary top-dressed applications of nitrogen. A small amount of nitrogen (20kg/ha) is commonly included with phosphorus and potassium at drilling. Further supplementary nitrogen is often applied late in the season depending on rainfall, possibility of irrigation and any indication—either visual or from leaf analysis that—nitrogen is deficient. However because of the mobilisation of nitrogen from soil organic matter, the accurate prediction of nitrogen response is often difficult on the krasnozems.

In a field experiment on krasnozem soil, an initial application of 20kg N/ha increased head yield by 100% at dry maturity with only a 5% increase in morphine concentration (Laughlin, 1983). In this trial 100kg N/ha increased head yield threefold with a 20% increase in morphine concentration. In other trials on the same soil type, responses have been very variable and this has been attributable to a range of soil and seasonal factors. In more recent trials, using leaf tissue analysis, a good correlation was established between leaf nitrate N at the 'hook' stage and morphine concentration response (Anonymous, 1992). An increase in capsule morphine was obtained if nitrogen was applied when the sap nitrate in fully expanded leaves fell below 800 ppm.

5.5.3.1 Forms of nitrogen

In a comparison between the effects of additions of ammonium sulphate, ammonium nitrate, potassium nitrate, calcium nitrate and urea, all forms of nitrogen were found to have similar effects on leaf nitrate and morphine concentration of capsules. Leaf nitrate at the hook stage was increased by about 28% and capsule morphine at dry maturity by 20% (Laughlin, 1983).

5.5.3.2 Time of nitrogen application

Nitrogen is a key element in the morphine molecule and the poppy capsule acts as a sink for morphine accumulation during the post-flowering stage. In a field experiment on krasnozem soil at Forthside, triple nitrogen applications were compared as follows: 14 days before flowering ('hook stage'); 28 days before flowering (early stem elongation); and 7 days after flowering (early capsule development). Nitrogen fertilizer in the form of ammonium nitrate was top-dressed at 0, 40 and 80kg N/ha and irrigation was applied immediately after the nitrogen application and continued at a 35mm deficit until leaf senescence (Laughlin, 1983). Applications at the hook and stem elongation times gave 31% and 21% higher morphine yields respectively than at post-flowering.

5.5.4 Boron

Although basaltic krasnozems are the preferred soil types for poppy production in Tasmania, competition from a wide range of vegetables and other crops has meant that some poppy crops are grown on a range of lighter soils. Some of these lighter alluvial soils are susceptible to boron (B) deficiency in a number of crops and the typical boron deficiency symptoms in poppies which have been recorded in European poppy growing areas (Zogg, 1946) have also been recorded in Tasmania. These

symptoms include lack of elongation of the internodes, rolling of the leaf lamina, inter-veinal chlorosis and blackening, and death of the growing point. In less severe cases of boron deficiency, stem elongation and flowering occur but the stem may be twisted and the capsules deformed (Laughlin, 1980b). Boron deficiency has often been more prevalent in dry conditions in European poppy growing areas and this has also been the case with poppies in Tasmania.

Boron deficiency symptoms have often been related to the liming of acid alluvial soils (Fox, 1968; Majewski *et al.,* 1969). In a greenhouse experiment with poppies on alluvial soil in Tasmania, the equivalent of 5t/ha of calcium hydroxide increased soil pH from 5.4 to 8.1 but reduced plant boron from 25 to 10ppm with severe boron deficiency symptoms (Laughlin, 1980b). In a field experiment on an alluvial soil in Tasmania 2kgB/ha were applied as sodium borate mixed and banded with the basal NPK fertilizer and banded 35mm directly below the seed at drilling. The same quantity of boron was also applied as a foliar spray at the hook stage two weeks before flowering. On a soil with a pH of 5.8 the band-drilled B gave a 700% increase in capsule yield and the foliar sprayed treatment a 500% increase over the nil boron control (Laughlin, 1980b). Boron is often applied at about 2kg/ha to commercial poppy crops on alluvial and other lighter soils in Tasmania and boron deficiency is now very uncommon in commercial crops. Boron deficiency symptoms are rarely seen on the krasnozems.

5.6 Weed Control

Because poppies are grown as part of a wide rotation of crops in Tasmania, a large range of weeds can be potential problems and a range of herbicides are routinely used. Herbicides are generally applied at the 4–6 leaf stage when the crop is in vigorous growth and the operation should be completed before plants are at the eight true leaf stage. In order to minimize damage the plants should not be suffering from any water stress and hence the herbicides should be applied soon after irrigation or rain and preferably not in full sun or high temperatures which can result in leaf scorch. Asulam, ethofumasate, diclofop methyl, diquat and sethoxydim are all registered and used for weed control in Tasmania. The weed poppies *Papaver dubium, Papaver hybridum* and *Papaver rhoeas* are specific problems in some situations because of their close relationship to *Papaver somniferum,* 'Stale' seed bed techniques with the cultivation of the area, early germination of the weed poppies and spraying prior to drilling commercial crops is one possible approach in this situation.

5.7 Growth Regulation

Although plant growth regulators are not used in commercial poppy production in Tasmania a number of those chemicals have been used in experimental programmes. In a field experiment on krasnozem soil at Forthside daminozide and indole acetic acid (IAA) were tested (Forbes and Laughlin, 1985). Daminozide was applied as a foliar spray at 0, 5000, 15000 and 20000ppm at early post-herbicide (seedling stage), rosette and early stem elongation. Daminozide at 20000ppm at the rosette stage was most effective in reducing plant height. This height reduction effect was most apparent

two weeks after full bloom when the daminozide-treated plants were 40cm shorter than the control plants. At dry harvest maturity the sprayed plants were 22cm shorter than the control. The incidence of lodging was 1.5% in the daminozide-sprayed plants compared with 13.5% in the control.

5.8 Irrigation

In Tasmania rainfall is at a minimum during the summer months (Table 1) and poppies, along with all other spring-sown crops, benefit from irrigation. Because of the topography, most poppy crops are irrigated by overhead water application either by pipes and sprinklers or travelling irrigators.

An irrigation application of 50mm of water is commonly applied commercially to poppy crops during the hook to flowering stages of growth. Some growers may also apply further irrigation after flowering depending on the competing demands of other crops in the mixed farming enterprises of which poppies form a part.

The effects of partial to complete irrigation have been studied in detail in two drought years in Tasmania (Chung, 1987). A field experiment at Forthside compared four irrigation treatments: nil irrigation (NI); irrigation up to 50% flowering (IF); irrigation up to 100% capsule formation (IC); and full irrigation up to 90% leaf senescence (IS). Irrigation increased the leaf area index, delayed leaf senescence and increased the yield of all plant components in both years of the study (Figure 5). When irrigation was continued until leaf senescence (IS) the total capsule morphine yield was increased by 5–20 kg/ha compared to no irrigation (NI) (Table 2). The effect of irrigation on yield increases was attributed to the increase in the number and yield of lateral heads, the yield of terminal heads and increases in morphine concentration of both terminal and lateral capsules (Table 2). Maximum morphine yield is achieved under Tasmanian conditions with one irrigation of 50mm at the 50% hook stage and further irrigation (50mm) at 50% flowering, at the end of flowering and two weeks after the end of flowering.

Compared with a common commercial practice during the development period of the industry (irrigating only until 50% flowering) yield increases of 4–13kg/ha of morphine were obtained with the addition of the two later irrigations (Chung, 1987). Continued overhead irrigation or heavy rainfall after flowering may reduce the concentration of morphine (Loftus Hills, 1945; Bunting, 1963; Laughlin, 1985) and other alkaloids (Hoffman and Menary, 1984). However, in the field experiments of Chung (1987), although the post-flowering irrigation treatments gave lower morphine concentrations in the capsules than when irrigation ceased at flowering, the large increase (56% over two experiments) in head (capsule and seed) yield more than compensated for the decrease in morphine (Table 2). These conclusions have been adopted by growers and irrigation is now commonly applied up to the green capsule stage.

5.8.1 Irrigation and Nitrogen Interactions

In the above experiments of Chung (1987) all the irrigation treatments were applied at a relatively low commercial rate of pre-drilled nitrogen fertilizer: 20kg N/ha in an NPK band. However, the interaction effect of higher rates of nitrogen fertilizer applied at a
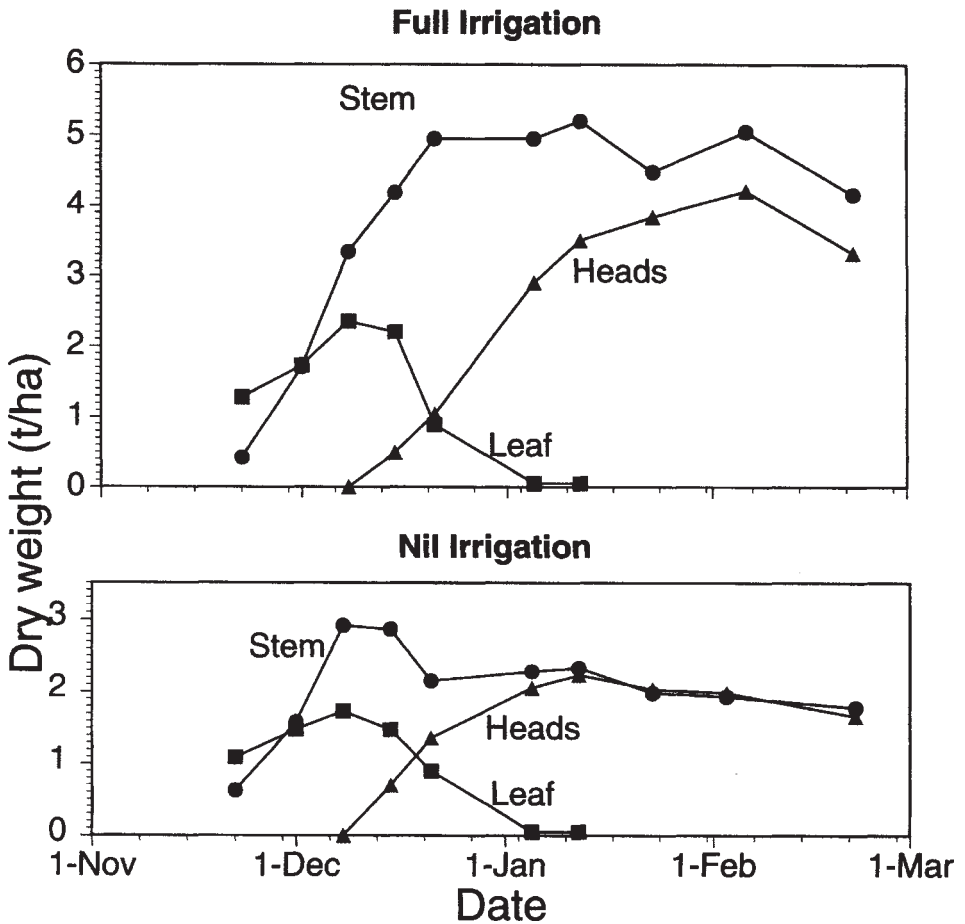
Figure 5 The effect of irrigation on the growth of poppy plants components for full irrigation of 256 mm (irrigated to 90% leaf senescence) and nil irrigation.●, stem; ▲, heads; ■, leaf (adapted from Chung, 1987)

range of irrigation regimes has also been studied in Tasmania (Laughlin and Chung, 1992). In a field experiment on krasnozem soil at Forthside, nitrogen fertilizer in the form of ammonium nitrate was both banded by pre-drilling and top-dressing at the hook stage two weeks before flowering at 20, 60 and 100kg N/ha. Irrigation was applied at an estimated soil water deficit of 35mm by overhead sprinklers and continued until leaf senescence to give treatment totals of 0, 95, 216 and 297mm. There were no effects of either banded N (Figure 6) or top-dressed N (Figure 7) on capsule morphine concentration at nil irrigation and no response to 20kg N/ha pre-drilled at any irrigation level. The application of 60kg N/ha pre-drilled increased morphine concentration by about 10% at 95 and 216mm and 100kg N/ha pre-drilled gave a maximum increase

Table 2 The yield and yield components at dry maturity harvest in the two seasons (after Chung, 1987). NI, nil irrigation; IF, irrigation (64ml 1977–1978, 128ml 1983–1984) applied up to 50% flowering; IC, irrigation (160ml 1977–1978) applied up to 100% capsules; IS, irrigation (192 ml 1977–1978, 256ml 1983–1984) applied up to 90% leaf senescence

	1977–1978					1983–1984			
	NI	*IF*	*IC*	*IS*	*S.E.*	*NI*	*IF*	*IS*	*S.E.*
Number of terminal heads/m^2	39	45	45	50	2.0	65	58	63	5.5
Weight of terminal head (g)	3.7	3.7	3.8	3.9	0.19	2.9	3.2	5.0	0.19
Terminal capsule yield (kg/ha)	495	598	635	718	36.7	763	719	1266	63.6
Terminal seed yield (kg/ha)	938	1062	1071	1240	64.3	1153	1144	1865	138.1
Terminal capsule/head (%)	34.5	36.0	37.2	36.7	1.76	39.8	38.6	40.4	0.96
Terminal morphine concentration (%)	1.14	1.34	1.16	1.22	0.059	1.95	2.4	2.05	0.71
Terminal morphine yield (kg/ha)	5.6	8.0	7.4	8.8	0.58	14.9	17.3	26.0	1.49
Number of lateral heads/m^2	32	35	52	57	3.1	7	23	34	9.5
Weight of lateral head (g)	2.0	2.0	2.4	2.2	0.11	0.8	2.2	3.2	0.47
Lateral capsule yield (kg/ha)	236	257	459	499	27.7	39	213	467	79.4
Lateral seed yield (kg/ha)	412	459	786	750	49.4	15	291	614	94.9
Lateral capsule/head (%)	36.4	35.9	36.9	40.0	1.57	72.2	42.3	43.2	10.2
Lateral morphine concentration (%)	1.04	1.40	1.24	1.28	0.055	1.95	2.40	2.05	0.71
Lateral morphine yield (kg/ha)	2.5	3.6	5.7	6.4	0.42	0.8	5.1	9.6	1.88
Harvest index (%)	31.4	30.4	32.8	34.9	1.44	35.0	28.6	33.6	1.1
Total head yield (kg/ha)	2081	2376	2951	3207	140.9	1970	2367	4212	32.7
Total morphine yield (kg/ha)	8.1	11.6	13.4	15.2	0.84	15.7	22.4	35.6	0.86
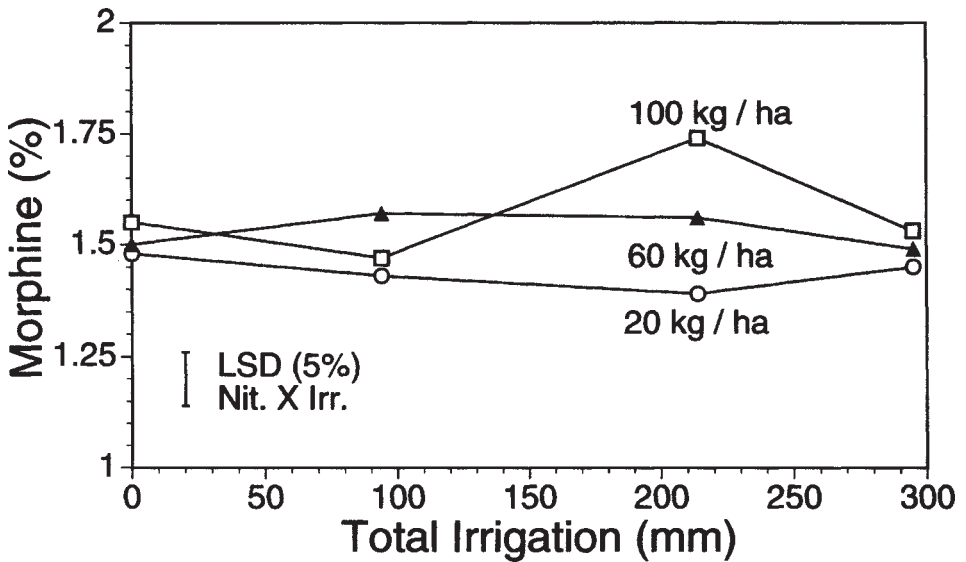
Figure 6 The effect of irrigation at three rates of banded nitrogen fertilizer on capsule morphine concentration (%): ○, 20; ▲, 60; □, 100kg N/ha (after Laughlin and Chung, 1992)
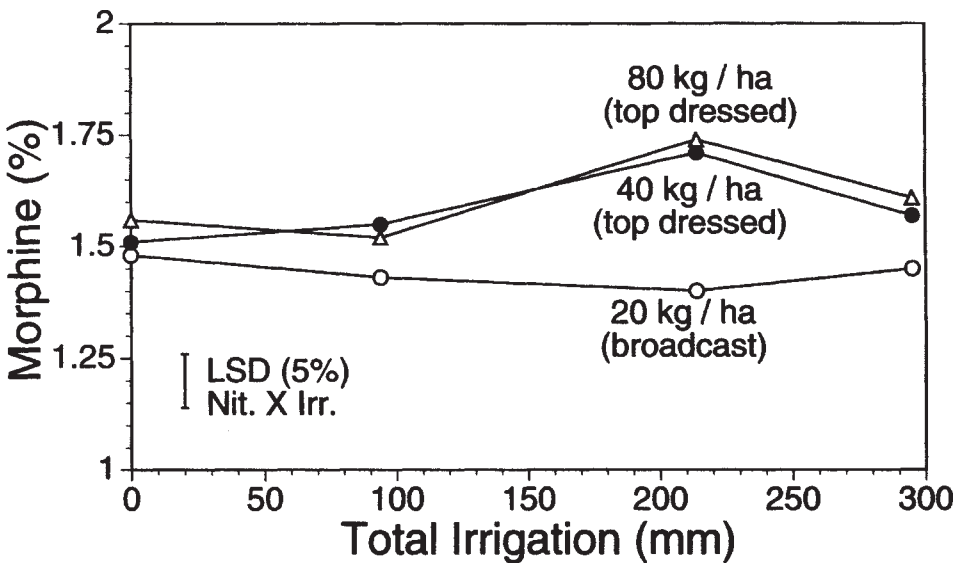
Figure 7 The effect of irrigation at two rates of top-dressed nitrogen fertilizer on capsule morphine concentration (%): ●, 40 and Δ, 80kg N/ha. Broadcast nitrogen was superimposed on basal banded nitrogen at 20kgN/ha (after Laughlin and Chung, 1992). LSD (5%)=least significant difference at $p<0.05$

of 24% at 216mm (Figure 6). The effect of top-dressed N was similar to pre-drilled N with a maximum increase in capsule morphine of 24% at 216mm (Figure 7). However, this effect with top-dressed N was achieved at 60kgN/ha with no further increase with 100kg N/ha. With both pre-drilled and top-dressed N, the morphine concentration of the capsules decreased with 297mm of irrigation to about the level of the nil irrigation control. These results may imply either leaching of nitrogen out of the root range of the poppies or leaching of morphine out of the capsules, or a combination of both.

In this study of the interaction between nitrogen and irrigation, nitrogen fertilizer had no significant effect on the dry matter yield of capsule or seed but gave 10% and 12% increases in capsule morphine yield at the highest rate of banded and broadcast nitrogen respectively. In contrast, irrigation had a very large effect on the dry matter yield of both capsules and seeds. Yields increased to a maximum with 216mm of water where there was a 40% increase above the nil irrigation control. At this point morphine yield was 53% greater than the nil irrigation control. Neither nitrogen nor irrigation had any effect on the ratio of seed to capsule at maturity (Laughlin and Chung, 1992). The lack of response to 20kg N/ha banded and the fact that 40kg N/ha top-dressed at the hook stage gave the same increase in morphine concentration (Figure 7) as that achieved with 100kg N/ha banded at drilling (Figure 6) probably implies extensive leaching of the early drilled nitrogen application.

5.9 Plant Population Density and Rectangularity

Plant population density and rectangularity (the ratio of intra-row to inter-row spacing) have an important influence on the commercial harvest yield of poppy heads. In Tasmania inter-row spacings of 300-350mm were originally used when inter-row cultivation was needed for weed control. However, with the advent of effective herbicides (Baldwyn, 1977) narrow row spacings of 150-175mm have commonly been used commercially and high densities of 100-200 plants per square metre or greater were often seen. The effects of plant population density and rectangularity on poppy yields were studied in Tasmania, initially by Frappell (1979) and in more detailed recent experiments by Chung (1990).

5.9.1 Plant Density and Lodging

Growth, yield and the influence of lodging (falling over) of poppies were studied in Tasmania at densities of 10, 25, 50, 100 and 200 plants/m^2 in factorial combination with rectangularities of 1:1, 4:1 and 10:1 in field experiments on krasnozem in 1983-1984 and 1988-1989 (Chung, 1990). In 1983-1984 the amount of wind was 24% more than the long-term average. The incidence of lodging increased as density increased to more than 50 plants/m^2 with 25% lodging on a plant number basis and 16% lodging on a head yield basis at 100 plants/m^2. Generally, the smaller capsules on proportionately thin-stemmed plants were the plants most likely to lodge. There was an asymptotic relationship between plant population density and total head (capsule plus seed) yield at dry harvest maturity. However, because of lodging the relationships between plant population density and harvestable head yield and harvestable morphine yield (Figure 8) were both markedly parabolic in 1983-1984.
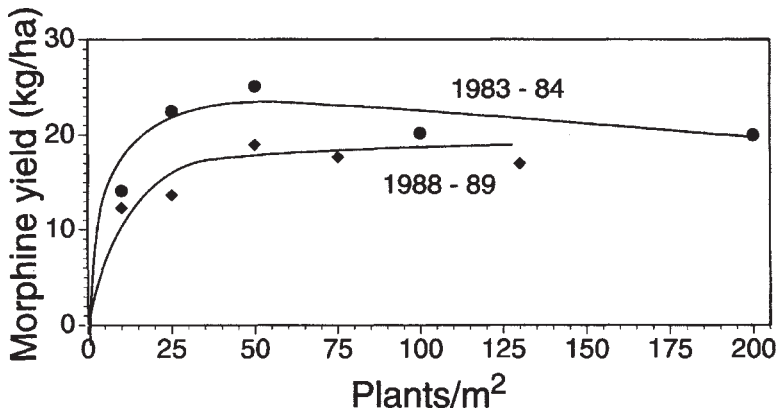
Figure 8 The relationship between plant population density (mean of three rectangularities) and erect (machine-harvestable) morphine yield of poppies for: ●, 1983-1984 ($1/\omega=3.736523 + 0.319259p+0.000740p^2$; ◆, 1988-1989 ($1/\omega=0.426000+0.049910p$). ω is the mean weight per plant (g) and ρ is the plant population density (number/m²). (Adapted from Chung, 1990.)

