Handbook on Drugs from Natural Sources
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<td>Indian Price</td>
<td>1175</td>
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<td>125</td>
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<td>Pages</td>
<td>480</td>
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<td>ISBN</td>
<td>978817831331</td>
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<td>Asia</td>
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<td>Pacific Business Press Inc.</td>
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Natural products have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various materials including terrestrial plants, terrestrial microorganisms, organisms etc. Historical experiences with plants as therapeutic tools have helped to introduce single chemical entries in modern medicine. About 40% of the drugs used are derived from natural sources. Most are pure substances which are isolated from various organisms & used directly or after chemical modification. Natural products will continue to be important in three areas of drug discovery: as targets for production by biotechnology as a source of new lead compounds of novel chemical structure and as the active ingredients of useful treatments derived from traditional systems. Biotechnology will contribute more new natural products for medicinal use. Plants provide a fertile source of natural products many of which are clinically important medicinal agents. Natural products have traditionally provided most of the drugs in use. Despite the achievements of synthetic chemistry and the advances towards rational drug design, natural products continue to be essential in providing medicinal compounds and as starting points for the development of synthetic analogues. With the increasing power of screening programs and the increasing interest in the reservoir of untested natural products, many future drug developments will be based, at least in part, on natural products. The major contents of the book are plant products produced in cell culture, application of genetic engineering to the production of pharmaceuticals, anti transpirants and plant growth regulators based, the potential and the problems of marine natural products, marine sterols, plants as a source of anti-inflammatory substances, anti hepatotoxic principles in oriental medicinal plants, immune stimulants of fungi and higher plants, amanita muscaria in medicinal chemistry, ergot alkaloids and their derivatives in medicinal chemistry and therapy, development of drugs from cannabinoids, etc. This book contains development of new drugs from plants, work on some Thai medicinal plants, plant growth based on Jasmonates, marine sterols, bleomycin and its derivatives, drugs from cannabinoids, bioactive compounds from nature, fungi and higher plants, biological active compounds from British Marine, microbial phytotoxins as herbicides and many more. This book will be very helpful to its readers, upcoming entrepreneurs, scientists, existing industries, technical institutions, druggist etc.

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35. Bot. : Acacia Arabica Hindi : Babul, Sanskrit : Babbula
37. Bot: Acacia catechu Willd. Eng: Cutch tree, M: Khair, H: Khair
42. Bot: Aloe barbadensis Mill. M: Korphad, H: Ghikanvar
43. Bot: Argemone mexicana Linn. Eng: Prickly poppy, Mexican poppy, M: Satyanaashi; Pivla Dhotra, H: Sialkanta
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59. Bot: Coriandrum sativum Linn. M: Dhane, H: Dhaniya
63. Bot: Cucumis melo Linn. Eng: Musk melon, Sweet melon, M: Chibud; Kharbuza, H: Kakani
64. Bot: Curculigo orchioides, Hindi: Kali Musli, Sanskrit: Talmuli
67. Bot: Glycyrrhiza glabra, Hindi: Mulethi,
Sanskrit : Yashtimadhu
69.Bot : Hyoscyamus niger, H. : Khursani, ajvayan,
Sanskrit : Yavani
71.Bot : Melia azedarach Linn. Eng. : Persian Lilac, Bead tree,
M : Bakaanaanimb, H : Bakain
72.Bot : Pterocarpus santalinus, Hindi : Lal Chandan,
Sanskrit : Raktachandan
73.Bot : Raphanus sativces, Hindi : Muli, Sanskrit : Moolaka
74.Bot : Trigonella foenum-graceum,
Hindi : Methi, Sanskrit : Methinee
75.Bot : Viola odorata, Hindi : Banaphasha, Sanskrit : Banaphasha
76.Bot : Withania somnifera, Hindi : Asagandh :
Sanskrit, Ashawgandha

Sample Chapter:
An Introduction to Drugs from Natural Products

Archaeological evidence reveals that drug taking is an extremely old human phenomenon. By necessity, the drugs used in ancient civilizations were extracts of plants or animal products, with a few inorganic salts. In India, the Ayurvedic system of medicine developed an extensive use of medicines from plants dating from at least 1000 BC. The earliest Chinese records give descriptions of diseases but not medicines: illnesses were thought to be godly punishments and they were treated by prayers and offerings. The earliest recorded Chinese prescriptions after about 500 BC show the beginning of the use of natural products as drugs. The first classic texts in Chinese medicine appeared in AD 25-220, and some of their formulae remain in use. Similarly, the Egyptian Ebers papyrus (around 1550 BC) contains descriptions of several active ingredients (notably purgatives) that are still used today.