In this experiment the maximum morphine yield was obtained at a density of 70 plants/m². At 200 plants/m² the yield of morphine was 17% less than the maximum due to lodging and the effect of density *per se* which decreased capsule morphine concentration by 9% below that at 70 plants/m² (Chung, 1990).

A similar experiment was carried out later at Forthside in 1988-1989 in a year in which the amount of wind was 15% below average. In this experiment lodging was less than in 1983-1984 with 18% lodging on a plant number basis and 9% on a head yield basis at a density of 130 plants/m². The relationship between morphine yield and plant population density was asymptotic with a maximum yield at 130 plants/m² (Figure 8). However, the fact that such calm wind conditions occur in Tasmania only once every four years suggests that densities such as these are too high a risk for commercial use (Chung, 1990).

5.9.2 Rectangularity

In 1983-1984 a rectangularity of 1:1 gave higher total plant dry matter production, total head yield, yield of machine-harvestable erect heads and morphine yield from erect heads than those obtained for rectangularities of 4:1 and 10:1. Quite often in Tasmania higher seed sowing rates have been used than the optimum as an insurance against herbicide damage, but improved weed control techniques have greatly improved survival rates and this has led to commercial densities of 100-150 plants/m². The results of these experiments suggest that commercial poppy crops in Tasmania should be drilled in 150-175mm rows and at a seed rate to give 70 plants/m². This results in a rectangularity of 2:1 which is very close to the optimum (Chung, 1990).

5.9.3 Plant Density and Nitrogen Interactions

Although nitrogen fertilizer application has been shown to increase morphine concentration and yield of poppy capsules in Tasmania (Laughlin, 1983) the perceived fear of lodging has made some growers reluctant to use more than a minimal amount of nitrogen. The effect of nitrogen top-dressing at a range of plant population densities was studied in 1983-1984 (Laughlin, 1987). In a field experiment on a krasnozem soil at Forthside, ammonium nitrate was top-dressed at the hook stage two weeks before flowering to give 0, 40 and 80 kg N/ha in factorial combination with densities of D_1=25, D_2=50, D_3=100 and D_4=200 plants/m^2.

In a year of 24% above average wind, lodging was strongly affected by plant density. With nil top-dressed nitrogen only 1% of plants lodged at D_1, 10% at D_2, 15% at D_3 and 68% at D_4. Nitrogen had no effect on lodging rates at $D1$ or D_2, but doubled the number of plants lodged to about 30% at D_3 (Table 3). This pattern of response to nitrogen top-dressing was reflected in both head and morphine yields. Nitrogen had a small effect on morphine concentration—giving a 7% increase—but because of the nitrogen-density interaction effect on lodging at D_3, both head and morphine yield decreased by 10% and 25% at 40 and 80kg N/ha respectively. Head yields at D_4 were dominated by the lodging effect with no further impact of nitrogen application. Generally these results confirm that nitrogen additions can aggravate the effect of lodging at high densities of 100 plants/m^2 and above but are probably safe at the recommended densities of 50-70 plants/m^2.

5.9.4 Irrigation and Plant Density Interactions

Irrigation up to flowering is commonly practised in the Tasmanian poppy industry but some growers have been reluctant to apply more extensive irrigation up to leaf senescence because of the perceived fear of the resultant lodging. Lodging does occur in Tasmanian poppy crops but most commonly such lodging has been associated with crops grown at high plant densities (>100 plants/m^2). The effect of irrigation at a range of plant population densities on lodging and machine harvestable (erect) yield of poppy heads was studied on a krasnozem soil at Forthside in 1983-1984 (Chung, 1992).

Three irrigation treatments were applied at a 35mm estimated soil water deficit, namely: non-irrigated; irrigation up to flowering (128mm applied); and irrigation

Table 3 Effect of top-dressed nitrogen at various plant densities on the percentage number of plants lodged (percentages of total numbers) (after Laughlin, 1987)

Nitrogen (kg/ha)	Density (plants/m^2)			
	$D_1 = 25$	$D_2 = 50$	$D_3 = 100$	$D_4 = 200$
0	0.9	10.7	15.0	68.2
40	2.3	7.9	31.0	58.1
80	1.2	8.6	33.3	62.8
LSD 5% = 7.9; 1% = 10.8				

through to 50% leaf senescence (256mm applied). These irrigation treatments were applied at densities of 25, 50, 100 and 200 plants/m². In a season which was drier and with 24% more wind than average, irrigation had a large effect on poppy yield. Irrigation up to flowering resulted in a 26% increase in machine harvestable (erect) heads over nil irrigation. When irrigation was continued through to 50% leaf senescence the head yield was 90% greater than the nil irrigation control (Figure 9). The irrigated treatments produced 22% of plants lodged—less lodging than the non-irrigated control with 28% lodging. The irrigation and plant density effects of this field experiment were independent. However in contrast to the asymptotic machine-harvestable yield responses of both poppy heads and Morphine, the high incidence
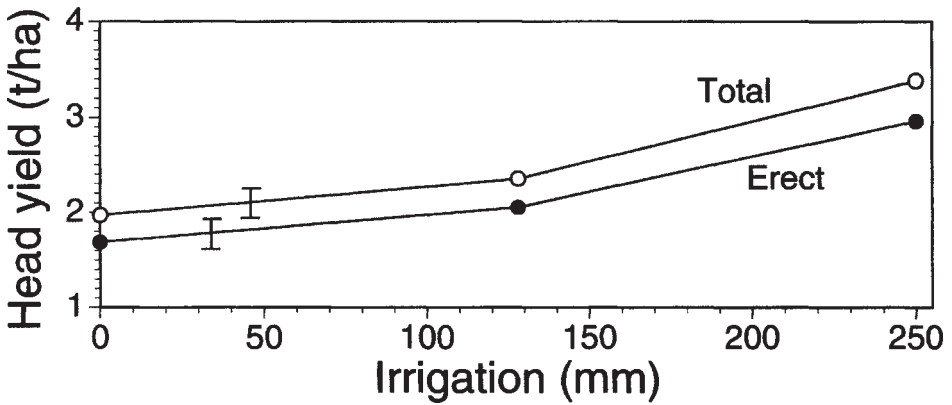
Figure 9 The effect of irrigation on the head yield of poppies: ○, total; ●, erect. The vertical bars represent the SED (after Chung, 1992). SED=standard error of difference
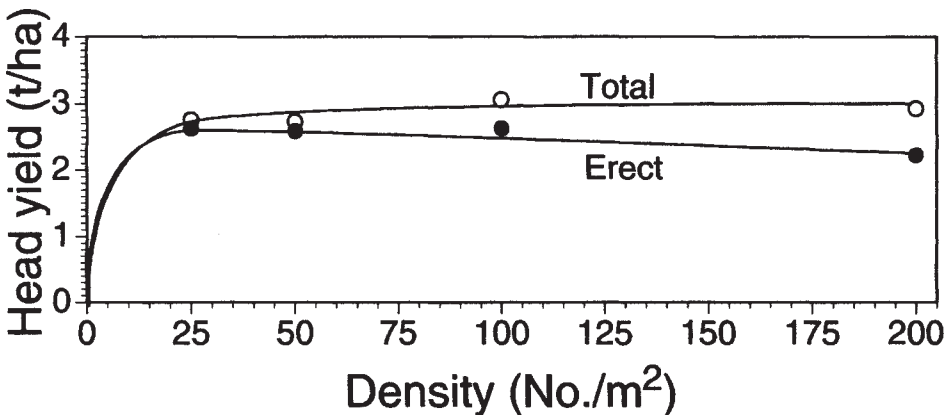
Figure 10 The effect of plant density on the head yield of poppies: ○, total; ●, erect (machine-harvestable) (after Chung, 1992)

of lodging (49%) at a density of 200 plants/m^2 gave a parabolic yield density response effect ([Figure 10](#)).

6 PLANT DISEASES

Although a number of fungal diseases of poppies which can have an impact on morphine concentration and yield have been recorded in Tasmania, their incidence has generally been low and fungicides are not commonly applied. These diseases have included poppy fire *(Pleospora papaveraceae)*, *Sclerotinia* wilt *(Sclerotinia sclerotiorum)* and poppy leaf smut *(Entyloma fuscum)*.

In exceptional seasons the morphine concentration of capsules has been reduced to about half the normal average. The fungi involved on this occasion were identified as *Dendryphion penicillatum* (Corda) Fr. the conidial stage of *Pleospora papaveraceae*, *Alternaria alternata* (Fr.) Keissler, *Cladosporium macrocarpon* and *Stemphyllium vesicarium* (Laughlin and Munro, 1982). In these field and laboratory experiments, associations were drawn between the degree of fungal cover of the capsules and their morphine concentration. The morphine concentration of capsules which had been colonized by fungi to the extent of >30% of their surface area were 20% lower in morphine than those capsules with a light fungal colonisation of <10%. The colonisation of these capsules was generally localized in the top half and the morphine concentration of the top half was about 20% less than the bottom half. In contrast, glasshouse-grown capsules which were free of fungal colonisation had similar morphine concentrations in the top and bottom halves of each capsule (Laughlin and Munro, 1982). These associations between fungal colonisation and morphine concentration were similar to those found in European poppy growing areas in Poland. In the Polish study (Miczulska, 1967), the morphine concentration of capsules was inversely proportional to fungal cover with a reduction of 50% morphine in capsules with more than a 50% cover of fungi.

In the field studies of Laughlin and Munro (1982) the development of fungal colonisation was often related to capsule tissue which had suffered physical damage and often to the removal of the waxy bloom which covers intact capsules (Hoffman and Menary, 1980). This damage and the removal of surface wax is often caused when capsules are chafed by the sharp stigmatic discs of neighbouring capsules but can also be a result of the abrasive effect of wind-blown sand and dust (Martin and Juniper, 1970) and rain (Ebercon *et al.,* 1977). In addition to allowing fungal colonisation, the removal of wax from the surface of capsules also facilitates the loss of morphine by leaching (Laughlin, 1985). In other Tasmanian studies, Hoffman and Menary (1984) also showed that losses of capsule codeine and thebaine were aggravated by loss of the surface wax from capsules.

6.1 *Sclerotinia sclerotiorum*

Sclerotinia stem infection by *Sclerotinia sclerotiorum* (Lib.) de Bary can occur relatively frequently with poppies in Tasmania. Although this infection does not cause any overall reduction in morphine levels in capsules it can weaken the stem and make the

plants more susceptible to lodging by wind. The typical symptoms of infection by *S. sckrotiorum* are that lesions with concentric dark and light zoned areas appear on the surface of the main stem in the lower third of the plant, most typically at the point of attachment of a senescent leaf. Later, the infected area of the stem shows a bleached appearance. Less commonly, lateral branches may show the same symptoms and on rare occasions the capsules may also be infected. The incidence of *Sclerotinia* stem infection and its effects on the dry matter and morphine production of poppies were assessed in a field experiment on krasnozem soil at Forthside (Laughlin and Munro, 1983). *Sclerotinia* had the effect of redistributing morphine within the plant but did not cause any reduction in total plant morphine. The concentration of morphine in terminal capsules was reduced by about 13% compared with non-infected plants but the concentration in lateral capsules was increased by a comparable amount and there were no effects in capsule dry matter. Similarly, there were no effects of *Sclerotinia* on the dry matter yield of stem and leaves but there was a marked increase of 75% in the morphine concentration of stem and leaves. This redistribution of morphine within the plant was interpreted as a disruption of normal translocation of morphine by the latex vessels to the terminal capsules. The effect on lateral capsules was further interpreted as the influence of *Sclerotinia,* altering the normal apical dominance within the plant so that lateral rather than terminal capsules became the main sink for morphine (Laughlin and Munro, 1983).

Although *Sclerotinia* infection does not appear to affect morphine production of poppies, there are practical considerations other than the effect on plant lodging which should be considered. In Tasmania, poppies are often grown as part of a rotation which includes a range of processing vegetables of which green beans *(Phaseolus vulgaris)* are particularly susceptible to *Sclerotinia*. The position in which poppies are placed in the rotation should be carefully considered as a means of controlling *Sclerotinia* in subsequent vegetable crops.

6.2 *Entyloma fuscum*

Poppy leaf smut *(Entyloma fuscum)* has appeared periodically in Tasmanian poppy crops and can be controlled by the application of propiconazole and chlorothalonil. Light infections of poppy leaf smut are not considered to significantly affect crop production in Tasmania. However, severe infection early in the growing season with premature defoliation may have very adverse effects on yield. The effects of partial and complete defoliation simulating *Entyloma* damage at various stages of plant development were studied in a field experiment on krasnozem soil at Forthside (Laughlin and Beattie, 1987). Complete defoliation at the hook and full bloom stages had drastic effects on capsule and morphine yields and the photosynthetic capacity of the stem did little to sustain growth. Complete defoliation at the hook stage reduced capsule yield and morphine concentration at dry harvest maturity by 86% and 50% less than nondefoliated plants respectively. The comparable reductions from complete defoliation at full bloom were 51% and 25% respectively. Complete defoliation at maximum volume of green capsules (two weeks after full bloom) had little effect on capsule yield or morphine concentration. Partial defoliation of either top or bottom leaves at the hook or full bloom stages resulted in a 25% reduction in capsule and

seed yields at dry maturity, but had no effect on capsule morphine concentration. Detailed symptoms of *Entyloma* infection in poppies and weed poppies in Tasmania have been recorded (Wiberg, 1990).

6.3 Insect Pests

Insect pests of poppies are not a major problem in Tasmania. There have been sporadic infestations of springtails *(Collembola sp.)* which can defoliate very young seedling plants on occasions. Spraying with methoate has been shown to be effective but this operation is only carried out routinely by a minority of growers. In some seasons in Tasmania damage to seedling poppy crops which had initially been attributed to insect pests was ultimately revealed to be caused by the European Skylark *(Alauda arvensis)* (Walker *et al.,* 1977). Damage caused by the European Skylark has been quite serious in some autumn-sown crops.

Native bud worm *(Heliothis punctigera)* is also an occasional poppy pest in Tasmania and damage usually occurs from the green capsule stage to the time of dry commercial harvest. A typical symptom of native bud worm attack is a small round hole in the capsule wall. The net result is a relatively small decrease in capsule yield but, more importantly, a more significant loss of seed. Insecticides can only be effectively applied by aerial spraying for this particular pest but this is rarely necessary or used.

7 HARVESTING AND PROCESSING

7.1 Time of Harvest

In Tasmania poppies are generally harvested when the crop is dry (12% moisture content) and the poppy alkaloids in the latex have formed a dried deposit on the walls of the capsules. Harvesting (Figure 11) is carried out with specialized headers, or more commonly, modified forage harvesters which take the poppy heads and a small quantity of stem (about 15cm). Capsules and seed are then separated in a later operation by sieving. Field experiments in Tasmania have studied the effect of earlier times of harvest on the dry matter and morphine yields of both capsules and stem and leaves (Laughlin, 1980a). In a field experiment on krasnozem soil, poppy plants were harvested at weekly intervals commencing ten days after full bloom and continuing until four weeks after dry commercial harvest (about eight weeks after full bloom). The changes in percentage dry matter over this period are shown in Figure 12. The changes in yield of dry matter and morphine content of capsules, stem and leaves and total plant over the same period are set out in Table 4.

7.1.1 Changes in Dry Matter

The dry matter yield of the total plant and of all the components other than seed gave maximum values two to three weeks after full bloom and then decreased. The decrease in total plant yield between maximum dry weight and commercial harvest (eight weeks after full bloom) was 26% (Table 4). The decrease for total capsules was 29% and for stem+leaves 39%. Other work has shown that the individual stem and leaf components
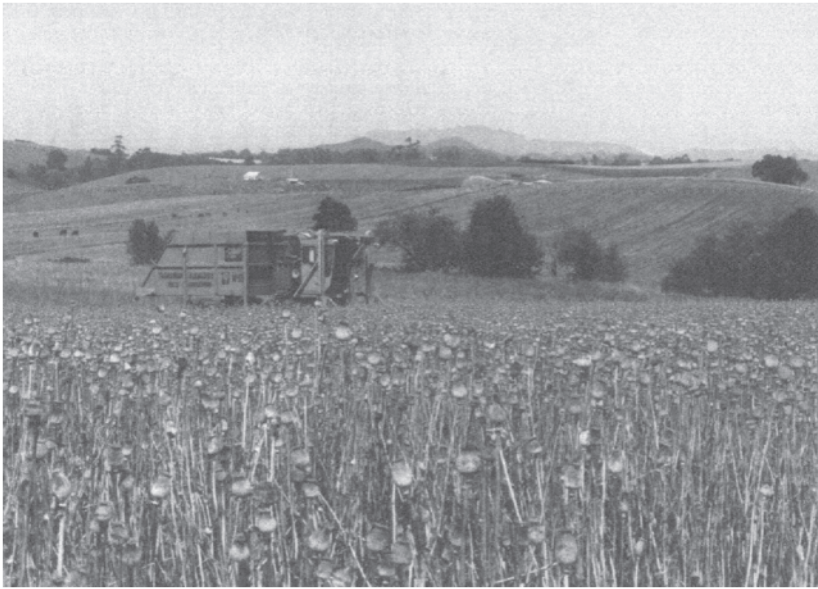
Figure 11 A field of dry poppies at harvest time (February) in North West Tasmania. Large mechanical headers and modified forage harvesters with a bin capacity of up to three tonnes are utilized. (The use of this photograph was facilitated by Mr Stan Blake, Poppy Industry Consultant, Devonport, Tasmania.)
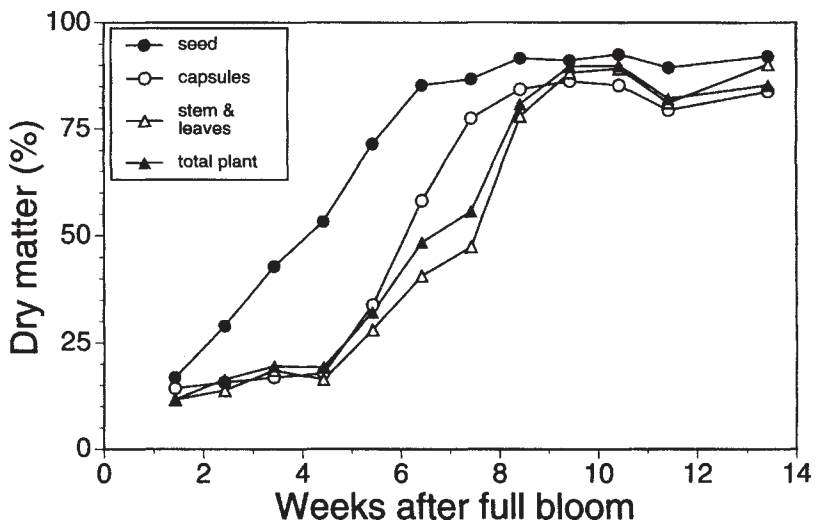
Figure 12 The effect of time of harvest between full bloom and dry maturity on the percentage dry matter of: ●, seed; ○, capsules; △, stem and leaves; and ▲, total plant (adapted from Laughlin, 1980a)

Table 4 The effect of time of harvest on dry matter and morphine yields of capsules, stem+leaves, seed and total plant (adapted from Laughlin, 1980a)

Time of harvest (days after full bloom)	Capsules			Stem + leaves			Total plant			Seed
	Dry matter (kg/ha)	Morphine (%)	(kg/ha)	Dry matter (kg/ha)	Morphine (%)	(kg/ha)	Dry matter (kg/ha)	Morphine (%)	(kg/ha)	Dry matter (kg/ha)
10	1251	0.70	8.73	8.42	0.09	7.86	10.11	0.16	16.59	442
17	1946	0.68	13.29	9.04	0.06	4.91	12.11	0.15	18.20	1127
24	1777	0.77	13.81	8.44	0.09	7.46	11.71	0.18	21.25	1488
31	1646	0.77	12.79	7.23	0.08	5.49	10.81	0.17	18.28	1929
38	1423	1.02	14.64	6.74	0.10	6.40	10.15	0.21	21.05	2000
45	1446	1.08	15.87	6.83	0.08	5.06	10.52	0.20	20.86	2120
52	1415	1.06	14.98	6.47	0.06	3.65	9.95	0.19	18.63	2069
59	1378	0.97	13.39	5.52	0.04	2.20	9.00	0.17	15.59	2099
66	1315	0.83	11.03	5.87	0.05	2.03	9.12	0.15	14.05	1950
73	1338	0.88	11.99	5.73	0.03	3.03	9.27	0.15	14.01	2186
80	1273	0.85	10.85	5.78	0.04	2.40	8.87	0.15	13.25	1803
94	1212	0.85	10.51	4.16	0.06	2.33	7.30	0.17	12.84	1917
SE of diff.	120.5	0.052	1.573		0.011		0.773		1.900	

both decline to a similar extent over this period (Chung, 1982). In contrast to these decreases, the total seed dry matter yield achieved a maximum by four weeks after full bloom and then remained constant at later harvests (Table 4).