Western medicine continues to show the influence of ancient practices. Current examples are the use of cardiac glycosides from the purple foxglove Digitalis purpurea and related plants, opiates from the opium poppy Papaver somniferum, reserpine from Rauwolfia species, and quinine from Cinchona species. More recently, there has been interest in other products from traditional systems of medicine: artemisinin is an active antimalarial compound isolated from Artemisia annua, a constituent of the Chinese antimalarial preparation Qinghaosu (Huang, 1984), and forskolin was isolated from Coleus Forskohlii, a species used in Ayurvedic preparations for cardiac disorders. A new standardized preparation, artemether has recently been introduced for treatment of drug-resistant malaria, and new analogues of forskolin are being tested for a variety of uses.

Plant products can also be useful as starting material for the semisynthetic preparation of other drugs. The main examples are plant steroids for the manufacture of oral contraceptives and other steroidal hormones. Diosgenin from several species of yams (Dioscorea) and hecogenin from sisal leaves (Agave sisalana) are the main compounds used.

Plants are not the only natural products used as a source of drugs. Microorganisms have been extensively screened for antibiotics since Fleming’s discovery of the antibacterial activity of Penicillium notatum. More recently discovered antibiotics of fungal origin are griseofulvin and cephalosporin C. Many antibiotics are produced by bacteria of the Streptomyces genus, these include streptomycin, neomycin, tetracycline and chloramphenicol.

Animal products were also used extensively in most ancient systems of medicine, although few of the traditional preparations have been shown to be active. However, some drugs in present use are prepared from animal sources. These include the anticoagulants ancrod (from the venom of the Malayan pit viper Calloselasma rhodestoma) and batroxobin (from the Venom of the South American viper Bothrops atrox) and hormones such as bovine and porcine insulins, and human growth hormone.

In addition to pharmaceuticals, natural products have also been used as agrochemical agents. The pyrethroid insecticides were developed from the naturally occurring compounds found in the flowers of various species of Chrysanthemum, the insecticidal properties of which had been known for many years. Plant growth regulators are also being used, including ethylene, abscisic acid, and, more recently, jasmonates and there is the hope that novel insecticides may develop from leads provided by some spider toxins.

CURRENT CONTRIBUTION

Although interest in natural products as a source of new biologically active compounds decreased in the last few decades as synthetic chemistry programmes expanded, natural products continue to form a significant proportion of drugs in current use and of those under investigation. It has been estimated that
56% of the lead compounds for medicines in the British National Formulary are natural products or are derived from natural products. Of the top 20 best-selling pharmaceutical products in 1990, four were derived from natural products (amoxycillin, cefaclor, ceftriaxone, and lovastatin), and two others (captopril and enalapril) resulted from leads provided by a natural product.

With developments in the techniques of molecular biology, there has been a great increase in interest in the use of naturally occurring proteins as potential therapeutic agents. Although the wilder expectations of the "therapeutic protein revolution" have not been fulfilled, several genetically engineered natural products have had a significant impact, and more than 20 biotechnology-derived products are now on the market. Tissue plasminogen activator is used as a thrombolytic after myocardial infarctions, erythropoietin is used to treat anaemia associated with renal failure, and several colony-stimulating factors have been marketed recently for use in cancer treatment.

CONTINUING DEVELOPMENTS

Natural products will continue to be important in three areas of drug discovery: as targets for production by biotechnology, as a source of new lead compounds of novel chemical structure, and as the active ingredients of useful treatments derived from traditional systems of medicine.