7.1.2 Changes in Poppy Alkaloids

The morphine concentration of capsules reached a maximum value of 1.1% at six weeks after full bloom and then declined by about 10% at the stage of dry harvest. The morphine concentration of stem+leaves also reached a maximum of 0.1% at six weeks after full bloom but decreased rapidly after this and had halved by dry commercial harvest stage. The compensating factor of decreasing dry matter yield and increasing morphine concentration gave very similar total plant morphine yields at all times of harvest from two to seven weeks after full bloom. The morphine extracted from the whole plant during the period two to seven weeks after full bloom was approximately 50% greater than that obtained from capsules alone at the dry commercial harvest stage (Laughlin, 1980a).

Generally then, the morphine content of capsules relative to leaves and stem dictates that it is economical to harvest only capsules in Tasmania. Under average seasonal weather conditions at dry commercial harvest (eight weeks after full bloom) the small decrease (10%) in capsule morphine content from the maximum is more than compensated for by the fact that there is limited need for artificial kiln drying.

In other sequential harvesting field studies in Tasmania, morphine, codeine and thebaine in capsules were assayed at weekly intervals from full bloom for a thirteen-week period (Hoffman and Menary, 1979). The changes in morphine were substantially similar to those described above (Laughlin, 1980a). Morphine and codeine both reached maximum concentrations about five weeks after full bloom but the thebaine concentration was at a maximum one week after full bloom and decreased by 48% during the second week.

7.1.3 Effects of Rain and Delayed Harvest

Although weather conditions at harvest time are generally good in Tasmania, continued periods of wet weather can occur, causing delays in the harvest. The effect of delayed harvest on the morphine concentration of poppy capsules has been studied in Tasmania (Laughlin, 1985). The morphine concentrations in poppy capsules were measured in four different seasons during which plots of poppies were left for four weeks after the normal time of dry commercial harvest (Figure 13). Morphine reduction in the capsules increased weekly and the relative reduction between seasons was strongly associated with total rainfall. Similar associations have been drawn in other studies (Loftus Hills, 1945; Pfeifer and Heydenreich, 1962; Bunting, 1963; Schröder, 1965; Hoffman and Menary, 1979). In some of these studies overhead irrigation was found to cause reductions in morphine content similar to those experienced as a result of rainfall, with distinct cultivar differences (Loftus Hills, 1945; Laughlin, 1985).

Although significant reductions of morphine in poppy capsules have been associated with rain or overhead irrigation, these reductions may not always be the result of leaching or movement of morphine out of the capsule. Other influences, such as chemical conversions of morphine within the walls of the capsule (Kopp,
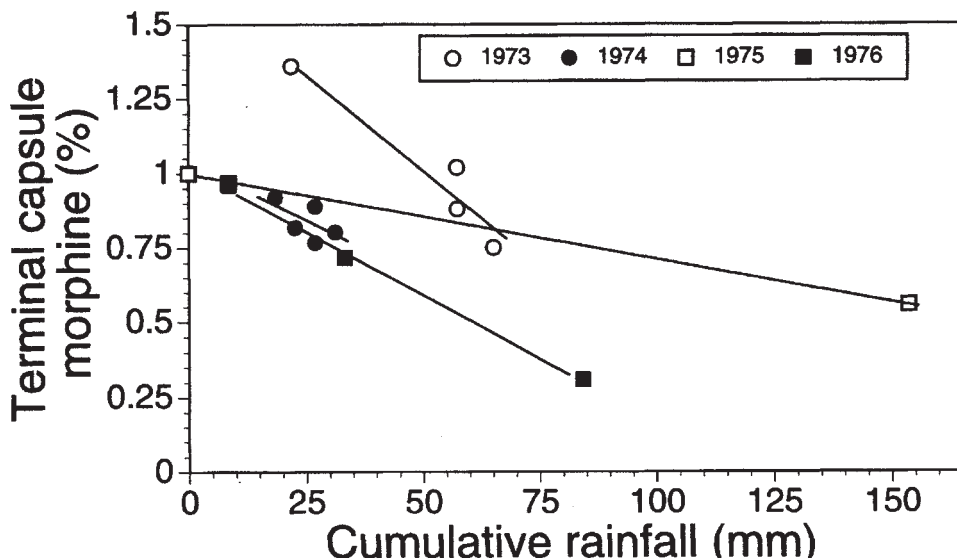
Figure 13 Relationship between cumulative rainfall *(x)* and morphine concentration of terminal capsules *(y)*. The data points represent mean values.
1973 (○):*y*=1.6718–0.0131*x*, *r²*=0.82; 1975 (□):*y*=1–0096–0.0027*x*, *r²*=0.99;
1974 (●):*y*=1.0587–0.0076*x*, *r²*=0.43; 1977 (■):*y*=1.0261–0.0084*x*, *r²*=0.99.
(Adapted from Laughlin, 1985.) *r²*=coefficient of determination

1957; Phillipson *et al.,* 1976) and metabolic conversion by capsule fungi (Laughlin and Munro, 1982), may also exert an effect. In a study in which intact capsules were immersed in deionized water in a glass receptacle for varying lengths of time up to 300 min, morphine was recovered from the immersion water. However the morphine detected in the immersion water only represented 25% of the actual decline in capsule morphine (Laughlin, 1985). In addition to the effects of rain, the battering of plants against each other—particularly the rubbing of the serrated stigmatic discs present at the top of adjacent capsules—causes removal of the waxy bloom which covers the capsule and this may also aggravate morphine loss (Laughlin, 1985). For all of these reasons every effort is made to harvest commercial poppy crops in Tasmania as soon as they reach the dry commercial harvest stage of 12% moisture in the capsules.

7.2 Drying and Storage

In certain unusually wet seasons in Tasmania poppies have been harvested at capsule moisture contents well above 12%. If the moisture content of the capsules is 16% or greater, artificial drying is used in order to prevent the development of moulds and fungi during bulk storage or the possible loss of morphine and other alkaloids by other chemical changes (Laughlin and Munro, 1982). The moisture characteristics

of the individual components of poppy capsules harvested at various times from green capsule to dry harvest maturity have also been studied in Tasmania (Nash, 1981). Generally lateral capsules had a higher moisture content than terminal capsules. Of the capsule components the placentae had the highest moisture contents in both terminal and lateral capsules. Even at seven weeks after full bloom the placentae of the lateral capsules had a mean moisture content of 17.5–20.5%. One of the inferences of this experiment is that if there were a relatively large moisture contribution from the lateral capsules then these may be the cause of damp patches and moulding during bulk storage. However, any attempt to virtually eliminate lateral capsules by the use of high-density strands would predispose the crop to lodging. In addition, at the optimum density of 70 plants/m^2 (Chung, 1990), a very significant part of the increase in head yield with irrigation is attributable to the effect on the number and yield of lateral capsules. In this situation the occasional possibility of having to resort to artificial drying is outweighed by the economics of the increase in total head yield.

ACKNOWLEDGEMENTS

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4. AN ALTERNATIVE RAW—THE CULTIVATION AND BREEDING OF *PAPAVER BRACTEATUM*

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1 INTRODUCTION

The species *Papaver bracteatum* is considered to be a potential alternative to opium poppy for codeine production due to its high content of thebaine and complete absence of morphine (Böhm, 1981). Thebaine is a precursor of codeine and can be easily converted to codeine by the pharmaceutical industry; thebaine is also a starring material for a number of narcotic antagonist drugs, such as naloxone and naltrexone, and of Bentley compounds (McNicholas and Martin, 1984). In some populations of this species, the capsules and roots contain almost exclusively thebaine; thus the extraction and purification of the raw material is relatively easy.

A world-wide effort was invested during the 1970s to domesticate and develop this species as an alternative source to the opium poppy for codeine production.

2 BOTANY AND DISTRIBUTION

Papaver bracteatum Lindl. is one of the three species belonging to the section *Oxytona*; two other perennial species *Papaver orientale* and *Papaver pseudo-orientale* of this section are closely related to *P. bracteatum*. Plants of these species were first brought to Europe early in the eighteenth century by Tournefort and were introduced as 'oriental poppy' (Goldblatt, 1974).

P. bracteatum is perennial, and produces every year during the winter a rosette of long dissected leaves. In the spring, when the leaves are fully developed, the plants produce non-branched 50–80 cm long flowering stems, with a single large flower. Three to eight floral bracts are formed at the base of the bud and are characteristic to this species. The bracts remain attached to the open flower and to the mature capsule. The flowers have four to six petals of a deep dark red colour with a black mark at their base. The plant usually starts flowering in the second growing season, and produces about 3–5 flowers. In the following years, up to 25 flowers may be produced by one plant.

The fruit is a dry capsule about 3cm long and 2g in weight, covered with a large flat stigmatic disc. At maturity, the stigmatic disc separates from the capsule enabling

scattering of the seeds through pores of dehiscence. After maturation of the capsules, the aerial parts dry out and the plant remains dormant during the summer until a new vegetative growth resumes in the following winter.

   P. bracteatum is naturally distributed in high altitudes from 1500 to 2500m. The species is found in three distinct areas: the Alborz mountains north of Tehran, in the Iranian Kurdistan and on the Northern slope of the Caucasus. It is an out-crossing species with gametophytic self-incompatibility; the prominent flowers of the plant attract several insects, bees and beetles which perform pollination (Goldblatt, 1974).

3 CHEMISTRY AND PHARMACOLOGY

The chemistry of *P. bracteatum* has been studied for more than 50 years. In the early reports, different alkaloids were isolated from various plant materials named '*P. bracteatum*'. Because of the confusion between the three species of section *Oxytona* which often occurred, the plant material was not characterized properly in some of these studies (Theuns *et al.*, 1987). Therefore, only studies where appropriate characterization of the species were performed are included in this review.

   Alkaloids can be found in different concentrations in most of the plant tissues. The alkaloid spectrum and content differs during plant development. In general,
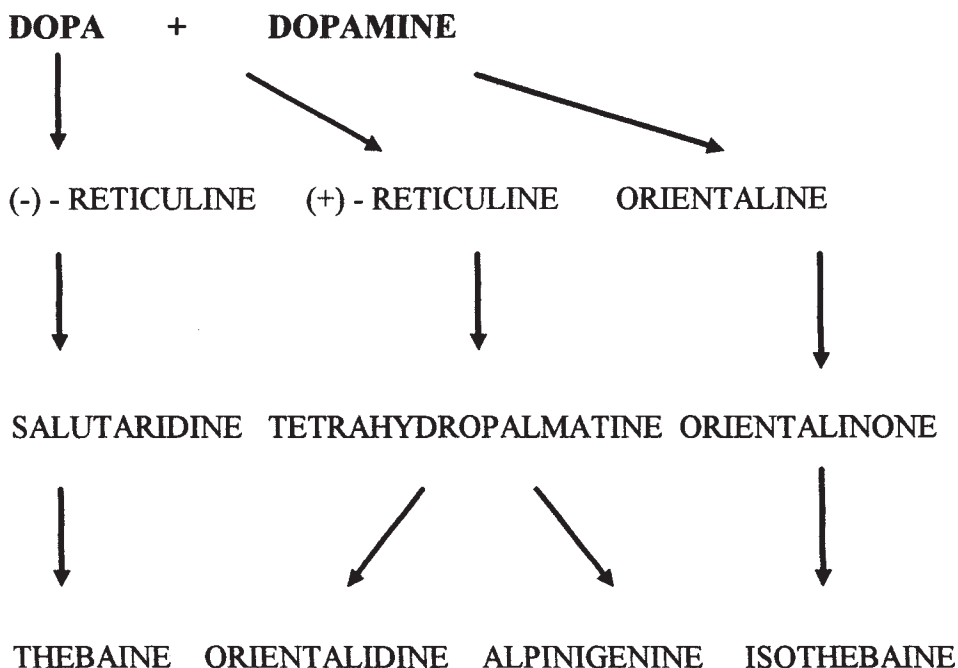
Figure 1 Biosynthesis pathways of some major and minor alkaloids of *P. bracteatum*

several alkaloids which are found during the young vegetative stage of the plant disappear after three to four months. The highest alkaloid content is found in the capsules or in the latex where it might reach 26% (Sharghi and Lalezari, 1967).

Several studies on the chemical composition of *P. bracteatum* were conducted during the seventies (Böhm, 1981; Fairbairn and Hakim, 1973; Meshulam and Lavie, 1980). These results are summarized in Figure 1 which presents the biosynthetic links between the major and some of the minor alkaloids in this species. The biosynthetic pathway for the formation of thebaine is similar to that in *P. somniferum* (Hodges *et al.*, 1977), in which this compound is further demethylated to codeine and morphine. Thebaine is also the precursor of oripavine, the major alkaloid of *P. orientale*. As mentioned earlier, *P. bracteatum* is unable to demethylate thebaine to either one of these morphinane alkaloids.

The chemical race Halle III which contains thebaine as predominant alkaloid (98% of total alkaloids) was first reported by Neubauer and Mothes in 1963, who found a thebaine content in the roots of 0.7–1.3% of dry matter. This profile was confirmed in 1967 by Sharghi and Lalezari, who reported 26% thebaine and no morphine in the latex of plants growing in the Alborz mountains in North Iran. Böhm (1967, 1971) found another alkaloid (E), which was identified by Lalezari *et al.* (1973) as alpinigenine in the Halle III population. In 1974, a population called Arya II with a thebaine content of 3.6% of dry mater, was found in Western Iran by Lalezari *et al.* (1974).

4 PHYLOGENETIC RELATIONSHIPS IN SECTION *OXYTONA*

Considerable confusion has prevailed in the systematic classification of the section *Oxytona* resulting in insufficient characterization of the species Yasui (1936). Goldblatt (1974) reviewed the taxonomy, chemistry and ecology of the section and included three species in it: *P. bracteatum* Lindl., diploid 2*n*=14; *P. orientale* L., tetraploid 2*n*=28; and *P. pseudo-orientale* hexaploid 2*n*=42. Based on morphological characters Goldblatt (1974) suggested that *P. orientale* is an allotetraploid, originating from a cross between *P. bracteatum* and another unknown alpine diploid species. Later on, a backcross of *P. orientale* to *P. bracteatum* gave a triploid plant from which, following chromosome doubling, the hexaploid species *P. pseudo-orientale* was obtained. According to his theory *P. pseudo-orientale* (*n*=3X) has two sets of chromosomes of *P. bracteatum* and one set of the unknown alpine ancestor of *P. orientale*. Ojala *et al.* (1990) proposed a different allopolyploid composition for the *Oxytona* section which is partly in agreement with Goldblatt's model.

Milo *et al.* (1986, 1988) addressed this issue using various experimental approaches. Cytogenetic analysis revealed the formation of multivalents at diakinesis, in the polyploid species *P. pseudo-orientale* and in its hybrid with the diploid species. This finding demonstrates the autoploid nature of *P. pseudo-orientale* and shows that *P. bracteatum* is its ancestor. The similarities found in isozyme variation and in chloroplast DNA restriction patterns between and within the three species of section *Oxytona* strongly indicate the autoploid nature of this section.

5 CULTIVATION ASPECTS

5.1 Effect of Environmental Factors on Seed Germination and Seedling Establishment

The effect of temperature on the germination of newly harvested seeds is shown in Table 1. Both the germination rate and speed were influenced by temperature. High temperatures increased the rate of germination. Treatment of seeds with GA$_3$ increased the germination rate and speed markedly (Palevitch and Levy, 1992).

5.2 Exploitation of Capsules

P. bracteatum is known to flower during the second growing year. However, breeding lines flowering during the first growing season have been developed. Early sowing is important to ensure a high flowering rate and capsule yield per plant as demonstrated in Table 2.

5.2.1 Direct Sowing vs. Transplanting

The effect of seedling type and direct sowing on the capsule yield components was studied in a synthetic cultivar selected for early flowering (Palevitch and Levy, 1983).

Flowering rate, number of capsules per plant and their yield were much higher in direct sowing than in transplanting. A higher flowering percentage and yield of capsules were obtained from the seedling transplants than from bare-root transplants (Table 3).

Table 1 Effect of temperature on germination (%) of fresh seeds (Palevitch and Levy, 1992)

Temperature (°C) (*night/day*)	Days after sowing			
	11	24	37	50
20/20	2.5 a*	10.5 a	11.5 a	15.0 a
10/20	0.0 b	0.0 b	12.5 a	15.0 a
20/30	0.0 b	3.0 b	53.0 b	61.0 b

* Within the columns, values followed by the same letter do not differ significantly at $p \leq 0.05$.

Table 2 Flowering rate (%) and yield of capsules of *Papaver bracteatum* from three different planting dates (Palevitch and Levy, 1983)

Plantting date	Flowering rate (%)		Capsules/plant	Yield/plant (g)
	24 *April*	4 *June*		
19 August	57.6 a*	92.1 a	2.2 a	5.1 a
29 August	50.3 a	88.6 a	2.0 b	4.8 a
13 September	27.6 b	74.2 b	1.1 c	2.2 b

* Within the columns, values followed by the same letter do not differ significantly at $p \leq 0.05$.

Table 3 Flowering rate (%) and yield of capsules of *Palaver bracteatum* from three different seedling types in the first growing season (Palevitch and Levy, 1983)

Seedling type	Plants/m^2	Flowering (%)	Capsules/plant	Capsule yield/m^2
Direct sowing	6.1 a*	76.6 a	2.2 a	36.8 a
Seedling transplant	5.0 b	27.3 b	1.5 b	16.1 b
Bare-root transplant	4.4 c	20.9 c	1.4 b	11.6 c

* Within the columns, values followed by the same letter do not differ significantly at $p \leq 0.05$.

On the other hand, Levy *et al.* (1988) found that plant densities from 3.4 to 6.3 per m^2 had no effect on the thebaine yield from capsules during two consecutive growing years. An increase in the number and weight of capsules per plant compensated for the low plant density resulting in the same capsule and thebaine yield per unit area. Fairbairn and Helliwell (1977) estimated that at a plant density of 6.2 plants/m^2 a yield of 25kg/ha thebaine can be obtained. Collection of latex would yield 58kg of dry material per hectare, compared with 26.5kg/ha from *P. somniferum* (Nyman and Bruhn, 1979).

5.2.2 Effect of Gibberellin on Flowering and Capsule Yield

Cold induction is necessary for flowering induction in *P. bracteatum*. Gibberellin (GA$_3$) can supplant vernalization in several plants species with such flowering patterns. The treatment of *P. bracteatum* plants with 250mg/l gibberellin enhanced flowering in late-flowering clones and significantly increased the number and weight of capsules. The thebaine concentration in the capsules was not significantly affected by GA$_3$; however, the thebaine yield per plant was greatly increased as a result of the higher number and weight of capsules (Levy *et al.,* 1986).

5.3 Exploitation of the Roots

The presence of thebaine in the roots of *P. bracteatum* enables growth of this crop under a wider range of climatic conditions, and not only in locations with a cool climate. Moreover, the root biomass is much higher than the yield of capsules, and can compensate for the lower thebaine content of the roots. Substantial yields of thebaine are obtained from the roots of the plant.

5.3.1 Plant Densities

Variations in plant density had no significant effect on the thebaine yield of the roots (Levy *et al.,* 1988). An increase in plant density from 2.4 to 4.5 plants per m^2 had no effect on the dry root yield (310 and 325 g/m^2 respectively) or on the thebaine yield (1.9 to 2.0g/m2).

5.3.2 Thebaine Fluctuations During Plant Development

Böhm (1967) studied the thebaine content in roots of Halle III strain. He observed a steady increase of thebaine content during growth from germination to four-month

old plants. The same pattern of linear increase in thebaine content was found in an Iranian plant population (Nyman and Bruhn, 1979). The onset of flowering was followed by a significant decrease of the thebaine content in the roots (Fairbairn and Helliwell, 1977; Aynehchi and Jaffarian, 1973). In the developing capsule, the alkaloid content reached a peak about one month after flowering, and decreased rapidly in the following weeks. A marked increase of thebaine content in the leaves of flowering plants was reported by Saco and Lopez-Belmonte (1987) with a significant negative correlation between precipitation (mm) and thebaine content. Unfortunately, the authors did not study the effect of rain fall on the production of the leaf biomass and thebaine yield.

The thebaine yield components of the roots were studied at various stages of plant development (Levy *et al.,* 1988). The highest concentration of thebaine was obtained at the start of flowering. However, the dry weight of the root and the thebaine yield increased until full flowering. Significant differences in the thebaine and dry matter distribution between various parts of the roots were found: the lower part had a higher concentration of alkaloids than the upper one. A high thebaine yield of 2.5 g/m^2 was obtained in the first growing season. It is therefore possible to exploit *P. bracteatum* both for capsule and root production. Alternatively, the capsules could be harvested during the first two or three years and then the roots.

6 GENETICS AND BREEDING

Studying the genetic control of thebaine formation and accumulation, along with other agronomic characteristics is essential for developing appropriate breeding methods for *P. bracteatum*. Böhm (1970) was the first to suggest that self-incompatibility prevails in *P. bracteatum*. This was later confirmed by Palevitch and Levy (1983), who suggested its gametophytic nature. Mentor pollen was found to be effective in overcoming the self-incompatibility: 23% of the treated capsules set seeds. However, the selfed capsules contained only small number of seeds (up to 173 seeds per capsule) compared with 1000 seeds that are normally produced by open pollination.