Biotechnology will contribute more new natural products for medicinal use. These will be additional therapeutic proteins and oligosaccharides, and new molecules from microbial fermentation. In the latter case, there will be expanded interest in the nonantibiotic activities of microbial products.

The discovery of new chemical structures with biological activities largely depends on chance, and it will, therefore, be made more likely by screening large numbers of compounds. Empirical screens based on functional bioassays have been established in several laboratories for many years. Such broad-based screening can detect novel effects, but it is slow, expensive, and not suited to screening of very large numbers of samples. Simpler, in vitro screens are more useful, although these have generally been restricted to detection of defined activities, such as anticancer properties. Even then, a very comprehensive battery of tests may be required, as shown by the development of the in vitro screening programme by the National Cancer Institute in the United States. However, such a screening programme did discover the activity of taxol.

More recently, screening has been based on biochemical assays and receptor-ligand binding assays. With the increasing availability of human receptors from molecular cloning, extracts and compounds can be tested for binding directly to the presumed therapeutic target protein. With further refinements brought about by applications of molecular biology, cloned receptors can be expressed in a functional state linked to reporter proteins in simple cells, such as yeasts.

When combined with robotic machinery and computerized data analysis, such assay Systems can be used for tens of thousands of samples each year. The keys to successful drug discovery become the adequate supply of a wide range of test chemicals and the appropriate choice of potential therapeutic targets for the assay system. Given the vast range of chemical structures provided by natural sources, screening of libraries of natural Products seems a good bet. There are estimated to be at least 250 000 species of higher plants and around 30 million species in total; most of these have not been tested for biological activity. However, the World Wide Fund for Nature estimates that at least 50 000 species are being lost annually through destruction of tropical forests. More efforts to maintain biodiversity are clearly necessary. There is increasing interest in traditional systems of medicines from non-Western societies. The expectation is that at least some of the medicinal products will be efficacious, and the hope is that traditional use will be based on products with low toxicity. It is not clear whether either of these assumptions is justified. Some particular examples of compounds with predicted activity being isolated from plant medicines are described by Waterman. However, the results from the large-scale screening programme of
the Central Drug Research Institute in Lucknow, India are worth noting. Almost 2000 plants were screened for a wide range of biological activities; more than 500 of the plants were associated with traditional medicinal uses; pharmacological activity was detected in 18.3% of the medicinal plants, and in 18.9% of the other plants. Thus, there is little evidence that working with plants used in traditional medicines greatly enhances the chance of finding biological activity.

There have also been doubts about the assumed low toxicity of products in traditional uses. For example, there have been reports of jaundice associated with the use of Chinese herbal medicines for eczema and cardiotoxicity fromaconite in herbal mixtures.

Natural products have traditionally provided most of the drugs in use. Despite the achievements of synthetic chemistry and the advances towards rational drug design, natural products continue to be essential in providing medicinal compounds and as starting points for the development of synthetic analogues. With the increasing power of screening programmes and the increasing interest in the reservoir of untested natural products, many future drug developments will be based, at least in part, on natural products.

Natural products from plants: bioassay-guided separation and structural characterization

Many important drugs are derived from compounds originally discovered in plants, and there is now increasing interest in searching for new therapeutically useful molecules from natural sources. The naturally occurring compound will, in all likelihood, only provide the starting point for development of analogues, but the most important step in the discovery process is the identification of this source of interesting biological activity. In an earlier review several approaches to discovery of activities were outlined:

(a) The ethnobotanical route,
(b) Bioassay-guided serendipity,
(c) Straight serendipity,
(d) Exploitation of chemotaxonomic knowledge, and
(e) Investigations based on chemical ecology,

This chapter will review some examples using the different approaches. Largely, it will draw on work being undertaken at Strathclyde University under the auspices of the Strathclyde Institute for Drug Research. The examples are taken from higher plants, but it should be remembered that bioactive molecules can be found from many other sources: pteridophytes, fungi, algae, marine organisms, and animal venoms. For further discussion on the study of microbial products, see the accompanying chapter by Berry and for a discussion on venom proteins, see the chapter by Dufton. Thus, the resource of biological material for investigation is virtually unlimited. With higher plants alone, current estimates are that the world contains between 250 000 and 350 000 species, while in total there may be as many as 30 million species of all types. None of these can be said to have been completely investigated because there are always new bioassays appearing to provide new test systems, and because improvements in Phytochemical methods allow identification of compounds in smaller and smaller concentrations. Furthermore, Wink demonstrated that many plants not normally regarded as producers of particular alkaloids do produce them in trace amounts, and must, therefore, have the genetic material for their synthesis. Therefore, many plants may have the capacity for producing many more secondary metabolites than they normally do. The possibilities of stimulating quiescent synthetic pathways could lead to a further expansion of the natural products library.

ROUTES TO DRUG DISCOVERY

The ethnobotanical route

Man, and perhaps some of his closer relatives, has always made use of plants to treat illness, and many of these remedies have real beneficial effects. The need to document plant usage and to attempt to confirm their efficacy remains urgent and must be undertaken with the same rigour as would any other scientific study.
Various approaches can be taken to using information about traditional medicinal plants. It is necessary to verify that the traditional knowledge has been accurately passed from generation to generation, and it is probably sensible to concentrate on regions with a diverse flora. Perhaps not surprisingly there has been a concentration on Chinese traditional medical products, but there is increasing interest in other ancient systems of medicine (e.g. the Ayurvedic in India) and those found in more primitive societies. It is hard to establish the success rate of a screening approach based on ethnopharmacological information. Few wide-ranging studies have been performed, and it is not known whether negative results are published as enthusiastically as positive ones. Additionally, it must be remembered that ethnopharmacological investigations may lead to the discovery of unusual biological activities or unique chemistries, which may, in turn, lead to the development of analogues suitable for pharmaceutical development. As an example, forskolin has unique actions to activate adenylate cyclase, and analogues may be useful in the treatment of glaucoma, and as cardiotonics. Forskolin is the major active component of the Ayurvedic plant makandi (Coleus forskohlii). In another recent example, a plant (Homalanthus nutans) used in Samoa to treat the viral disease, yellow fever, has provided a phorbol ester, prostratin, that was discovered by the National Cancer Institute to have anti-HIV activity.

One ethnobotanical lead that we developed was the recognition of the antimicrobial activity of the leaves of Premna schimperi (Verbenaceae) from Ethiopia and the subsequent identification of 12-oxo-10b,17a,19a,20a-cleroda-3,13(16)-dien-15-oic acid I as the active principal. This diterpene proved to have appreciable activity against some important Gram-positive pathogenic bacteria. Although not particularly exciting as an antibacterial, the value of this extract for local use is confirmed. As a consequence of this finding, we initiated a study of other Ethiopian Premna species. From P oligotricha, we have obtained the lactone 2, which proves to be a more active antibacterial than 1 and the structurally unusual peroxide 3. The latter has no antimicrobial activity but does have structural novelty.

Plant Products Produced in Cell Culture

It is well known that plants provide a fertile source of natural products many of which are clinically important medicinal agents. In numerous instances, the present source of the medicinal drugs still derives from large plantations propagated in appropriate areas of the world where climatic conditions, etc., are optimum for growth. In such cases, the supply and cost of the active agent is dependent upon the complexity of the plant extract from which it is isolated, the concentration which, in turn, may vary depending upon the season of plant collection, etc. Within certain areas of medicinal applications, for example, cancer chemotherapy, lack of the target compound may be associated with difficulties of plant collections due to geographical locations or even political problems. In order to alleviate such situations, various laboratories have recently addressed themselves to studies with tissue cultures derived from such plants in the hope that this technique would afford methodology for laboratory-controlled production of selected medicinal agents.