6.1 Genetic Variation in Thebaine Yield Components, Heritability and Selection Response

A large variation in thebaine yield components was found in populations of *P. bracteatum* (Böhm, 1967; Vincent *et al.,* 1977; Levy *et al.,* 1979).

Marked differences in the thebaine yield components were obtained in different clones of Arya I and Arya II populations (Levy *et al.,* 1981): In the second growing season, the capsule number per plant varied between 8.6 and 26.0 with thebaine content from 1.1% to 3.4% of dry weight. Heterosis above the best performing parent was detected in two F1 combinations. A significant increase in the thebaine yield (more than three times) than that of the best parent in the cross, was found in one F1 hybrid. All the other hybrids had an average performance of the parental clones.

Heterotic effects can be used in a breeding program aimed at increasing the thebaine yield. This species is suited for the production of hybrids because of the very large

quantities of seeds that are produced in each capsule. Self incompatibility can be used for this purpose.

No correlation was found between the thebaine content and yield in capsules and in the roots (Levy *et al.,* 1981). Therefore, both characteristics can be improved by independent selections and individuals with a high alkaloid content in both organs can be bred.

The heritability (h^2) of thebaine content was found to be significantly higher than for the other yield components. This indicates that thebaine content is the most reliable characteristic for efficient selection.

6.2 Effect of Polyploidy on Thebaine Content and Yield

Wold *et al.* (1983) reported an increased thebaine content of the capsules in a population of plants raised from colchicine-treated seeds. A later report from this group (Laane *et al.,* 1988), includes a cytogenetic study of the colchicine-treated plants. The plants seem to be chimeric, with diploid and tetraploid sectors. No morphological differences were found between the treated plants and the original population. Remarkable differences in thebaine content were reported between plants raised from the colchicinetreated seeds in the seven years of that study. Furthermore, 24 plants originating from one tetraploid plant by vegetative propagation exhibited a large variation between plants from 3.1% to 17.9% thebaine in mature capsules. Unfortunately, data concerning the capsule yield and the variation found in the original plants were not included in this report.

In another study, Milo *et al.* (1987) evaluated the thebaine content and yield in capsules of colchicine-induced autotetraploid plants, its diploid source and the autotriploid hybrid. A significant increase in the thebaine content was found in the tetraploid plants during two consecutive growing seasons. Thebaine contents of 4.9% and 4.78% of dry weight were found in the tetraploid plants compared with 2.4% and 2.15% thebaine in the diploid plants. On the other hand, the number of capsules per plant was significantly reduced in the tetraploid (5.0) and triploid plants (8.3) in comparison with the diploid plants (13.2). The triploid plants had a very high thebaine content in the first growing year (8.8%) but these plants had only a few very small capsules. In the second year, the thebaine content decreased to the same level found in the diploid plants. The increase in the thebaine content obtained in the tetraploid plants is of primary value, although the thebaine yield was slightly reduced; indeed, the concentration of the active compound is much more important economically than the plant biomass component, because it affects the efficiency of the extraction of the compound.

6.3 Chemical Spectrum of Inter-specific Hybrids

P. bracteatum and *P. orientale* are characterized by the high content of the morphinane alkaloids thebaine and oripavine, respectively. If the biosynthesis of these alkaloids is blocked, chemical races having salutaridine and alpinigenine are found (Theuns *et al.,* 1987). *P. pseudo-orientals* always has isothebaine as a dominant alkaloid, and morphinane alkaloids may be found in trace amounts (Böhm and Nixdorf, 1983). The minor alkaloids that may or may not be present in these species are: protopine,

orientalidine, codeine, neopine, bracteoline mecambridine and any of the alkaloids mentioned as major ones.

The inter-specific hybrid between *P. bracteatum* and *P. pseudo-orientale* has an intermediate phenotype. It has the alkaloid spectrum of the polyploid parent, with an increase in the thebaine content and reduced isothebaine compared with *P. pseudoorientale* (Levy and Milo, 1991).

On the other hand, the alkaloid profile of the inter-specific hybrid between *P. bracteatum* and *P. orientals* was different from both parents and contained only thebaine and oripavine (Milo *et al.,* 1990). The oripavine concentration was up to ten times higher in the hybrid compared with the *P. orientals* parent. The thebaine content was much lower than in *P. bracteatum.*

The inter-specific hybrid between *P. bracteatum* and *P. somniferum* was studied by Nyman and Bruhn (1979) and Pyysalo *et al.* (1988). This hybrid contained 1.5% morphine, only 0.1% thebaine and traces of alpinigenine in dry capsules. In this case as for the hybrid with *P. orientals* the thebaine produced by the *P. bracteatum* genome was converted into oripavine or morphine by the demethylating-reducing system of the *orientale* or *somniferum* genome respectively.

6.4 Mutants in Agronomic and Chemical Traits

A spontaneous shattering-resistant mutant of a dominant nature was isolated in an experimental field of *P. bracteatum* (Levy, 1985). There were no marked differences between the mutant and normal plant in the dimensions and weight of the capsules, except for the size and shape of the stigmatic disc. The normal capsule is topped by a large flat stigmatic disc with pores of dehiscence under it. In the mutant, the smaller stigmatic disc is concave and does not detach from the capsule at maturity, preventing the formation of dehiscence pores. This mutant can be very useful in the cultivation of *P. bracteatum* for the production of seeds and oil, which are of similar compositions as those of *P. somniferum* (Nyman and Bruhn, 1979). Furthermore, the thebaine content of this mutant was higher than that of the normal plants.

7   TISSUE CULTURE FOR ALKALOID PRODUCTION
AND VEGETATIVE PROPAGATION

Tissue cultures of *P. bracteatum* have been studied for thebaine production. Unfortunately, little success has been achieved in producing the desirable alkaloid in substantial amounts in cell or tissue culture. As for *P. somniferum,* a different alkaloid profile is obtained in culture, compared with plants. Thebaine was present in only trace amounts in cell cultures (Kamimura and Nishikawa, 1976) or absent (Lockwood, 1981; Kutchan *et al.,* 1976; Hook *et al.,* 1988; Alkhimova *et al.,* 1993). On the other hand, dopamine, the precursor of the morphinane biosynthetic pathway in *Papaver* species, and sanguinarine, an alkaloid which is rarely detected in plants, were produced in significant amounts in culture (Cline *et al.,* 1988).

Rush *et al.* (1985) demonstrated the correlation between the appearance of laticifer cells and the detection of thebaine in germination seedlings. It seems that for thebaine production in cell cultures, differentiation is needed.

Day *et al.* (1986) were successful in regenerating plants from embryogenic callus culture of two varieties of *P. bracteatum*. The regenerated plants were transplanted into soil and grown in a greenhouse for seven weeks; these plants produced a thebaine concentration similar to the original variety. Using tissue culture as a method for mass micropropagation in *P. bracteatum* might be useful for rapid multiplication of superior individuals. The self-incompatibility system in this species does not allow the creation of pure lines through selfing.

8 CONCLUSIONS

The most widely used opiate in medicine, codeine, is mainly produced from the opium poppy *P. somniferum*. However, the plants major alkaloid, morphine, and its highly addictive derivative heroin are also used illegally as drugs. Codeine can also be produced from thebaine, the major alkaloid of *P. bracteatum*. The addiction potential of thebaine or its derivatives, and of the minor alkaloids reported for *P. bracteatum* is negligible and no cases of abuse or illicit production of thebaine have been reported (Theuns *et al.,* 1986).

The cultivation of *P. bracteatum* for commercial purposes has been limited by sociopolitical considerations which reflect the fear of negative consequences for the economy of traditional growers of opium for the pharmaceutical industries. Whenever the climatic conditions are suitable for the growth of *P. bracteatum* it may advantageously replace the traditional *P. somniferum*.

Higher yields of codeine per unit area can be obtained from some of the best performing breeding lines of *P. bracteatum* in comparison with *P. somniferum*. Moreover, higher yields of codeine per unit area can be obtained from exploitation of the capsules and roots of the plant.

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VI. PHARMACOLOGY OF POPPY ALKALOIDS

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1 INTRODUCTION

The latex obtained by the incision of unripe seed capsules of *Papaver somniferum* and which is known as opium is the source of several pharmacologically important alkaloids. Dioskorides, in about AD 77, referred to both the latex *(opos)* and the total plant extract *(mekonion)* and to the use of oral and inhaled (pipe smoked) opium to induce a state of euphoria and sedation. Since before the Christian era the therapeutic properties of opium were evident, with the first written reference to poppy juice by Theophrastus in the third century BC. Powdered opium contains more than 40 alkaloids which constitute about 25% by weight of the opium and are responsible for its pharmacological activity.

In 1803 the German pharmacist Sertürner achieved the isolation of morphine as one of the active ingredients of opium. Morphine, codeine, thebaine, papaverine, narcotine and narceine are the most important bases, with many of the remaining (minor) alkaloids occurring only in traces.

2 MAJOR OPIUM ALKALOIDS

2.1 Morphine

Morphine has long occupied an eminent position on the list of useful drugs. As a pure alkaloid, it has been employed for over a century and a half and, as the most important constituent of opium, it has contributed to the comfort of the human race since very early times. Morphine has greatly facilitated the practice of medicine by furnishing the physician with a potent, reliable and relatively inexpensive analgesic agent. (-)-morphine, the naturally occurring enantiomer combines selectively at many recognition sites through the body to produce pharmacological effects.

Extensive structure-activity studies involving hundreds of compounds have established firm stereochemical structural requirements for morphine-like activity (Braenden *et al.,* 1955; Casy and Parfitt, 1986; Eddy *et al.,* 1956; Fürst *et al.,* 1995).

Studies of the binding of various ligands over the past 50 years in brain and other tissues suggested the existence of multiple and distinct receptor types that can interact with opioid drugs. There is reasonably strong evidence for three main categories of opioid receptors in the brain, designated μ, κ and δ (Casy, 1989; Martin, 1984; Paterson *et al.,* 1983). The affinity of morphine for μ-receptors is about ten times that for δ

and κ-receptors. A fourth receptor, the s-receptor is more controversial but may be related to the dysphoric, hallucinogenic and cardiac stimulant effects of certain opioids.

Although biochemical and pharmacological evidence indicates that the μ-, δ- and κ-receptors are distinct molecular entities, all three classes of opioid receptors share a number of characteristics. They are usually found on presynaptic nerve terminals, where their action results in decreased release of excitatory transmitters. They all appear to be coupled to guanine nucleotide binding regulatory proteins (G-proteins), and elicit their actions via the cAMP system (Collier and Roy, 1974a, b). Collier and Roy also reported the inhibition of the accumulation of cyclic AMP in rat brain homogenates. In the neuroblastoma×glioma hybrid cell line, NG108–15 was observed to be rich in opiate receptors that are coupled as inhibitory modulators to adenyl cyclase (Sharma et al., 1975).

Morphine exerts its effects either by hyperpolarizing or inhibiting postsynaptic neurones, probably by increasing K_+ efflux, or by reducing Ca^{2+} influx into presynaptic nerve endings and thereby reducing transmitter release, including acetylcholine, norepinephrine, dopamine, serotonin and substance P (Katzung, 1995).

(-)-Morphine produces its main action, analgesia, primarily through interaction with μ-receptors. The antipode, (+)-morphine is devoid of anti-nociceptive activity, although it showed some central action when administered intracerebrally (Jacquet et al., 1977).

Other consequences of μ-receptor activation include euphoria, respiratory depression, miosis, constipation and dependence. Two apparently distinct types of μ-receptors have been detected, based on their relative affinities for agonists: μ-1, having a higher affinity to agonists, postulated to mediate supraspinal analgesic action, and μ-2, having a lower affinity, postulated to mediate spinal analgesia, dopamine turnover, growth hormone release, respiratory depression, gastrointestinal actions, inhibition of guinea pig ileum contractions, bradycardia and reversal of endotoxic shock (Casy, 1989; Reisine and Pasternak, 1996).

κ-receptors might contribute to morphine analgesia at the spinal level and mediate sedative actions as well (Horwell, 1988; Rang et al., 1995).

The three main receptor types have been isolated and cloned (Katzung, 1995). It was demonstrated that the contraction of the coaxially stimulated longitudinal muscle strip of guinea pig ileum (GPI) was inhibited by morphine (Paton, 1957; Paton and Vizi, 1969). In this preparation the ID_{50} value means the concentration of drug required to inhibit the twitch (induced by the release of acetylcholine) height by 50% and is a measure of agonist potency. Bioassays measuring in vivo morphine analgesia or inhibition of electrically induced contraction of guinea pig ileum induced by morphine clearly showed good correlation and similar dose-dependent effects with a well defined maximal response (Kosterlitz and Waterfield, 1975; Leslie, 1987).

It was found that a mouse vas deferens (MVD) preparation behaved similarly to the GPI and can also be used for detection of agonist activity of morphine-like substances (Henderson et al., 1972). Morphine inhibits transmission in postganglionic adrenergic fibres in small doses (Trendelenburg, 1957).

Morphine produces an increase in muscle tone (Straub's phenomenon) and a marked increase in spontaneous motility in the mouse. In laboratory animals morphine induces catalepsy accompanied by marked rigidity, which is antagonized by morphine antagonists (Ahtee, 1978).

Pharmacologically, morphine is active in all the standard bioassays for analgesia both in animal or humans (Geller and Axelrod, 1968; Kuhar and Pasternak, 1984). It is able to change both pain perception and the reaction to pain. It is prescribed for the symptomatic relief of severe pain in humans.

μ-opiate receptor agonists, like morphine, are approximately equipotent in heat (hot plate, tail flick) and non-heat (acetic acid writhing and pression) induced nociception. The anti-nociceptive potency ratios of morphine in animal heat tests were found to be similar to those for analgesia in man (Tyers, 1980).

Morphine-induced analgesia is due to actions at several sites within the central nervous system (CNS): both spinal and multiple supraspinal sites have been identified. The sites of morphine analgesic action are the periaqueductal grey matter (PAG), the dorsal horn of the spinal cord and probably the limbic system. Morphine selectively inhibits various nociceptive reflexes and induces analgesia when administered intrathecally or instilled locally into the dorsal horn of the spinal cord, into the third ventricle or in the midbrain and medulla, most remarkably in the PAG and the nucleus raphe magnus (Yaksh, 1981).

Brain loci involved in the transmission of pain and in the alteration of reactivity to nociceptive stimuli appear to be primary, but are not the only sites at which morphine acts. Recently, evidence has begun to accumulate regarding the possibility on peripheral opioid anti-nociception (Katzung, 1995; Stein, 1994).

Morphine is readily absorbed when taken orally, injected subcutaneously (s.c.) or intramuscularly (i.m.). It is also easily absorbed from the mucosal surfaces of the nose or mouth. Morphine gains access to the brain with difficulty, being an amphoteric agent (Burgen and Mitchell, 1985; Oldendorf, 1978).

Its effects are seen in about half an hour and begin to pass away after 3–5 hours but may last for at least 12 hours. The bioavailability of morphine taken orally may be considerably reduced because of significant first-pass metabolism by glucuronidation in the liver (Oguri, 1980; Oguri et al., 1970; Yeh et al., 1977). Morphine is also N-demethylated in the liver. A certain amount is excreted into the stomach and probably into other parts of the alimentary canal (Burgen and Mitchell, 1985; Rang et al., 1995). Recent findings indicate that morphine-6-glucuronide possesses greater analgesic properties than morphine (Katzung, 1995; Pasternak et al., 1987).

Morphine exerts its most important actions on the CNS, where it causes depression and excitation of certain centres. The parasympathetic portion of the oculomotor nucleus is stimulated and the pupils became contracted—in cases of morphine poisoning they may be of pinpoint size. Miosis is also a pharmacological action to which little or no tolerance develops. This action can be blocked by atropine and by opioid antagonists (Martin, 1984). Morphine depresses acetylcholine turnover in parietal and occipital cortices, hippocampus and nucleus accumbens (Loh and Ross, 1979; Szekely and Ramabadran, 1990). Other effects mediated via the CNS include a feeling of heaviness in the limbs, a dry mouth, itching and the reduction of hunger sensations.

Morphine disrupts normal REM and NREM sleep patterns. It exercises a biphasic action on cerebral electrical activity at low doses in humans, which correspond to analgesic doses in the rat. Electrical tracings tend toward synchronization with an increase of slow waves and spindles. In the cat, desynchronization can be seen. High

doses of morphine induce EEG convulsive manifestations (Iwamoto and Martin, 1981; Martin, 1984).

The average convulsive and fatal dose of morphine for nearly all animal species depends on several factors, such as age, diet, and degree of hydration (Eddy, 1939; Reynolds and Randall, 1957).

Drowsiness and mental clouding usually occurs after morphine administration. It has been proposed that morphine simultaneously activates two different processes resulting in opposite changes in spontaneous motility: hyperactivity or hypoactivity (Schnur and Hang, 1985). In contrast to humans, a number of species (cats, horses, cows, pigs) may manifest excitation rather than sedation when given morphine.

Morphine depresses the cerebral cortex and reduces the powers of concentration and fear. Morphine causes a sense of satisfaction and well being (euphoria) and freedom from anxiety and distress. In addition, pain, particularly prolonged as opposed to acute pain, is reduced and these actions produce a feeling of contentment. Its intravenous (i.v.) administration has been reported to result in a sudden 'rush' similarly to 'abdominal orgasm'. Euphoria appears to be mediated by μ-receptors (Katzung, 1995).

Microinjection of morphine into the ventral tegmentum activates dopamine neurones to project to the nucleus accumbens. This pathway might be a critical element in the reinforcing effects of morphine and morphine-induced euphoria.

Various centres in the medulla are affected by morphine. Morphine activates the brain stem chemoreceptor trigger zone to produce nausea and vomiting. Also, morphine has an action of vestibular apparatus. The vomiting centre and associated centres for salivation, sweat and bronchial secretion are stimulated first, though they became depressed by large and subsequent doses. Sweating is associated with vasodilatation of the skin vessels, so that administration of morphine also increases heat loss (Burgen and Mitchell, 1985).

Respiratory depression is another undesirable effect of morphine. In man, respiration is depressed by doses which are below the narcotic threshold. Large doses are fatal by stopping respiration altogether (Rang *et al.,* 1995; Reisine and Pasternak, 1996). Morphine therapy must therefore be used with particular care in obstetrics where foetal respiration may be affected and in respiratory ailments such as bronchial asthma. Morphine interacts with respiratory modulator processes principally by decreasing the responsivity of the respiratory centre to CO_2 and may have some selectivity in depressing neuronal modulation of the respiratory centre. Opioid-induced respiratory depression is mediated by μ-2 receptors.

Among the peripheral actions of morphine, constipation is one of the most important. The constipation produced is unaffected by denervation of the intestine or by atropine and is largely due to an increase of the tone of the gut and sphincters and an inhibitory action on the Auerbach plexus. Other factors that probably increase this action of morphine are inhibition of the secretion of the intestinal glands and depression of the reflexes responsible for defecation (Burgen and Mitchell, 1985; Katzung, 1995; Rang *et al.,* 1995).

Morphine has anti-diuretic action (i.e. it inhibits urinary output) because it may increase the tone and amplitude of contractions of the ureter, inhibiting the urinary voiding reflex. Urine retention is observed even with therapeutic doses. Morphine stimulates the release of anti-diuretic hormones such as prolactin and somatotropin

but inhibits the release of luteinizing hormone (Reisine and Pasternak, 1996). Morphine also causes retention of bile by closing the sphincters. It raises the pressure in the common bile duct and may cause biliary colic (Burgen and Mitchell, 1985; Katzung, 1995).

Morphine might produce hypotensive action in subjects whose cardiovascular system is stressed. This hypotensive effect is probably due to peripheral and arterial dilatation, which has been attributed to various factors, e.g. release of histamine and central depression of vasomotor stabilizing mechanisms.

Morphine affects cerebral circulation minimally, except when P_{CO2} is increased. This increased P_{CO2} leads to cerebral vascular dilatation, a concomitant decrease of cerebral vascular resistance, an increase of cerebral blood flow and an increase in cerebrospinal fluid pressure (Katzung, 1995).

Morphine inhibits the formation of rosettes by human lymphocytes. The administration of morphine to animals causes suppression of the cytotoxic activity of natural killer cells and enhances the growth of implanted tumours (Goodmann Gilman's 1990 *The Pharmacological Basis of Therapeutics* (see Reisine and Pasternak (1996); Katzung, 1995).

Tolerance to morphine occurs, usually takes 2–3 weeks to acquire on normal therapeutic doses and it applies mostly to the depressant action of the drug. The effects on the pupils and on the intestine remained unchanged during chronic administration.

Humans or animals receiving morphine regularly are liable to become physically dependent on morphine. When this does occur, withdrawal of the drug produces symptoms within 15–20 hours. In addicts, morphine antagonists (e.g. naloxone) can produce withdrawal signs within 30 minutes. The withdrawal symptoms commence with yawning, sweating, and running of the eyes and nose, restlessness, mydriasis, the appearance of 'goose bumps', cramps, nausea, insomnia, vomiting and diarrhoea. Tolerance to morphine is rapidly lost during this period and the withdrawal symptoms can be terminated with a suitable dose of morphine (Katzung, 1995; Martin, 1984). Thyreotrop-releasing hormone inhibits the tolerance to and dependence on morphine (Bhargava, 1980).