In addition, plant tissue cultures also provide excellent media for detailed biosynthetic investigations and, via cell-free extracts derived from such systems, potentially important sources of enzymes for various studies of interest to the scientific community. The present lecture will summarize the recent

Studies with Catharanthus Roseus

Vinblastine (3), one of the clinically important anti-tumor agents isolated from C. roseus, represents an important member of these complex natural products and synthetic routes to (3) from more readily available starting materials have been under study for some years. The development of the â€œbiogeneticâ€ approach in our and other laboratories and involving the coupling of catharanthine N-oxide with vindoline provide an important route to the bisindole alkaloid system. Furthermore, in a parallel study in our
laboratory and utilizing cell-free extracts from C. roseus, we were able to demonstrate that 3', 4â€’Manhydrovinblastine (7) is also formed in the enzymatic process via the coupling of catharanthine (1) and vindoline (2) (Fig. 2). Subsequent enzymatic transformation of this bisindole system (7) to the clinical drug vinblastine (10, R = CH3) and the bisindole alkaloids leurosine (8) and catharine (9) (Fig. 3) is also achieved with the enzymes obtained in the cell-free extract. A simultaneous independent study by Scott provided results analogous to those summarized in Fig. 2. These various studies clearly demonstrated the overall importance of the 2 monomeric alkaloids vindoline and catharanthine and consequently the production of these compounds via the tissue culture methodology.

Callus grown from anthers generally originated at the cut of the filament and in the anther walls, i.e., diploid tissue. When grown to a size of 1-2 g freshweight, about 2 cm in diameter, the callus was cut into small pieces and serially subcultured on fresh agar medium or transferred to liquid medium (Gamborgâ€™s B5 medium) giving rise to a cell suspension. For large-scale production Zenkâ€™s alkaloid production medium was employed.

The alkaloid production varied with the cell line and age of the subculture and ranged from 0.1â€“1.5% of cell dryweight. The relative amounts of alkaloids produced was fairly constant under conditions given and appeared cell-line specific. Monitoring of alkaloid production is achieved by HPLC so that not only relative concentrations of total alkaloids produced can be ascertained but comparative concentrations of specific alkaloids, for example structures 11-24, with time, etc., can be accurately determined.

All subcultures of cell lines grown in 7.5 liter Microferm bioreactors followed essentially the pattern shown in Fig. 4. After incubation with actively growing cell-suspension, the mitotic index (MI) dropped to zero within 24 h and remained there for 2-3 days. Thereafter, the index rose sharply and reached its maximum (MI 1.8-3.0) within 2 days and declined again gradually over the following 10-15 days to zero. The cell dryweight over the culture period increased by a factor of 8 to 10 while the variation in pH stayed within half a unit. During an 8 week culture period alkaloids have been found as soon as 2 weeks after inoculation. Most cell lines showed a maximum accumulation of alkaloids in the 3rd-5th week of culture. Having established a large number of cell lines capable of alkaloid production, we proceeded to a more detailed study with several of the more promising lines. The results from 2 such lines coded as â€œ953â€œ and â€œ20OGW- are summarized below.

a. The 953 line

Studies with this selected line were performed both in shake flasks and bioreactors employing the IB5 medium for inoculum growth and then Zenkâ€™s alkaloid production medium. Detailed accounts of these experiments are published so only a brief summary is provided. On harvesting the culture, the water is removed by freeze drying and the alkaloids are extracted in the conventional manner to provide the data summarized in Table 1. The crude alkaloid mixtures were fractionated by intermediate-scale reverse phase high-performance liquid chromatography (HPLC). Final purification by analytical reverse-phase HPLC allowed the isolation of the following alkaloids, characterized by their physical and spectral data and by comparison with authentic materials: ajmalicine (11), yohimbine (12), isositsirikine (13), vallesiachotamine (14), strictosidine lactam (15), lochnericine (16), horhammericline (17), horhammeriniline (18), vindolinine (19), 19-epivindolinine (20), 19-hydroxy-11-methoxytabersonine(21), 19-hydroxy-11-methoxytabersonine(22). and dimethyltryptamine (23).