2.2 Codeine

Codeine is the 3-O-methyl ether of morphine. In *in vivo* animal tests it is less potent than morphine and *in vitro* it is less potent still. The ID_{50} of codeine in guinea pig assay was found to be 10 300nM. A discrepancy between its depressant effect in the guinea pig ileum and its analgesic actions in the whole animal or in man has been noted—in the former assay it has only 0.7% of the effect of morphine whereas its analgesic effect in man is about 10% of that of morphine (Kosterlitz and Waterfield, 1975; Kosterlitz *et al.,* 1972). In the mouse hot plate test, or in the rat bradykinin induced flexor reflex (Takagi and Satoh, 1978), codeine has about one seventh the activity of morphine as an anti-nociceptive agent, and this is reflected in the human parenteral dose, where 60–120mg of codeine is equivalent to 10mg of morphine (Krueger, 1955; Martin, 1984). It has been suggested that the virtual inactivity of codeine *in vitro* is because it is not converted to morphine under these conditions (Kosterlitz and Waterfield, 1975).

It has been demonstrated that codeine-induced analgesia is mediated via morphine, and depends, at least in part, on centrally formed morphine (Chen *et al.,* 1990). Codeine is used orally for the relief of mild to moderate pain and as an anti-tussive (Eddy *et al.,* 1969). Codeine has been used to depress pathological coughs and in patients in whom it is necessary to maintain ventilation via an endotracheal tube. Tolerance to the cough depressant actions of codeine can occur (Katzung, 1995).

It is frequently combined with mild analgesics. Since its action is much weaker than that of morphine it appears less likely to elicit nausea, vomiting, constipation or respiratory depression. It also has a lower potential for the development of tolerance and physical dependence than morphine (Hoffmeister, 1984). Similarly to morphine, the toxicity of codeine in different animal species displays great variation, depending on the strain of the animals used and on dietary conditions. It is noteworthy that in animals particularly susceptible to the convulsant effects of codeine, this substance is more toxic than morphine.

A significant difference between the activities of morphine and codeine is that the latter retains much of its activity after oral administration relative to its parenteral effect. The olive oil/water partition coefficient of codeine is 0.25 and when the brain uptake of this compound was measured during a single rat brain passage 25% of the codeine was cleared (Oldendorf, 1978).

Codeine, unlike morphine, is not destroyed in the body, but is mostly excreted in the urine (Oguri, 1980).

2.3 Narcotine

Narcotine (noscapine) belongs to the phthalideisoquinoline alkaloid group of opium, fails to produce anti-nociceptive activity, although it has central anti-tussive action (better than that of codeine) by inhibiting the cough reflex (La Barre and Plisnier, 1959; Put *et al.,* 1974). Narcotine as an anti-tussive agent lacks anti-convulsant activity and fails to reduce responses to N-methylaspartate on rat spinal neurones *in vivo* following microelectrophoretic administration (Church *et al.,* 1989). On the other hand, narcotine appears to be less toxic than codeine (Aurousseau and Navarro, 1957; Winter and Flataker, 1961).

It resembles papaverine in its pharmacological actions more closely than any of the other opium alkaloids. Like papaverine and many other isoquinoline alkaloids, narcotine exhibits mild local anaesthetic properties. It has no significant actions on the CNS in doses within the therapeutic range.

Narcotine has a relaxant effect on smooth muscle, similar to, but about ten times less than, that of papaverine. Contrary to codeine it does not cause constipation. A weak convulsant action of narcotine has been observed in dogs (Mercier and Mercier, 1955).

It was found that narcotine did not increase the analgesic action of morphine (Ota *et al.,* 1964). In mice the analgesic effect of narcotine was very weak compared to morphine, but its toxicity was greater (Szegi *et al.,* 1959). It also has a sedative action. Aldehyde reductase I enzyme has been found to be inhibited by narcotine (Paulová *et al.,* 1987).

The metabolism of narcotine has been studied in detail (Goeber *et al.,* 1977; Tsunoda and Yoshimura, 1979, 1981).

2.4 Papaverine

Papaverine is an important member of the benzylisoquinoline group of opium alkaloids. Unlike the alkaloids of the phenanthrene skeleton, the effects of this alkaloid on the CNS are not prominent, at least with ordinary doses. Papaverine is only slightly narcotic and large doses tend to increase reflex excitability; it displays weak analgesic properties by parenteral or oral administration.

In 1914, Pal demonstrated on animals *in vivo,* that papaverine decreases the tone of the smooth muscle and is a very effective agent against pathological spasms of the smooth muscle. Papaverine was found to inhibit the tonic phase of contractions of guinea pig ileum and rabbit duodenum induced by acetyl choline, nicotine, heart glycosides, histamine, urea, barium chloride and copper ion. In patients with gastric and duodenal ulcers papaverine decreases the bioelectrical potential of the stomach.

On the human pregnant and non-pregnant uterus, papaverine has a strong spasmolytic effect. It has a vasodepressive effect on the vessels of perfused human placenta.

The effects of papaverine on respiration, blood pressure in dogs and cats, on isolated vessels, on the intestine *in situ* and *in vitro* as well as its toxicity in rats are not affected by nalorphine (Preininger, 1975). Papaverine was found to potentiate the analgesic action of morphine (Bowman and Rand, 1980).

Papaverine has a stimulant action on dopamine receptors in the central nervous system (Bowman and Rand, 1980). It has been shown in cats that papaverine readily penetrates the hematoencephalic barrier. Papaverine inhibits vomiting induced by apomorphine and produces vasodilatation of cerebral vessels.

Papaverine has a statistically significant palliative effect on experimentally induced pruritus.

Subcutaneous administration of papaverine to guinea pigs prevented bronchospasm induced by inhalation of an aerosol of acetylcholine or histamine. The bronchodilatory effect of papaverine was increased by alkalosis and decreased by acidosis.

Papaverine increases blood flow in the coronary arteries and causes their dilation, followed by an increase in the formation of creatine phosphate. Besides its strong coronary vasodilating effect, papaverine diminishes the tendency of the development of ventricular fibrillation.

Papaverine has a marked vasodilating effect upon the vessels: the dilatation is more significant in atherosclerotic than in intact vessels, or when the tension of the vessel walls is increased by epinephrine.

In isolated porcine coronary strips, K^+-induced contractions were approximately 10000 times more sensitive to the relaxing effects of nisoldipine, nitrendipine, the Ca^{2+} antagonist, than to those of papaverine (Fleckenstein *et al.,* 1989).

Papaverine failed to reduce responses to N-methylaspartate on rat spinal neurones *in vivo* following microelectrophoretic administration (Church *et al.,* 1989).

Intracavity injection of papaverine to impotent man induces penile swelling, attributable the smooth muscle relaxant action of this drug (Szasz, 1987).

Papaverine has also been found to inhibit cyclic GMP-stimulated nucleotide phosphodiesterase (Yamamoto *et al.,* 1983).

The mechanism of relaxant action of papaverine differs from that of amytal. Papaverine was found to relax the taenia coli even in a sodium-free solution, although its relaxant activity was markedly reduced, while amytal failed to reveal relaxant action in the same condition. Amytal was found to be more sensitive against the re-introduction of small amounts of sodium ions to the sodium-free solution than papaverine. Papaverine induced muscle relaxation with synchronous acceleration of [45]Ca, while amytal did not. The cellular uptake of [45]Ca was inhibited by papaverine, but not by amytal (Sunagane *et al.,* 1983).

Caffeine-induced contractions of guinea pig taenia coli are attributed to mobilization of calcium ions from intracellular store sites. Papaverine decreased these contractions at 32°C, while lower temperatures were noted to inhibit papaverine's action (Sunagane *et al.,* 1982).

Using several *in vitro* biochemical assays—related to smooth muscle excitation—contraction coupling, binding to β_1, β_2, and a-adrenergicreceptors, antagonismofcalcium accumulation—papaverine was observed to be inactive, except as a phosphodiesterase inhibitor (Greenslade *et al.,* 1982).

The mechanism of action of papaverine was studied by investigating the correlation between inhibition of cyclic AMP phosphodiesterase, antagonism of endogenous adenosine and relaxation of guinea pig tracheal smooth muscle (Fredholm *et al.,* 1979). Papaverine's action seems to involve a combination of phosphodiesterase inhibition (as with methylxanthines) and block of Ca channels (Rang *et al.,* 1995).

The systemic haemodynamic and myocardial effects of papaverine administered directly into the left coronary artery were determined in anaesthetized dogs. Papaverine caused profound increases in left ventricular diastolic pressure /dt and arterial hypotension/ in the non ischemic state. In the presence of segmental ischemia papaverine proved to be significantly less potent in this respect (Higgins and Bookstein, 1977).

The actions of papaverine on the CNS were studied by influencing sleep (Bauer and Kadlecova, 1972).

Papaverine was found to be an effective histamine liberator (Feldberg and Paton, 1951). Papaverine, similarly to methylxanthines, relaxes smooth muscle presumably by inhibiting phosphorylation of myosin and by preventing breakdown of cAMP so, that myosin light chain kinase is converted to its less active form (Creed, 1994). Papaverine potentiates the relaxing response to adenosine in isolated canine cerebral arteries (Fujiwara and Muramatsu, 1994).

3 MINOR OPIUM ALKALOIDS

The pharmacology and biology of minor opium alkaloids have been surveyed previously in two comprehensive reviews (Preininger, 1975; Reynolds and Randall, 1957).

3.1 Thebaine

The pharmacology of thebaine was summarized by Reynolds and Randall in 1957 and studied comprehensively by a WHO Advisory Group in 1980.

The pharmacological actions of thebaine in various isolated organs have been studied. Thebaine can induce a temporary decrease in blood pressure in anaesthetized dogs and this depressor effect showed a marked tachyphylaxis. In isolated guinea pig atrium, thebaine decreased the heart rate and contractions depending on the concentration. In isolated rabbit ileum it decreased the peristaltic movement and contractions (Teraoka, 1965). The predominant effect of thebaine is stimulation of the central nervous system. In the mouse, rabbit, cat and dog increases in motor activity and reflex excitability were observed at doses around 2–10mg/kg s.c. or i.m. The Straub-tail response was noted only occasionally. The effects of thebaine on body temperature and respiration have also been studied. Convulsions were observed in almost all species of animals including the frog, pigeon, mouse, guinea pig, cat and dog. Transient tremors, restlessness and convulsions were observed in the rhesus monkey. The convulsant action of thebaine was studied by electrophysiological analysis (Corrado and Longo, 1961). Naloxone antagonized the convulsions induced by thebaine in mice, but it was ten times less effective versus thebaine than it was versus heroin (Gilbert and Martin, 1975; WHO Advisory Group, 1980). Thebaine was noted to produce a moderate decrease of catecholamine levels in heart and brain (Sloan *et al.,* 1962).

Detailed analgesic studies were performed in mice (Szegi *et al.,* 1959). Compared to morphine, thebaine is a more effective narcotic but a weaker analgesic. Thebaine is inactive in the tail flick and writing tests, but it is active in the hot plate and Nielsen tests. However, doses in the higher range of the dose—response curves produced convulsions. In isolated organ preparations (GPI and MVD) thebaine possesses 0.3 times the potency of morphine. The actions of thebaine are partially reversed by naloxone, but these effects are presumed not specific opioid-like effects (WHO Advisory Group, 1980).

Repeated administration of thebaine for six weeks in rhesus monkeys did not result in the development of tolerance to convulsant effects.

In rats the physical dependence potential of thebaine was very low. Thebaine did not precipitate morphine withdrawal signs in chronic spinal dogs (Gilbert and Martin, 1978). Nevertheless naloxone produced very mild withdrawal signs in spinal dogs treated chronically with thebaine. Thebaine did not substitute for morphine in morphinedependent rhesus monkeys. On the other hand, definite withdrawal signs were observed upon abrupt withdrawal of thebaine in the monkeys treated with i.v. thebaine for 31 days (WHO Advisory Group, 1980).

The toxicity of thebaine was examined in rabbits. These studies indicated that thebaine exhibits marked convulsive effects (Eddy, 1939). Thebaine is far more toxic than morphine (WHO Advisory Group, 1980; Teraoka, 1965).

The metabolism of tritium-labelled thebaine was studied in rats and several metabolites were detected. Codeine, norcodeine, normorphine, morphine and 14-hydroxycodeinone were identified as minor metabolites. Oripavine was the major metabolite (Misra *et al.,* 1973, 1974). Metabolism was also examined in rhesus monkeys (Yamazoe *et al.,* 1981). Five substances were detected and separated by means of thin layer chromatography in the urine. Oripavine and N-nororipavine have been identified by gas chromatography—mass spectrometry (GC—MS) analysis. It was reported that oripavine was the major metabolite of thebaine *in*

vitro (Mikus *et al.,* 1991). Thebaine was incubated with rat liver microsomes in these experiments. The transformation of thebaine to oripavine, codeine, and morphine has been reported in rat liver and kidney (Kodaira and Spector, 1988). Opioid receptor binding of thebaine was studied in rat brain membranes, but it had very weak affinity (Chen *et al.,* 1991). Thebaine displays some cytotoxic effects *in vitro* (Nassiri *et al.,* 1991).

3.2 Oripavine

The pharmacology of oripavine was the subject of a comprehensive study by a WHO Advisory Group (1981).

Single doses of oripavine administered intravenously manifested a decrease in spontaneous motor activity in rats and in rhesus monkeys. Additionally, tremors and vomiting were also observed in monkeys.

Its analgesic potency in mice is much higher than that of thebaine and is comparable to that of morphine in both tail flick and writhing tests in which thebaine is reported to be inactive. The analgesic activity of oripavine was also studied in the mouse and rat with the hot plate method after subcutaneous drug administration. Peak analgesic effects in the mouse and rat were observed 20 minutes after drug administration and the effects lasted for about 40–60 minutes. Oripavine appears to have analgesic potency of the same order of magnitude as morphine in these species, but has a low therapeutic index because of its high toxicity. Signs of oripavine toxicity in both species were clonic-tonic convulsions followed by death. The toxicity of oripavine and morphine in the mouse did not appear to be antagonized by pre-treatment with naloxone. Toxicity does not appear to be mediated at the opioid receptor, however oripavine did show some cross tolerance with morphine, but did not appear to suppress morphine abstinence in the mouse and rat (Yeh, 1981).

Oripavine possesses a weak morphine-antagonistic property, as evidenced by its partial precipitation of morphine withdrawal signs in morphine-dependent nonwithdrawn monkeys.

The administration of oripavine resulted in the development of physical dependence in rats. Obvious morphine-like withdrawal signs were precipitated by naloxone. Oripavine did not suppress the withdrawal signs of morphine-dependent rhesus monkeys. However, it is known that the physical dependence potential of morphine antagonists or partial agonists may not be demonstrable because the antagonistic property of these drugs may prevent the suppression of morphine withdrawal signs.

The metabolism of oripavine has not yet been reported.

3.3 Neopine

The actions of neopine closely resemble those of codeine. Clinical trials, however, have shown this drug to be less effective than codeine when employed in doses of 15–30mg. In frogs, slight drowsiness followed by increased reflexes is seen with small doses, and larger doses produce tetanus. In rabbits some narcotic effect is seen, but with doses of 80mg or more, convulsions and death occur. No changes were observed in the size of the pupil. In the dog the bronchioles become constricted (Reynolds and Randall, 1957).

3.4 Salutaridine

Salutaridine does not display significant effects on human platelet aggregation (Panosyan *et al.,* 1986). Racemic Salutaridine can be considered as a partial agonist of the GABA (γ-aminobutyric acid)-benzodiazepine receptor complex (Kardos *et al.,* 1984).

3.5 Pseudomorphine

Pseudomorphine is very insoluble in saline fluids and exerts little or no action when administered by the oral or subcutaneous route. However, when given intravenously even in quite small doses, very definite effects are produced, particularly on the circulation. The drug exhibits practically none of the direct actions of morphine on the central nervous system. Some general depression and uncoordination have been described, but neither true narcosis nor primary respiratory failure have been observed (Schmidt and Livingston, 1933; Travell, 1932).

Conflicting results have been reported with regard to the occurrence of convulsions after the administration of pseudomorphine; at any rate, the convulsant action seems to be much weaker than that of morphine. Emesis is a common symptom and defecation usually follows soon after injection. As already stated, the most pronounced effects following intravenous injection are exerted on the circulatory system, and indeed some investigators attribute most of the acute effects of pseudomorphine to acute circulatory depression resulting chiefly from peripheral vasodilatation. The vascular effects of this compound appear to be qualitatively similar to those of morphine, but are much more intense. In the dog and cat, the abrupt fall in blood pressure seems to be the result of marked dilatation of muscular and cutaneous blood vessels; the depressor effect does not depend on the integrity of the medulla. The isolated heart is somewhat depressed although coronary flow seems to be slightly increased. Hypotension is not observed in rabbits, rats, or guinea pigs.

Pseudomorphine readily produces acute tolerance to the circulatory effect, not only to itself, but to morphine, codeine, and heroin as well; this acute tolerance is limited to the circulatory effects. The intravenous injection of pseudomorphine results in symptoms which superficially resemble those observed during withdrawal of morphine from chronically treated tolerant dogs (Drevon and Richard, 1936; Leulier and Pommé, 1930; Richard and Drevon, 1936). Pseudomorphine is one of the metabolites of morphine (Misra and Mule, 1972; Yeh, 1973) and it is converted into a less toxic substance in the mouse (Fichtenberg, 1946).

3.6 Laudanosine

Laudanosine has a convulsant action in anaesthetized curarized dog and this effect was suppressed by pentobarbital (Mercier and Mercier, 1955). Anaesthetic drugs administered before the convulsive stimulus increased the dose of laudanosine necessary to produce seizures (Al-Muhandis *et al.,* 1991). The pharmacology of laudanosine has been studied extensively because laudanosine is a principal metabolite of atracurium (Stenlake *et al.,* 1981). EEG effects of laudanosine were examined in an animal model of epilepsy. It was found that no increase of seizure activity was

produced by mean laudanosine concentrations and the routine use of atracurium is unlikely to provoke seizures, even in the presence of an epileptogenic focus (Tateishi *et al.,* 1989).

Laudanosine enhanced the release of ^{3}H-noradrenaline in isolated right atria of guinea pigs (Kinjo *et al.,* 1989). This effect of laudanosine may explain some of the unwanted effects seen following administration of atracurium.

Laudanosine crosses readily the blood—brain barrier and can produce hypotension. It is excreted unchanged by the kidneys and its metabolites are excreted by both the kidneys and liver (Hennis *et al.,* 1986). Metabolism of racemic laudanosine was studied in the dog, rabbit and man. Codamine and laudanine were detected among the metabolites (Canfell *et al.,* 1986).

Aldehyde reductase or alcohol dehydrogenase enzymes have been found to be inhibited by laudanosine, protopine and berberine (Paulová *et al.,* 1987).

3.7 Laudanine

In frogs, the effect of laudanine is similar to that of strychnine. Laudanine is noted to induce convulsions and larger doses cause paralysis. Similar effects were observed in pigeons. Small doses produced acceleration of respiration in rabbits, dogs and cats but higher doses induced tetany. Laudanine in small doses was also reported to cause a sudden rise in blood pressure (Preininger, 1975).

3.8 Reticuline

S-(+)-reticuline showed negative ionotrop effects in the papillary muscles of guinea pig heart (Kimura, I. *et al.,* 1989). Anti-inflammatory effects were also detected by the pouch granuloma method in mice (Kimura, M. *et al.,* 1985).

(-)-reticuline produces catalepsy and a decrease in locomotor activity in mice. It blocks locomotor activation and rotational behaviour induced by apomorphine, but not those induced by methamphetamine (Watanabe *et al.,* 1981).

Reticuline was reported to inhibit specific ^{3}H-dopamine binding to dopamine receptors in tissue homogenates from rat corpora striata. The blockade of apomorphineinduced climbing behaviour was observed by reticuline in mice. Reticuline also blocked amphetamine-induced circling behaviour in mice, but it did not produce catalepsy at doses which blocked circling behaviour (Banning *et al.,* 1980). The administration of reticuline exerted a uterine inhibitory effect mainly related to a decrease in the concentration of cytosolic Ca^{2+} available for contraction (Martin *et al.,* 1988). Reticuline shows a low affinity to catecholamine receptors (Nimitkitpaisan and Skolnick, 1978). It has an inhibitory effect on indirectly stimulated contractions in frog sciatic nerve—sartorius muscle preparation, but produces almost no effect on directly stimulated contractions (Kimura *et al.,* 1983).

Reticuline shows no anti-microbial activity (Liao *et al.,* 1978; Villar *et al.,* 1987).

3.9 Protopine

Protopine—HCl (given intravenously in rats or rabbits) decreased the atrioventricular and intracardiac conductivity leading to a decrease in the frequency of cardiac

contractions. A decrease in contractions induced by $BaCl_2$ was also observed in the isolated intestinal segment of rat. Protopine displays anti-arrhythmic activity and is more effective than quinidine or novocainamide for $CaCl_2$-induced and aconitine-induced cardiac arrhythmia in rats (Burtsev *et al.,* 1978). It has been suggested that the mechanism of the anti-arrhythmic effect of protopine is due to the suppression of the foci of heterotropic stimulation, a decrease in the excitability of myocardial cells and normalization of the catecholamine content in the myocardium.

In addition to its anti-arrhythmic effect, protopine also shows short-term hypotensive, ganglion-blocking, and spasmolytic properties (Aliev and Zakirov, 1970). Protopine is slightly weaker as a smooth muscle relaxant than papaverine. The smooth muscle relaxant mechanism of protopine may be due to inhibition of intracellular Ca^{2+} release (Huang, Y. *et al.,* 1991). In small doses it was reported to retard heart activity, decrease blood pressure and produce a sedative effect. However, large doses caused excitation and convulsions in the animals studied. Protopine showed inhibitory action in the isolated heart and muscle of frog, but it had a stimulating effect in the intestine of guinea pigs.

Protopine displays some inhibitory effect on tumours associated with considerable cytotoxic side effects (Sokoloff *et al.,* 1964). The effects of protopine on the aggregation of platelets have been reported (Ko, F.N. *et al.,* 1989; Matsuda *et al.,* 1988a,b; Teng *et al.,* 1991). The mechanism of the action of protopine on rabbit platelet aggregation has been investigated in detail (Shiomoto *et al.,* 1990, 1991).

It was also observed that protopine is an antagonist versus acetylcholine on mouse small intestine and an anti-spasmodic effect on the uterus was detected (Kitabatake *et al.,* 1964; Pandey *et al.,* 1971). Protopine exhibits a significant decrease in intestinal muscle contractions and considerable cardioinhibitory, anti-arrhythmic, hypotensive and anti-pyretic effects were also found (Hilal *et al.,* 1989). Antagonism of the lethal effect of histamine was observed in the guinea pig (Dil, 1973; Ustunes *et al.,* 1988).

3.10 α-Allocryptopine

The anti-arrhythmic action of α-allocryptopine was compared with quinidine. It was more effective than quinidine in preventing and treating aconitine-induced arrhythmia in rats (Akbarov *et al.,* 1972a). α-allocryptopine prevented $CaCl_2$-induced cardiac fibrillations in ~20% of the rats studied (Akbarov *et al.,* 1978). The alkaloid has a local anaesthetic effect. The inhibitory effects of allocryptopine on the growth of tumours has been reported (Aizenman *et al.,* 1963). Allocryptopine produced no detrimental effects on peripheral blood or histology of organs and tissues after long-term administration in mice (Ashrafova *et al.,* 1974). It was reported that allocryptopine, cryptopine and protopine enhance ^{3}H-γ-aminobutyric acid binding to rat brain synaptic membrane receptors, suggesting that these alkaloids have diazepam-like activity (Kardos *et al.,* 1986). Allocryptopine has also been reported to have some anti-bacterial activity (Abbasoglu *et al.,* 1991).

3.11 Cryptopine

In mice cryptopine produced depression and asphytic twitches. Arterial blood pressure was lowered in cats and dogs and atropine could not prevent this effect.

Nerve impulse conduction through cardiac vagal ganglia was blocked by cryptopine and the conduction through cervical sympathetic ganglia was not affected. Other effects of this compound were a transient stop of the heartbeat in frogs and an increase in the tone of uterine smooth muscle in pregnant rats (Tashbaev and Sultanov, 1967).

The anti-arrhythmic activity of cryptopine was compared with that of allocryptopine and the latter substance proved to be more effective in preventing the development of cardiac arrhythmia against aconitine (Akbarov *et al.,* 1972b). Cryptopine shows some anti-bacterial effect (Abbasoglu *et al.,* 1991).

3.12 Magnofluorine

This alkaloid produced a decrease of motility and a slight relaxation of voluntary musculature in rabbits and mice. At toxic doses the cause of death was the cessation of respiratory movements (Fakhrutdinov and Kamilov, 1967).

Magnofluorine and diacetylmagnofluorine have less curare-like activity than remerine hydroxymethylate (Fakhrutdinov, 1971) and they possess low hypotensive action (Shah *et al.,* 1989). Hypotensive effects are attributed to ganglion-blocking action (Inoue, 1957). Acetylation of magnofluorine resulted in an increase of toxicity. Ionotrop activity of magnofluorine was demonstrated on isolated and perfused rat heart by Cave *et al.* (1984). Magnofluorine was reported to decrease arterial blood pressure in rabbits and induce hypothermia in mice. It induced contractions in isolated pregnant rat uterus and stimulated isolated guinea pig ileum (El Tahir, 1991).

Magnofluorine displays cytotoxic (Wu *et al.,* 1989) and anti-inflammatory activity (Otsuka *et al.,* 1981). Magnofluorine and corytuberine show no anti-microbial activity *in vitro* (Villar *et al.,* 1987).

The inhibition of protein formation has also been observed (Gupta *et al.,* 1980).

3.13 Isoboldine

Isoboldine shows weak anti-microbial activity (Abbasoglu *et al.,* 1991; Simeon *et al.,* 1990; Villar *et al.,* 1987). It has high affinity for the α_1-adrenoreceptor in the binding assay (Huang *et al.,* 1988). Isoboldine inhibits adenyl cyclase (Sheppard and Burghardt, 1978) and aldol reductase (Nakai *et al.,* 1985).

3.14 Corytuberine

Corytuberine causes increased reflex irritability in the frog and tonic convulsions with slightly increased irritability in guinea pigs and cats. Death from lethal doses results from asphyxia during convulsive seizures. This alkaloid accelerates respiration, stimulates the secretion of tears and saliva, and slows the pulse by stimulation of the vagus.

Corytuberine does not act as a mitotic poison *in vitro*. The methiodide of corytuberine displayed curare-like activity and a hypotensive effect (Preininger, 1975).

The neuroleptic, anti-convulsant and analgesic actions of corytuberine have been studied in mice—the substance elicited catalepsy and hypothermia and was anticonvulsant against harman and picrotoxin. It did not reduce nociception in hot

plate and writing tests. However, in low doses corytuberine antagonized the anti-nociceptive effect of morphine in the hot plate test (Zetler, 1988).

3.15 Narcotoline

The anti-tussive properties of narcotine, narcotoline, O-ethylnarcotoline, and O-benzylnarcotoline were examined in cats and guinea pigs. All compounds displayed anti-tussive potency with narcotine and O-ethylnarcotoline being the most active (Put *et al.,* 1974). 3,4,5-trimethoxybenzoyl-narcotoline proved to be a cough reliever with mild depressive activity. It had no effect upon the respiratory centre (Toth, 1970).

3.16 Narceine

Intravenous administration of narceine to rabbits stimulates the respiratory centre, and accelerates the frequency and increases the volume of respiration.

Narceine has an anti-tussive effect similar to that of codeine in animal models (mice, dogs, cats and rabbits) but without its analgesic potency; its anti-tussive effect is less potent than that of narcotine (Kelentey, 1969). However, it has also been reported that narceine has no influence on the cough reflex of cats (Haas, 1955).

A considerable depressant action on blood pressure was observed and a stimulating effect on intestinal peristalsis was detected (Kelentey *et al.,* 1958). Narceine has no analgesic action (Szegi *et al.,* 1959) and no convulsant action in the anaesthetized curarized dog (Mercier and Mercier, 1955).

The toxicity of narceine was found to be similar in mice, rabbits and cats (Kelentey *et al.,* 1958).

3.17 Sanguinarine

The possible relationship of sanguinarine to glaucoma in epidemic tropical hydropsy which is frequent in India, has been the subject of numerous papers. Oil from *Argemona mexicana* L., whose seeds contain sanguinarine, is sometimes mixed with mustard oil which is commonly used in foodstuffs in India. It was found that the oil from *A. mexicana* causes glaucoma in epidemic hydropsy and this observation was corroborated by studies on rabbits and monkeys. Sanguinarine produced a decrease in intraocular pressure when injected i.v. into rabbits (Dyke, 1978; Hakim, 1954). The large distribution of sanguinarine in *Papaveraceae* plants was discussed and the relationship between the consumption of poppy seeds and the possible development of glaucoma was evaluated (Hakim *et al.,* 1961). It was also reported that the frequent incidence of glaucoma in epidemic tropical hydropsy is the result of the effect of mustard oil contaminated or adulterated with the oil of seeds from *A. mexicana* containing sanguinarine (Shenolikar *et al.,* 1974).

Sanguinarine has sympathicolytic, adrenolytic and local anaesthetic effects. It increases blood pressure, tonicity and intestinal peristalsis (Kelentey, 1960). Sanguinarine possesses a large spectrum of anti-microbial activity *in vitro* (Mitscher *et al.,* 1972; Stermitz *et al.,* 1975; Tolkachev and Vichankova, 1978; Vichankova *et al.,* 1969) and displays low toxicity in rats when applied orally or intravenously

(Becci *et al.,* 1987). The intercalating properties of sanguinarine with DNA have been examined in detail (Maiti *et al.,* 1982; Smekal and Kubova, 1984; Smekal *et al.,* 1984). Sanguinarine shows some anti-tumour activity (Ishii *et al.,* 1985; Stermitz *et al.,* 1973; Tin-Wa *et al.,* 1970).

The fraction of quaternary benzophenanthridine alkaloids from roots of *Chelidonium magus* containing sanguinarine has been tested for anti-inflammatory activity in rats. On the basis of its low toxicity, high anti-inflammatory activity and anti-microbial action it is recommended for medical use in the treatment of oral anti-inflammatory processes (Lenfeld *et al.,* 1981). Sanguinarine exhibits anti-plaque activity in humans. For its plaqueretentive properties in combination with anti-microbial and anti-inflammatory effects, sanguinarine has been a component of toothpastes and oral rinses sold in the United States since 1984 (Perdok *et al.,* 1990).

Sanguinarine inhibits liver enzymes and acetylcholine esterase (Stejskal *et al.,* 1985; Walterova *et al.,* 1981).

3.18 Dihydrosanguinarine

Dihydrosanguinarine and sanguinarine were reported to inhibit cyclic AMP phosphodiesterase (Moriyasu *et al.,* 1990), and the inhibition of reverse transcriptase activity was also observed by these alkaloids (Sethi, 1981). Dihydrosanguinarine displays a lower toxicity than sanguinarine in rats (Sarkar, 1948).

3.19 Scoulerine

Scoulerine shows sedative activity in mice (Sadritdinov and Rezhepov, 1982), and it is inactive as an anti-tussive. Scoulerine has been noted to prevent apomorphine-induced emesis in dogs (Khamdanov and Sadritdinov, 1977) and to decrease locomotor activity in mice. This effect is mediated primarily by the cerebral cortex and secondarily by direct effect on the muscles (Sadritdinov and Khamdanov, 1976). It has an affinity for the dopamine receptors in brain (Xu *et al.* 1989).

3.20 Canadine

Weak anti-bacterial effects of canadine (Abbasoglu *et al.,* 1991) have been reported. Canadine inhibits the liver alcohol dehydrogenase enzyme (Pavelka and Kovar, 1975). It has some sedative effect in mice (Sadritdinov and Rezhepov, 1982).

3.21 Stepholidine

The pharmacology of Stepholidine has been investigated in detail because this alkaloid has been detected in numerous medicinal plants in Japan and China.

Stepholidine binds to dopamine receptors in rat brain. It is an antagonist of D_1 dopamine receptors, but it behaves as an agonist in a supersensitive state of the receptor (Jin, 1987; Jin *et al.,* 1992). Stepholidine displays analgesic and anti-pyretic actions in mice and rabbits and it is interesting that tolerance to the analgesic effect of this substance did not develop. Prolonged administration of Stepholidine did not induce dependence (Chen, L. *et al.,* 1986).

The interaction of stepholidine with opioid analgesics has been studied. Stepholidine potentiated the analgesic effects of dihydroetorphine or pethidine (Bian *et al.,* 1986). Stepholidine lowered blood pressure in anaesthetized dogs and rats. This hypotensive effect is mainly due to stimulation of the presynaptic α_2-adrenoreceptors (Xiong *et al.,* 1987), but the regulation of central dopamine receptors may take part in the hypotensive action (Gu *et al.,* 1990).

Stepholidine does not show anti-microbial activity (Simeon *et al.,* 1990; Villar *et al.,* 1987).

3.22 Isocorypalmine

Isocorypalmine showed inhibitory action on blood platelet aggregation induced by collagen, arachidonic acid, and ADP *in vitro* (Matsuda *et al.,* 1988b).

3.23 Berberine

Berberine has some therapeutic value and is being used in the treatment of gastrointestinal disorders. The toxicity of berberine sulphate was studied in rats and was shown to display low toxicity by oral administration (Kowalewski *et al.,* 1975). Berberine sulphate produced a reversible hypotension in the anaesthetized rat, and it increased the mortality in guinea pigs and dogs receiving safe doses of histamine. Berberine potentiated apomorphineinduced emesis in dogs. It decreased the urine volume and urinary concentrations of Na^+, Cl^- ions in conscious saline-loaded rats. Berberine lowered the rectal temperature in normal rats and was three times more effective than sodium salicylate in decreasing fever induced by Brewer's yeast. This finding confirms its traditional use as an antipyretic (Sabir *et al.,* 1978). Berberine chloride displays anthelmintic activity in mice against *Syphacia obvelata* (Singhal, 1976). The pharmacokinetics of berberine were studied in rats, and after i.p. administration, its rapid distribution was observed (Mrozikiewicz *et al.,* 1980).

The anti-bacterial activity of berberine was evaluated (Kowalewski *et al.,* 1972; Sawada *et al.,* 1971) and cross resistance between berberine and antibiotics used in therapy was not observed. The anti-inflammatory effects of berberine were studied in rats injected locally with cholera toxin (Akhter *et al.,* 1977). This anti-inflammatory activity was also detected by several methods, e.g. fertile egg or cotton-pellet methods, by Otsuka *et al.* (1981). The hypotensive effect of berberine, followed by bradycardia, was observed in rats. This hypotensive effect may involve the depression of heart performance (Chun *et al.,* 1979; Kulkarni *et al.,* 1972).

Berberine displays weak cytotoxic activity on human and animal cell cultures *in vitro* (Hladon *et al.,* 1978). Berberine administered orally prolonged the latent period and reduced the frequency of purging in dogs. Since it did not precipitate serum albumin or egg-white, its anti-diarrhoeal effect cannot be due to any astringent action (Akhter *et al.,* 1979). The alkaloid inhibits electrogenic ion transport in rat isolated colon (Taylor and Baird, 1995). Berberine inhibits the formation of DNA, RNA, proteins and lipids. The inhibition of formation of macromolecules may reflect such primary actions as inhibition of glucose utilization and interaction with nucleic acids (Creasy, 1979).

Berberine (canadine, coptisine) inhibits the function of liver alcohol dehydrogenase (Pavelka and Kovar, 1975). Berberine was found to have an anti-secretory effect on rat ileum *in vitro*. This effect of mucosal berberine may be explained by stimulation of a NaCl-coupled absorptive transport process (Tai *et al.,* 1981). On the other hand, luminal berberine reduced the cholera toxin induced secretion of water, Na^+ ions and Cl^- ions in a concentration-dependent manner in rat ileum (Swabb *et al.,* 1981). Berberine also exhibits anti-malarial activity comparable to that of quinine *in vitro* (Vennerstrom and Klayman, 1988).

Berberine administered to rabbits anaesthetized with urethane produced a long-lasting dose-related decrease in blood pressure. This hypotensive effect of berberine was not influenced by vagotomy or pre-treatment with atropine. Berberine-induced hypotension is attributed to α-adrenoreceptor blockade, not a direct relaxant effect upon vascular smooth muscle (Ko and Lim, 1980).

3.24 Coptisine

The anti-inflammatory activity of coptisine (Otsuka *et al.,* 1981) was confirmed using the cotton-pellet method, the croton oil—granuloma pouch method, and the punch method. Antibacterial activity was also detected (Sawada *et al.,* 1971). The binding of coptisine to DNA was studied (Koudelka *et al.,* 1978). This alkaloid inhibits acetylcholinesterase (Ulrichova *et al.,* 1983b) and butyrylcholinesterase (Ulrichova *et al.,* 1983a) enzymes *in vitro*.

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VII. OVERVIEW OF WORLD TENDENCIES ON CULTIVATION, PROCESSING AND TRADE OF RAW AND OPIATES

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1 LICIT PRODUCTION OF POPPY

Morphine and its related compounds which are used in modern medicine are derived commercially from opium as well as poppy straw. The consumption of crude opium and its purified forms for their therapeutic effects is also well documented and officially accepted in many regions including some developed countries. However, in evaluating international data it becomes obvious that the estimated world production of opium is much higher than the demand for its use in medical applications. As an extreme example, the data of Bryant (1988) indicate that only 5% of the world's opium production is used for the licit (official) processing of opiates. There have been many attempts to quantify the difference between official requirements and actual world-wide production (INCB, 1981; UN Statistics, 1984).

The activity of the International Narcotics Control Board (INCB) has meant that reliable data on licit and illicit poppy production are available. The system of international control and monitoring of the licit movement of narcotic drugs, as embodied in the 1961 Convention and amended by the 1972 Protocol, has functioned in a generally satisfactory manner. The system has succeeded in limiting, for each country and territory and for the world as a whole, the licit cultivation of narcotic plants and the illicit production, manufacture, distribution and trade in narcotic drugs to the quantities required for medical and scientific purposes. In the next section data from the INCB and some more information taken from special estimates are evaluated. The main sources are: UN Statistics (1984), INCB (1981, 1993, 1994, 1995a, b, c) Gordon (1994) and Bryant (1988).

1.1 Raw Material Production

1.1.1 Opium

The total world area of licit cultivation of the poppy for opium production was about 15000 ha in 1989, as shown in Figure 1. A continuous decrease was observed after 1989, but an increased area was forecast for 1994 and 1995. The world production of opium shows a similar trend (Figure 2)-500000kg of opium was produced in 1989, and this amount had decreased to about 75% of the 1989 value in

CULTIVATION AREA
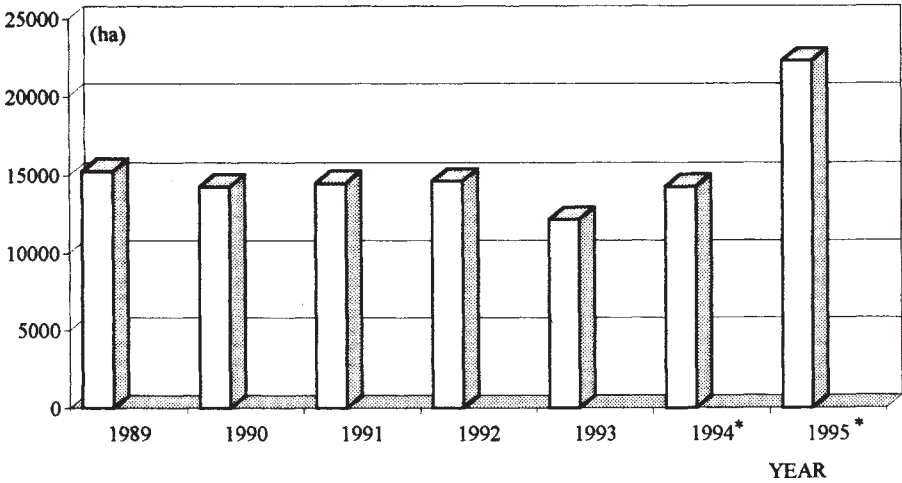
Figure 1 Official world cultivation area of *Papaver somniferum* for the production of opium in the period 1985–1995). * indicates data forecast by experts

OPIUM PRODUCTION
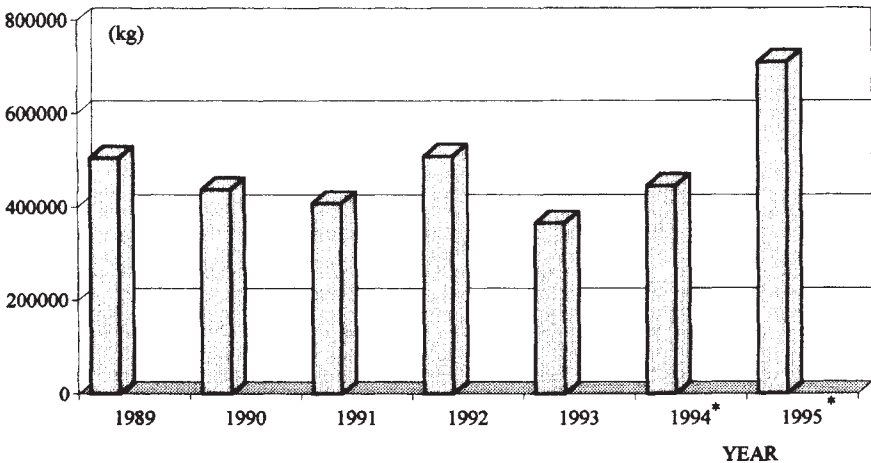
Figure 2 Official world opium production in the period 1989–1995 (INCB, 1995b). * indicates data forecast by experts

1993. Changes in climatic conditions may have contributed to the decrease but the major reason must be the reduced cultivation area.

There are only a few countries which are involved in the licit production of opium and the majority of the world's morphine requirement is satisfied by Indian opium.