As general alkaloid formation was not observed during the initial periods of rapid cell growth, it was decided to examine whether the appearance, disappearance or build-up of particular components could be observed over different time periods. The results are given in Tables I and II. These show that the percentage of alkaloid per gram of cell weight increases with time, with optimum production at 3-4 weeks. It
is also observed that maximum cell dry weight occurs during the same period and coincides with a zero value of the mitotic index. With respect to the earlier periods of culture growth, it is noted that there is a more rapid increase in the biosynthesis of ajmalicine (11) and yohimbine (12) (Corynanthe family) than in the biosynthesis of vindolinine (19) (Aspidosperma family).

b. The 200G W line
Another particularly interesting cell line under recent investigation is coded as â€œ200GWâ€. The general procedures concerning tissue propagation, HPLC analyses, etc., are very similar to those discussed above. However, this line is uniquely different from the 953 line and produces its own â€œspectrumâ€ of alkaloids as summarized in Table III. Of particular interest is the alkaloid catharanthine (1, 0.005% dry cell wt) isolated for the first time in our studies. This line originally provided this alkaloid in amounts ca. three times that normally obtainable from C. roseus plant material. Indeed, recent optimization studies with this line have shown even a further improvement.

c. Biotransformation studies
The above discussion has demonstrated the capabilities of different tissue-culture cell lines from C. roseus to produce various types of alkaloids. Another area of potential importance for the purpose of increasing cell yield of desired products, as well as for biosynthetic investigations, concerns the use of selected cell lines for biotransformation of appropriate substrates introduced into the culture medium at various stages of culture growth. Studies involving the transformation of various functional groups within organic compounds by plant tissue culture techniques have been reported but compared to the extensively studied area of microbial transformation, much research is still required with such cultures before a proper understanding of this method can be attained. To this end we have initiated some studies with selected C. roseus cell lines and appropriate substrates available from our earlier investigations.

The substrate 3', 4'-anhydrovinblastine (7) available from the synthetic route outlined in Fig. 1, was selected for our initial experiments. Several serially cultured cell lines have been propagated for the preliminary screening to determine their capability of biotransforming 7 into desirable products. From these studies we chose a line coded as â€˜916â€™ since this cell line was unique in that it exhibited satisfactory growth characteristics, etc., but did not produce any alkaloids. In the initial study with the 916 cell line, 3-5 mg of 3',4'-anhydrovinblastine (7) was incubated with the cells in shake flasks for 2, 6, 12, 18, 24, 48 and 72 h.

6-72 h contained a new, less polar compound. However, the highest concentration of this new product was observed in 24- and 48-h incubation samples. In the 72 h incubation samples the concentration of the new product was decreasing and degradation products appeared. From these samples the new Compound was subsequently isolated by TLC and HPLC methods and shown to be dimeric (C46H54,N4,O11) by mass spectrometry although no further studies were performed. Further large scale experiments (3', 4'-anhydrovinblastine, 300 mg added as the hydrogensulfate salt) involving an incubation time of 48 h were performed in a Microferm bioreactor (cell line 916, 5.5 l) and allowed a more detailed study of the biotransformation process. Based on the amount of recovered substrate, the transformation of 7 to leurosine (8) and catharine (9) was 25.5 and 16.3 % respectively, or, approximately 42% of 7 had been utilized by the cells. It should be noted, however, that no attempts have yet been made to optimize the yields of specific products.

The results also indicate that these high-molecular-weight alkaloids have passed through the cell walls since bisindole alkaloids were present in both the cell material and the culture medium.

Finally, the short period of time required for such biotransformations (24-48 h) is interesting, particularly when compared to plant cell culture production of alkaloids from nutrients present in the growth medium (usually several weeks). The inoculation of suspension cultures with biosynthetically â€œadvancedâ€ precursors which reduce time periods for the production of target compounds may provide an important avenue for the commercial production of such pharmaceutically important agents. Further studies are
From Toxins to Drugs—Venom Proteins as a Guide to the future Design of Protein Drugs

PEPTIDES AND PROTEINS AS DRUGS

The proposition that a substantial number of future drugs will be deliberately engineered peptides and proteins is an attractive one but, in reality, the “revolution” seems slow in coming. In terms of the specificity of action that could be achieved, the range of activities and the probable absence of toxic metabolites, protein drugs clearly have much to commend them. Set against these advantages, however, is the general complexity of such molecules, their vulnerability to enzymes, the tendency of the body to make antibodies to them, the economics of producing them in large quantities, and the almost overriding desire in most markets for drugs that can be administered orally. The goal of many drug design programmes, therefore, continues to be the small organic compound which, although it may imitate some of the chemical properties of a naturally occurring protein or peptide (i.e. it is a peptidomimetic), is relatively uncomplicated, synthesizable at reasonable cost, orally administrable and widely saleable.