Table 1 Licit opium production of the world based on 1993 estimates (selected from INCB data for 1993)

	Poppy cultivation area (ha)	Opium production (kg)	Yield (kg/ha)
India	11 907	345 708	29.0
China	240	19 870	82.8
Democratic People's Rep. of Korea*	91	415	4.5
Japan	0.7	5	6.8

*Data for 1991.
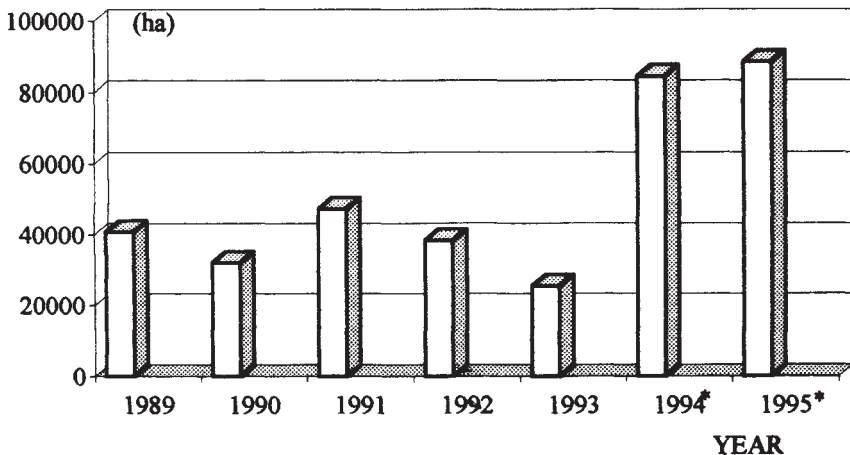
Figure 3 World area of licit *Papaver somniferum* cultivation for purposes other than production of opium. * indicates data forecast by experts

The cultivation of the poppy for opium is still licit in India and for this reason it is theworld's major producer, meeting both domestic and international demands. As shown in Table 1 the estimated cultivation area in India was 11907 ha in 1993, resulting in an opium yield of 345708kg. This is about 96% of the total world opium production. China, the Democratic People's Republic of Korea and Japan are also registered as producers, but their production areas and opium yields are very much smaller than in India and the opium produced only meets domestic demand.

1.1.2 Poppy Straw

In the second half of the twentieth century the importance of poppy straw has been increasing continuously. Figure 3 shows that the world area of poppy cultivation for purposes other than opium production was as large as 25000–40 000ha in the early

1990s and this was forecast to increase to some 80 000ha in 1994. This means that the cultivation area of poppy for straw is two to three times that of the area used for licit opium production.

While licit opium production is restricted to Asia, poppy fields for straw are mostly situated in Europe, where poppies have been cultivated for their seed for centuries. Australia is a relative newcomer to poppy production and cultivation there has been accelerated by industrial needs.

Although the area of poppy cultivation for non-opium purposes is changing from year to year, the spectrum of countries involved is rather stable (Table 2). Data from 1993 show that the main producers are the Czech Republic, Poland, Turkey, Australia, France, Spain, Hungary and Slovakia (data for Slovakia are not shown in Table 2 due to a lack of information). The data concerning straw yields reflect the practical problems of the statistics: some values are absent and some are much smaller than would be accepted from a professional point of view. The expected poppy cultivation area for non-opium purposes forecast by INCB (1995c) experts for 1995 shows a rearrangement in production rankings (Figure 4)—Turkey was expected to be the largest producer with about 45.5% of world straw production. Hungarian production was also expected to increase, exceeding that of France and Spain.

1.2 Processing of Raw Material

1.2.1 Extraction of Opium

Over recent years the total quantity of opium used annually for the extraction of alkaloids has been about twice the production values measured which means that the surplus must come from the stock of the opium producers or from the stock of countries involved in the continuous cultivation of the opium poppy. The increasing demand for alkaloids is well demonstrated by Figure 5 which shows that 457195kg of opium was processed in 1989 and nearly double that amount—898276kg—was extracted in 1993.

Table 2 Licit cultivation of *Papaver somniferum* for purposes other than production of opium, based on 1993 data (selected from INCB data for 1993)

	Area (ha)	*Poppy straw harvested* (kg)	*Yield* (kg/ha)
Australia	6026	5 108 000	848
Austria	551	—	—
Bulgaria*	400	—	—
Czech Republic	11 000	—	—
France	4161	3 166 000	761
Germany	3	—	—
Hungary	3500	610 000	174
Poland*	10 000	—	—
Romania	273	22 410	82
Spain	3930	707 061	180
Turkey	6930	2 684 536	387

* Forecast by experts for 1994.
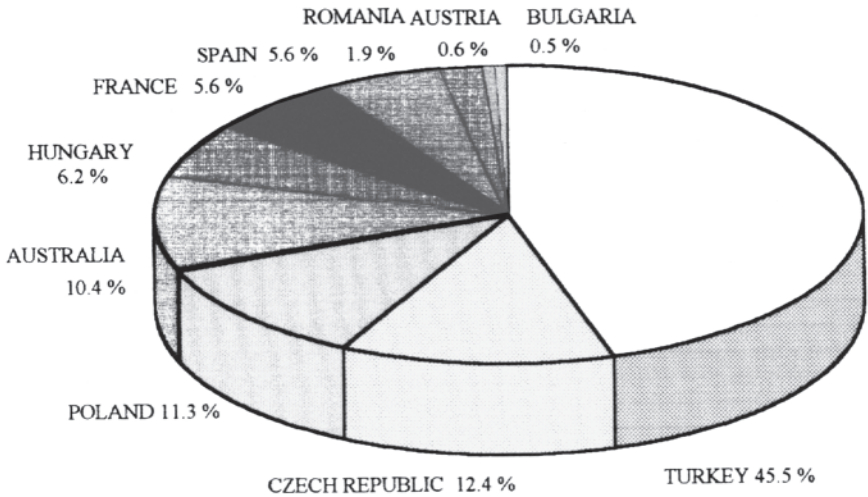
Figure 4 *Papaver somniferum* licit cultivation area for purposes other than the production of opium by country (forecast for 1995)
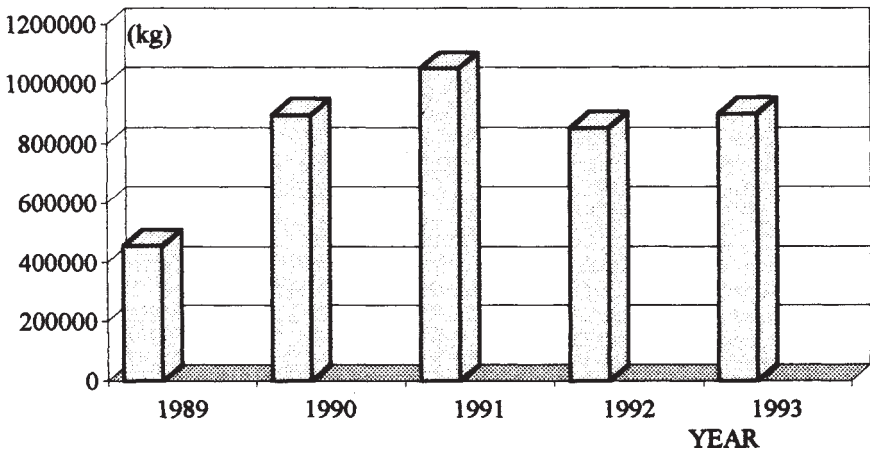
Figure 5 World opium usage for the extraction of opiates 1989–1993

The extraction of opium is undertaken in countries which both produce and import raw materials (Table 3). From this point of view the evaluation of Indian opium processing seems to be one of the most interesting questions. While India is the major opium producer, only 118370kg of opium is processed there (11.1% of the world total). The other extreme is the USA, which has no indigenous opium production but a high level of opium processing (65.3% of the world total). The bulk of opium

Table 3 Amount of opium used for extraction of morphine, codeine and thebaine in 1993
(selected from INCB data for 1993)

	Opium used (kg)	Alkaloids extracted (kg)		
		Codeine	Morphine	Thebaine
China	15 379	355	1364	5
France	14 398	310	1616	—
Hungary	90	—	7	—
India	118 370	—	8352	—
Iran	67 000	—	3365	—
Japan	81 590	1844	9817	667
Myanmar	1500	—	57	—
Poland	4974	—	559	—
Russian Federation	50 000	6842	470	33
United Kingdom	5977	358	1271	41
USA	538 998	12 124	48 308	4745

processed in the USA is sourced from India; the majority of the other opium processing countries (Russian Federation, France, Poland, Hungary, etc.) also have to import opium.

It is also obvious from Table 3 that the main goal of opium processing is for the extraction of morphine. Some countries (India, Iran, etc.) are specialized in morphine extraction only, but in the majority of cases codeine and thebaine are also extracted simultaneously from the raw material.

1.2.2 Extraction from Straw

Based on the data shown in Table 4, poppy straw and straw concentrate are more important than opium in the production of morphine. More than 50% of the world's morphine is now manufactured from these raw materials. However, the simple method of using dry capsules for direct extraction is practised only in some central European countries. The amount of poppy straw involved in the direct manufacture of morphine (Figure 6) was about 6558111kg in 1989 and decreased to 4029727kg in 1993. The leading countries processing by the direct extraction method are Hungary, the Czech Republic and Slovakia. The total amount of morphine produced by this technology was 19011kg in 1993.

1.2.3 Extraction from Straw Concentrate

The concentrate produced from straw makes the industrial manufacture of opiates more economical and the importance of this type of processing is increasing. The statistics usually consider concentrate with a 50% morphine extraction equivalent.

The quantity of morphine which is extracted from straw concentrate world-wide is continuously increasing. While 99,418kg of morphine was extracted by this technology in 1989, estimates show this had increased to 150112kg in 1993—an increase of 50%.

The United Kingdom is the largest producer of morphine from straw concentrate (Table 4). The second largest is Australia, producing 36082kg of morphine from

Table 4 Amount of poppy straw and straw concentrate used for extraction of morphine in 1993 (selected from INCB data for 1993)

	Poppy straw		Concentrate of poppy straw	
	Amount used for extraction (kg)	Morphine extracted (kg)	Amount used for extraction (kg)	Morphine extracted (kg)
Australia	—	—	72 165	36 082
Belgium	—	—	599	300
Czech Republic	3 472 769	6539	—	—
France	—	—	69 188	30 706
Hungary	1 106 120	7772	—	—
Italy	—	—	4842	2372
Netherlands	—	—	6238	2976
Norway	—	—	8885	4442
Portugal	—	—	449	368
Romania	7893	19	—	—
Slovakia	2 915 714	4681	—	—
South Africa	—	—	12 045	5971
Spain*	—	—	12 360	5644
Turkey	—	—	6210	4422
UK	—	—	92 910	46 293
USA	—	—	35 145	16 180

* Data from 1992
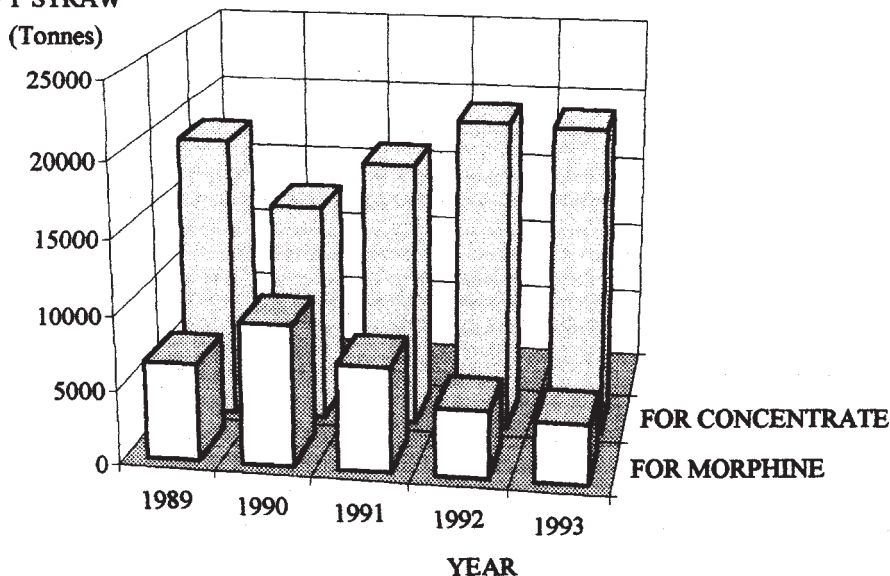
Figure 6 World poppy straw usage for the direct extraction of morphine or production of straw concentrate

concentrate in 1993. This considerable level of production is the result of the successful R&D activities which have been carried out in Australia to improve both agricultural and industrial aspects of poppy cultivation. The other major producers, including the USA, Spain, South Africa, Norway, the Netherlands and Italy, import straw concentrate for morphine extraction, with the exception of Turkey, which has its own poppy cultivation industry.

1.3 Manufacture of the Principal Narcotic Drugs

The manufacturing of the principal drugs from morphine occurs in pharmaceutical and chemical factories. Taking into consideration the quantity of morphine which is con verted into the three main chemical forms, the importance of codeine has to be emphasized (Table 5). Between 1989 and 1993 about 200000kg of morphine was used annually for world codeine production, with the actual amount increasing each year. Pholcodine is the second main chemical which is manufactured from morphine, however the amount of morphine used for this conversion (4000–6000kg) is considerably smaller. The production of ethylmorphine is of a similar order.

Taking into consideration the absolute production values of the most important eight chemical compounds derived from opium (morphine, codeine, hydrocodone, dihydrocodone, pholcodine, thebaine, oxycodone and ethylmorphine) the leading role of morphine, followed by codeine is obvious. Figure 7 shows the proportions of these eight compounds as percentages of annual world production in 1993. It is clear that the cumulative value of the six minor chemicals does not reach the percentage of either morphine or codeine; with the exception of hydrocodone, yearly global production of these minor compounds does not reach 10000kg.

1.4 World Stocks of Narcotic Drugs

From INCB data (1995a) the number of countries owning stocks of notable amounts of opiates is much higher than the number of producing and manufacturing countries. The stocks of narcotic drugs held by different nations are important in terms of both

Table 5 Amount of morphine used for conversion into codeine, ethylmorphine, pholcodine and other substances (selected from INCB data for 1989–1993)

	Amount of morphine used for conversion (kg)			
	Into codeine *	*Into ethylmorphine* **	*Into pholcodine*	*Into others*
1989	177 878	4455	7555	3121
1990	176 496	4405	5133	5654
1991	204 036	4139	4424	6671
1992	196 635	3348	4275	5624
1993	218 534	3193	6354	2699

Country ranking:
  * United States of America, United Kingdom, Australia, France, Japan, Hungary.
** France, India, Hungary.
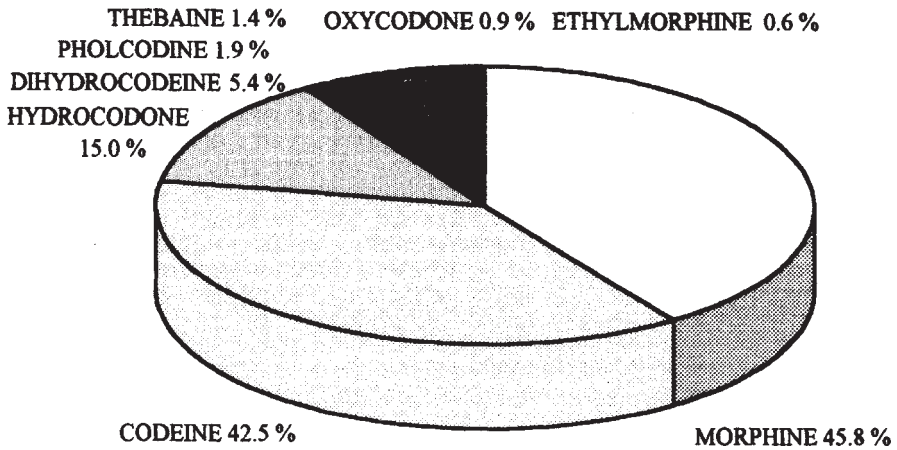
Figure 7 Proportions of principal opiates produced globally in 1993

health strategy and economics. Utilization of these stocks can have an effect on the cultivation, processing and price of poppy products.

World stocks of opium were extremely high in 1989 (Table 6), being similar to a three-year global requirement. This amount was reduced in subsequent years, and in 1993 stocks were half those of the 1989 value (1 342937kg). Straw concentrate obviously plays an important role in stock formation, however, the level of concentrate stocks has increased continuously since 1989, in contrast to the figures for opium stocks. While 84478kg of straw concentrate was stored in 1989, double this amount (163521kg) was held as stock in 1993.

Stocks of manufactured products show much more stability. The world stocks of these compounds varied slightly in 1989–1993 around the following levels: morphine 35000kg, thebaine 16000kg, dihydrocodeine 9300kg, pholcodine 3600kg, ethylmorphine 2600kg, hydrocodone 3100kg, oxycodone 2300kg and heroin 280kg.

Table 6 Total stocks of narcotic drugs in kg (selected from INCB data for 1989–1993)

	1989	1990	1991	1992	1993
Codeine	83 355	82 291	86 496	83 913	77 376
Concentrate of straw	84 478	78 838	95 933	171 103	163 521
Dihydrocodeine	7 996	7 280	9 585	9 590	12 087
Ethylmorphine	3 079	2 819	2 636	2 285	1 983
Heroin	173	178	268	398	405
Hydrocodone	2 071	2 702	2 832	3 611	4 174
Morphine	30 585	29 512	39 171	39 270	35 259
Opium	2 843 108	2 378 138	1 994 906	1 775 865	1 342 937
Oxycodone	1 747	1 938	2 159	2 756	2 881
Pholcodine	3 663	3 907	4 188	3 041	3 406
Thebaine	14 828	15 908	17 053	15 801	15 487

1.5 International Trade of Raw Materials and Manufactured Products

1.5.1 Export Activity

The export activity of raw materials and manufactured products has to be evaluated from different points of view, analysing both production background and consumption data. The complexity of the situation explains the values given in Tables 7 and 8. As a result of the regulation of national stocks, non-producing countries may occasionally appear in the world market either as exporters. For instance, Switzerland and Germany have no indigenous poppy cultivation yet appear in both export and import lists.

Among the raw materials the export of opium is of leading importance. A considerable amount of this raw—668208kg from 1993 estimates—is sold from India. The export of straw is more limited than that of opium because of the difficulties and extra costs arising from the transport of this less valuable material. However, the amount of straw exported from Spain in 1993 was considerable.

The global export of straw concentrate utilized for the industrial production of opiates is increasing. The total amount exported in 1993 was some 140000kg, for a possible extraction of morphine of about 70000kg. The increasing export of concentrate is due to the accelerated activity of Australia and Turkey in this respect.

In the case of manufactured products, export is dominated by the trade of codeine. The main exporting countries in this context are Australia, UK, USA, France and Hungary.

1.5.2 Import Activity

As shown by the data of Table 8, in 1993 there were at least 20 countries importing more than 25kg of opium, with the USA and Japan being the world's largest importers. The majority of Indian opium production is exported to these countries. The opium imports to Japan and the USA far outstrip the other nations involved and there were only three other countries buying more than 1000kg opium in 1993.

The import of poppy straw seems to be more specialized. France, Germany and Switzerland were the only countries who bought considerable amounts of this raw in 1993.

The import of straw concentrate is clearly more attractive to many countries. Large amounts of concentrate were imported by the UK (68523kg) and Japan (40134kg). The Netherlands, South Africa, Norway and France also buy considerable amounts of concentrate for industrial processing.

The number of countries importing opiates in processed forms is very large; the world-wide application of opiates is due to their well known therapeutic activity.

2 ILLICIT PRODUCTION OF POPPY

2.1 Opium Production Estimates

The opium poppy is cultivated illegally in many countries and this is coupled with unofficial processing and unregulated trade. However, in order to make any regulations

Table 7 Countries exporting raw materials and opiate products in 1993 (selected from INCB data for 1993)

	Raw materials (kg)			Manufactured products (kg)				
	Opium	Straw	Concentrate	Morphine	Codeine	Ethylmorphine	Dihydrocodeine	Pholcodine
India	668 208	9 208	—	—	—	—	—	—
Bulgaria	5 000	—	—	—	—	—	—	—
Germany	507	1 022	—	—	1 488	—	543	—
Italy	481	—	—	—	—	—	1 691	—
UK	324	—	—	3 674	12 822	228	2 809	1 661
France	243	—	13 464	1 848	7 757	163	—	384
Switzerland	62	3 644	—	—	744	27	—	14
Spain	—	650 000	12 000	201	—	—	—	—
Austria	—	92 161	—	—	964	—	—	—
Yugoslavia	—	57 789	—	—	—	—	—	—
Hungary	—	11 421	—	249	6 002	101	670	100
Australia	—	—	65 635	582	15 795	—	792	142
Turkey	—	—	41 173	—	1 924	51	—	—
Netherlands	—	—	8 537	2 242	4 420	13	609	268
Sweden	—	—	—	273	—	—	—	—
USA	—	—	—	—	11 780	—	—	—
Norway	—	—	—	—	2 077	99	—	203
Czech	—	—	—	—	2 069	—	—	—
Portugal	—	—	—	—	—	—	47	—
Others	138	—	—	462	626	6	33	8

Table 8 Countries importing raw materials of opiates for manufacturing or curing in 1993 (selected from INCB data for 1993)

	Opium (kg)	Poppy straw (kg)	Concentrate (kg)
USA	529 552	—	—
Japan	113 000	—	40 134
France	16 201	651 000	7 999
Poland	5 000	—	340
Hungary	2 005	—	—
South Africa	209	—	12 293
New Zealand	125	—	—
Denmark	103	—	—
Sri Lanka	100	—	—
Switzerland	92	14 804	—
Indonesia	85	—	—
Brazil	65	—	—
Finland	50	—	—
Tunisia	40	—	—
Morocco	30	—	—
Portugal	30	—	448
Senegal	30	—	—
Zimbabwe	25	—	—
Candada	25	—	—
Hong Kong	32	—	—
Germany	—	95 040	—
Netherlands	—	5 976	15 555
UK	—	—	68 523
Norway	—	—	8 584
Italy	—	—	4 846
Belgium	—	—	599
Others	110	—	—

or control this field reliable information is required. The estimates of the INCB, INM (US State Department), RCMP (Royal Canadian Mounted Police) (1991, 1992) and some independent evaluations (Smart and Archibald, 1980; Johnson, 1989; Whynes, 1991; Armstead, 1992; Gordon, 1994) help to widen our knowledge in this field.

2.1.1 World Area of Illicit Cultivation

Although there are large differences in the estimates, all agree that the production area of illicit cultivation is much larger than that used for licit production—based on the estimates of INCSR, the global area of unofficial opium poppy cultivation is larger by an order of magnitude. The area used for illicit opium production is considered to be more than 200 000–220 000ha. The changes of cultivation area based on estimates related to the five-year period between 1989 and 1993 are shown in Figure 8. According to the estimates the illicit cultivation area has increased
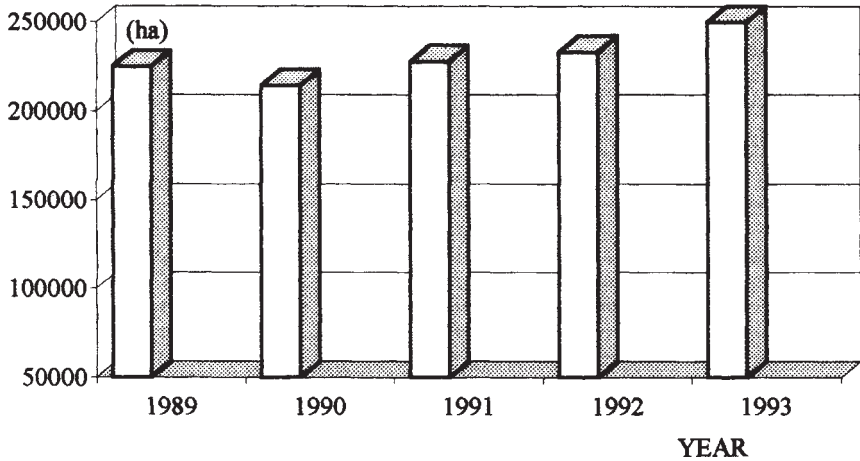
CULTIVATION AREA

Figure 8 Estimates of world cultivation area of illicit opium, from the data of INCSR

continuously and reached a maximum of 250428ha at the end of this period. For comparison, the official cultivation area for the same year was only 14241ha.

2.1.2 Opium Production

Although there are no reliable data available on illicit annual world opium production, all the estimates agree that its amount must be over 3000000kg. (For comparison, licit production registered by the authorities produces around 500000kg.) Opium production estimates for the period 1989–1993 are shown in Figure 9; it has increased steadily since 1990 with a maximum estimated value of 3879000kg in 1993.

2.1.3 Illicit Opium Production by Region

1993 data for the majority of the countries involved in the illicit production of opium poppy are summarized in Table 9. Of course, some countries are not included in the table due to a shortage of reliable estimates or because of their minor cultivation area.

2.1.3.1 East and southeast Asia (golden triangle)

In this region the opium poppy is cultivated mainly in Myanmar, along its borders. This country is considered to be one of the largest sources of illicit opium, producing about 2500000kg in 1993—about two thirds of the world's illicit opium. Opium production in the surrounding countries (Thailand, Laos and Vietnam) is also recorded. Effective steps to prohibit illicit opium production have been made in Thailand and Vietnam (Suwanwela *et al.*, 1978).

2.1.3.2 South Asia

The states in this region, with the exception of the Maldives, are parties to the 1988 Convention and although abuse of narcotics has been reported from the majority of
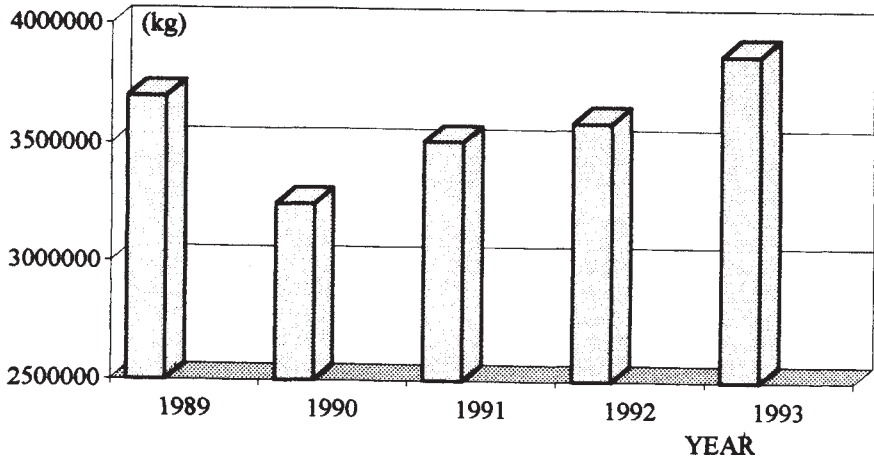
OPIUM PRODUCTION

Figure 9 Illicit opium production of the world, from the data of INCSR

the countries in this area, cultivation of the opium poppy is absent. Illicit plantations which were discovered were eradicated locally.

2.1.3.3 West Asia (golden crescent)

The Golden Crescent and the newly independent states in Central Asia are more affected by the illicit cultivation of poppy, along with its inherent abuse problems (Masood, 1979). This region is the second major illicit drug-supplying area of the world. Afghanistan is the leading country with an estimated production of 685 000kg opium in 1993 (Table 9). Production in Pakistan (140000kg) and Iran (35000kg) is also considerable. Recently, especially in Pakistan, increased control measures and law enforcement activities have been shown by the local authorities. According to

Table 9 Countries of main importance involved in illicit opium production, based on 1993 using estimates of INCSR

	Area harvested (ha)	Opium (kg)	Yield (kg/ha)	Yield* (kg/ha)
Afghanistan	21 080	685 000	32.5	49.1
Iran	3 500	35 000	10.0	20.0
Lebanon	440	4 000	9.1	15.0
Pakistan	6 280	140 000	22.3	44.1
Laos	26 040	180 000	6.9	27.5
Myanmar	1 65 800	2 575 000	15.5	17.0
Thailand	2 880	42 000	14.6	14.6
Colombia	20 000	200 000	10.0	10.0
Guatemala	438	4 000	9.1	10.1
Mexico	3 960	49 000	12.4	12.4

* Highest values of different estimates (Gordon, 1994).

the Iranian authorities, the success of these activities has led to the elimination of clandestine heroin and morphine laboratories in the Islamic Republic of Iran.

2.1.3.4 Africa
Egypt is the only country of this region where efforts to eradicate illicit poppy cultivation have been reported. There are no indications of illicit cultivation or production of opiates in other parts of Africa.

2.1.3.5 Central America
Illicit opium production has been recorded in two countries of this region—Mexico and Guatemala. Mexico is the larger producer of the two (Table 9), harvesting poppy on 3960ha in 1993, yielding 49000kg of opium. The INCB estimates that the plantation area used to be much larger, but is kept under control regularly by the authorities' Production and cultivation area in Guatemala are smaller by an order of magnitude.

2.1.3.6 South America
Illegal opium poppy cultivation is reported to be taking place in some South American countries, for example Colombia, Ecuador and Peru. However, there are no reliable estimates of the extent of cultivation. The data of Table 9 indicate that the cultivation area in Colombia is considerable—20 000 ha yielding 200000kg of opium in 1993.

2.2 Main Routes of Illicit Opium Traffic

Although an increase in heroin seizures was reported recently from Myanmar and from a number of other countries in East and Southeast Asia, this region (Golden Triangle) remains the major outlet of opium and its converted forms (heroin, heroin hydrochloride) which are produced in local laboratories. Favoured routes for transporting illicit drugs from this region are the sea routes along the Western coast of Malaysia and Thailand, with seaports and airports located in China, Hong Kong, Indonesia, the Philippines and Singapore. There is an alternative and increasing land route for transporting drugs via China and in response to this situation, law enforcement activities have been strengthened by the authorities.

The Golden Crescent is the other major illicit drug-supplying area of the world. Based on some reports, 75% of heroin seized in Europe and 25% in the USA are produced in this region. The majority of drugs abused in Africa and the Arabian Peninsula have the same origin. Turkey remains one of the main transport routes to Europe. With the collapse of the former Soviet Union, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan have become alternative routes for production and transport. In addition, because of the lack of satisfactory border controls, Central Asia has become a transit route for opium products originating in Southwest Asian countries destined for Europe. There are also reports which have tried to calculate the increasing role of the seaports of the Persian Gulf States in the transport of heroin.

There is evidence that illicit poppy cultivation and heroin manufacture in Colombia have developed only recently as the Colombian products started to be smuggled into the United States. Caribbean products have also appeared in Europe, as reported in seizures data from Italy.

Western Europe is the main destination of illicit heroin. However, it has been observed that the traffic routes of opiates inside Europe have diversified over recent years. It has shifted from Yugoslavia and its former republics to several alternative routes, e.g. sea routes through Southern Europe and land routes through Eastern European countries. The airports of these latter countries are also being increasingly used for smuggling.

3 OBJECTIVES OF INTERNATIONAL DRUG CONTROL

It was recognized even at the beginning of the twentieth century, that the struggle against drug abuse and illicit traffic needs to be harmonized at both national and international level. The results of the first international conference on narcotic drugs held in 1909 in Shanghai and the provisions of the International Opium Convention signed in 1912 in The Hague proved that it is possible to react against the unlimited availability of narcotic drugs in the world, on the bases of international consensus.

The Single Convention on Narcotic Drugs signed in 1961 has to be considered as a great advance in the history of the international co-operation in this field. This convention was based upon earlier national and international regulations controlling the cultivation and production of narcotic plants. The countries were also obliged to take measures against illicit processing, traffic and abuse of narcotic drugs. The diversification of narcotic drugs and the global distribution of synthetic products made completion of the former Convention necessary; this was realized in 1971 with the signing of the Convention on Psychotropic Substances.

The United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances, which was accepted in 1988 can be evaluated as a response to the increasing world activities observed in the illicit cultivation, production, manufacture and traffic of poppy products and other narcotic substances. The result of this Convention is remarkable. By the measures which were introduced in the Convention, a much simpler situation was created. The illicit and licit production areas were distinguished. Drugs produced and traded on illicit markets are no longer produced in official production areas which means that the international illicit drugs traffic is supplied mainly by illicit producers and laboratories.

A special board was brought into existence for checking and advising on global tendencies in this respect. The International Narcotics Control Board and its predecessors (Permanent Central Opium Board, Permanent Central Narcotics Board, Drug Supervisory Body) were established to limit—in co-operation with national governments—the cultivation, production, manufacture and use of drugs to an adequate amount required for medical and scientific purposes. Over the years the INCB has had much experience in monitoring the control of narcotic drugs, psychotropic substances, precursors, and the supply and demand for drugs for medical purposes. Evaluation methods and techniques were developed to analyse the world situation, as well as regional ones. The INCB has a right to recommend various forms of technical assistance that should be provided by the United Nations to other countries.

The International Narcotics Control Board—on the basis of international drug control

treaties—prepares an annual report which contains an analysis of the global drug control situation. From analyses of the data presented, the INCB can draw the attention of governments to weaknesses that occur in their national control and make suggestions to improve the situation.

ACKNOWLEDGEMENTS

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VIII. UTILIZATION OF POPPY SEED

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1 FIELDS OF UTILIZATION

Although much of the literature refers to the narcotic properties and applications of the capsules of poppies in ancient times, it seems obvious that the seed was also utilized. It was mentioned by Kritikos and Papadaki (1967) that Dioskourides (in the first century AD), in sorting several kinds of capsules, drew attention to the garden poppy, whose seeds were suitable in food preparation. Poppy seeds and oil are also mentioned in certain books of the Bible and, particularly, in the Talmud.

Poppy seed is now utilized by the majority of rural people living in regions where the cultivation of poppies for opium is the main objective. The consumption of seed is rarely mentioned, because seed is of much less economic and social importance than is opium for rural people. Harvesting the seed is, however, part of the cultivation process in opium producing areas. Singh (1982) reported that in India poppy plants are left for about 20–25 days after the last lancing of the capsules has taken place and exudation of latex has ceased. The capsules are then picked and the crop is harvested with sickles. The capsules are spread over open yards for drying and the seeds are then beaten from the capsules using a wooden rod. After separation the seed is used in India as an important culinary item and a small part is exported, mainly to other countries in Asia and Africa (Gupta, 1984). In many regions, especially in Europe, special cultivars have been selected for seed and oil production as a result of many hundreds of years of seed consumption in traditional dishes. In such regions the straw is considered to be a side product only, and is sold to chemical factories for further alkaloid extraction. These types of cultivars are frequently mentioned in the literature and are even called 'oil poppies' in some regions (Shuljgin, 1969).

The economics of poppy cultivation in Europe and in other developed countries of the world dictate that the seed has to be utilized (Bryant, 1988). The spectrum of its utilization is very wide, as a result of the essential chemical compounds which accumulate in the seed. Seeds are widely used in the food industry and seed oil is considered to be an important raw material in the manufacture of paints and varnishes (Balbi, 1960). There are many examples in the literature which detail the industrial utilization of poppy seeds and oil. The seed is commonly used for decoration of bakery products (Anonymous, 1985a,b; Benk 1987; Gorton 1993; Markuschenko *et al.,* 1983) or for filling 'poppy seed' products (Anonymous, 1982; Seibel *et al.,* 1977). Based on Austrian data (Steidl and Gressl, 1992) domestic demand in Austria is some 1700–2300 tonnes per annum, 30% of which is used in bakeries, industrial users account for a further 40% and 30% goes directly to food retailers. There are

also reports that the seed has a high nutritive value for animals (Statham, 1984; Kempen *et al.,* 1994) and birds (Karle *et al.,* 1989).

The biological value of seed products has been compared to other foods. Beare et *al.,* (1979) proved that poppy seed oil exhibited properties similar to those of sunflower oil and olive oil and considered it a promising oil for human consumption. In the experiments of Srinivas and Narasinga-Rao (1986a, b), the water and fat absorption capacity, emulsification capacity, and foam capacity and stability of de-fatted poppy seed meal were compared with those of soy-bean meal. The similarity and some advantageous characteristics of the poppy product were determined.

2 MAIN GROUPS OF UTILIZED CHEMICAL COMPOUNDS

2.1 Seed Oil

Poppy seed samples of different origins have been analysed for oil content and fatty acid composition by many research groups. Raie (1985) measured oil contents of 47% and 53% in the seeds of plants which were grown in Pakistan. The oil contents of selected populations and hybrids of opium poppy grown in Lucknow (India) varied in the similar interval with values of 41.4–49.1% (Singh *et al.,* 1990). In Sweden the differences between white- and blue-seeded varieties were also compared in this respect (Eklund and Agren, 1975)—the white variety contained 40% oil, while the blue seeds showed a considerably lower level (33%).

There are large differences in the fatty acid composition of oils even in seed samples taken from the same region (Table 1). Whilst the investigations of Sengupta and Mazumder (1976) determined well defined values under Indian cultivation conditions, the seed samples analysed by Singh *et al.* (1990) showed large differences. In this latter case the palmitic, stearic, oleic, linoleic and linolenic acid contents varied over wide ranges: 8.9–21.5%, 1.4–10.87%, 13.2–36.8%, 41.0–68.0%, and trace-9.4% respectively. The fatty acid composition of seeds of 18 domestic and foreign populations grown under diverse ecological conditions in Leningrad was also analysed by Yarosh and Megorskaya (1975). The fatty acid composition of the seeds ranged as follows: palmitic acid 7.8–9.6%; stearic

Table 1 Fatty acid content of seed samples of different origin

	RUSSIA (Yarosh and Megorskaya, 1975)	INDIA (Sengupta and Mazumder, 1976)	INDIA (Singh et al., 1990)
Palmitic acid (%)	7.8–9.6	12.0	8.9–21.5
Stearic acid (%)	1.7–2.4	3.0	1.4–10.8
Oleic acid (%)	14.7–16.2	20.0	13.2–36.8
Linoleic acid (%)	72.2–75.0	65.0	41.0–68.0
Linolenic acid (%)	0.0	0.0	Trace–9.4

acid 1.7–2.4%; oleic acid 14.7–16.2%; linoleic acid 72.2–75.0%. Distinguishing between cultivars, the late poppy seed varieties had even higher contents of linoleic acid, 78.5–80.0%. It was also concluded by Yarosh and Megorskaya that the proportion of linoleic acid decreased in seeds from characteristically dry and hot vegetation cycles.

The importance of the composition of fatty acids in relation to seed and oil quality, especially the occurrence of a 'burning/bitter' taste, has been proved in many investigations. The early appearance of the bitter taste may be the consequence of improper harvesting. The investigation of Meshehdani *et al.* (1990) showed that manually harvested seeds contained 3.3–4.8% damaged seeds and 0.4–1.5% free lipids on the surface of the seeds, which resulted in low peroxide values and a low percentage of oxidation products in the total free lipids (5.0–14.4%). There were 5.3–6.6% damaged seeds in the combine harvested product, showing 0.9–2.8% free lipids on the seed surface and high peroxide values, resulting in 7.4–16.5% of oxidation products in the total free lipids. It was concluded that seed damage during harvesting should be minimized to avoid the development of the bitter flavour which is due to the action of lipoxygenase on linoleic acid on the seed surface. The importance of the free linoleic acid and the role of lipoxygenase activity in generating the bitter taste have been proved by other authors. Oxidation processes also occur in poppy seeds used for baking during storage at room temperature (Grosch and Laskawy, 1984).

2.2 Seed Proteins

In their analysis of poppy seeds, Srinivas and Narasinga-Rao (1986b) stated that the seed is rich in aspartic and glutamic acids, arginine and methionine. In a more detailed investigation (Srinivas and Narasinga-Rao, 1987) gel electrophoresis showed that the proteins were heterogeneous and consisted of at least five protein fractions. The proteins contained 3.1% carbohydrate and no phosphorous. The composition of low molecular weight amino acids was different from that of the 10S protein in having higher amounts of cystine, glutamic acid, arginine and lower amounts of aspartic acid, leucine, isoleucine, valine, histidine, tryptophan and phenylalanine. The molecular weight of the proteins determined by the gel filtration technique was 14 500. The total protein content of seed was measured to be 21.1% in Turkey (Nergiz and Oetles, 1994). Large differences in protein content were measured by Swedish scientists in their study of different cultivars (Eklund and Agren, 1975)— white seeds were found to contain 27% protein, while the blue variety contained only about 21% protein.

2.3 Sterols

The composition and proportion of free sterols and sterol esters in poppy seed oil were determined by Johansson (1979). The proportions of free and esterified sterols were found to be 0.33% and 0.05% respectively. The sterols in poppy seed oil were composed almost entirely of campesterol, stigmasterol, sitosterol and 5-avenasterol, although their percentage distributions were remarkably different in the free and esterified fractions. Large variations in the ratio of different sterols occur during seed

storage, as was demonstrated by a decrease in delta-5-avenasterol by Johansson and Appelqvist (1979). The proportion of delta-5-avenasterol in the total sterols dropped from 25.3% to 16.9% in seed which had been stored for ten months.

3 WORLD TRADE OF POPPY SEED

Poppy seed is utilized for human consumption, industrial processing and in the manufacture of animal feeds. However, the quantity of seed and the ways in which it is utilized vary from country to country as a result of national traditions. There are particular regions (e.g. Central Europe and India) where poppy seed consumption has been traditional for many centuries, while, in other countries the seed is only used for industrial processing and food decoration.

According to data from the Food and Agriculture Organization (FAO), the number of countries playing a characteristic role in the world trade of poppy seed is somewhat limited. Table 2 shows export data from the FAO database and emphasizes the importance of the Czech Republic, Pakistan and Turkey in the trade of poppy seed. Export from these countries is mainly based on indigenous production. Table 2 also shows that there are other large seed exporters, such as France, Germany, and the Netherlands, whose export obviously has to include seed sourced from other countries.

The import data (Table 3) show that Germany, the Netherlands, Denmark, Austria and the USA are the larger consumers. India also imports a relatively large amount of seed, which indicates that Indian production, which is relatively significant, does not satisfy the home demand at all.

Table 2 Countries which play an important role in poppy seed export (countries which exported more than 100 tonnes per annum in two or more years of the five-year period investigated). Data selected from the 1996 FAO database

	Export of poppy seed in metric tonnes				
	1990	1991	1992	1993	1994
Australia	3013	3153	4688	5654	6904
Czech	0	0	0	5386	20 288
Denmark	740	559	506	661	605
France	391	809	2224	3220	1998
Germany	666	2607	332	711	428
Hungary	0	2988	1151	650	829
Netherlands	3423	3850	4931	10 285	12 161
Pakistan	6406	4419	1391	3596	9038
Slovakia	0	0	0	243	569
Spain	1259	272	1671	1612	710
Sweden	25	59	8	122	137
Turkey	3971	5066	6866	4995	6039
USA	278	97	80	118	388

Table 3 Countries which play an important role in poppy seed import (countries which imported more than 100 tonnes per annum in two or more years of the five-year period investigated). Data selected from the 1996 FAO database

	Import of poppy seed in metric tonnes				
	1990	1991	1992	1993	1994
Austria	1695	1514	1398	1640	1662
Canada	447	685	722	711	1548
Croatia	0	0	151	163	216
Denmark	1625	2095	1593	1786	1755
France	297	273	755	1505	232
Germany	5921	6022	7297	7560	6710
Hungary	0	210	398	477	577
India	2083	129	3192	3525	3525
Israel	467	421	200	0	0
Italy	99	112	90	139	172
Japan	367	372	221	301	332
Malaysia	254	467	344	285	461
Netherlands	3821	4671	3897	3929	5283
Norway	174	224	207	229	220
Pakistan	922	37	322	392	311
South Africa	48	109	74	146	146
Sweden	874	914	875	820	1142
UK	836	710	796	680	851
USA	3355	4989	4882	5162	5619
Yugoslav SFR	312	250	350	110	110

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