Some peptides and proteins have nevertheless found routine medical use. Perhaps the most common use is in replacement therapy, whereby a natural protein variant is used to counter a deficiency. The classic example is, of course, insulin, but other examples are orally administered digestive enzymes such as amylase, lipases and proteases. Streptokinase, urokinase, anistreplase and alteplase are used as fibrinolytics; these assist the conversion of endogenous plasminogen to plasmin and thereby enhance the breakdown of fibrin blood clots. Anistreplase is a chemically modified streptokinase-plasminogen complex while alteplase is a genetically engineered tissue plasminogen activator. Predictably, these enzymes and derivatives require intravenous administration. Examples of other prescribed peptides include growth hormones, vasopressin (which can be administered in the form of nasal drops as well as by injection), and porcine calcitonin, the latter being used for the reduction of plasma calcium in hypercalcaemia. Interestingly, salcatonin (synthetic salmon calcitonin), which amounts to an “engineered” version of porcine calcitonin, is preferable since it is less immunogenic.

These examples cover natural proteins, chemically modified proteins, genetically engineered proteins and in vitro synthesized peptides delivered by several routes, so the potential for protein drugs definitely exists. However, the basic logistics of cost, tissue delivery and source apart, it is the nature of proteins themselves that often make them unattractive for the purposes of drug design. A naturally occurring proteinaceous enzyme inhibitor may, for example, contain 60 amino acids, which gives tremendous potential for imitation, chemical modification, sequence engineering and natural variation. A comprehensive structure-activity study designed to optimize specificity, activity and behaviour towards non-target enzymes can hardly be completed on any reasonable timescale. In addition, it is likely that the mechanism is not fully understood, making uninformed modifications and sequence alterations to the protein a singularly unprofitable exercise. Above all, perhaps, the complexity of the molecule may mean that an effective patent cannot be framed once the expensive design programme has been completed. It is not surprising, then, that where peptides or proteins are exploited as drugs, they tend to be “off-the-shelf” variants obtained from a natural source (often close to man) to be used with little or no modification in a “niche” market. Current examples include aprotinin (to reduce blood loss), α-antitrypsin (against emphysema) and factor VIII (to control blood clotting). Even the use of a “natural” protein has its problems, however, especially if it is obtained by artificial means. It is essential that the product is pure and sequentially homogeneous, yet both in vitro and in vivo methods of synthesis can give rise to sequence errors and modifications which are...
VENOM PEPTIDES AND PROTEINS

As a counter to the basically negative arguments used above against the general feasibility of peptides and proteins as drugs, animal venoms are a dramatic demonstration of the power and potential of such molecules as pharmacological agents. Snake venoms especially reveal that, for the purposes of controlling a prey animal’s mood, movement, heart-rate, circulation, nervous transmission, and immunological defence mechanisms, and the digestion of its tissues, peptides and proteins are indeed the tools of the trade. To achieve all these effects in the victim quickly and in the right order, the venom proteins have to target particular enzymes, receptors, membranes, ion channels and cells while evading physical and chemical countermeasures.

Although the potential for drug design based on venom peptides and proteins has only started to become fully appreciated with the recent advances in biotechnology, the path has already been followed to great effect. The best known example is the story of captopril (produced by Squibb), which is essentially a dipeptide analogue derived from consideration of the bradykinin-potentiating peptides (BPPs) originally identified in the venom of the pit viper Bothrops jararaca. These small peptides are capable of inhibiting angiotensin-converting enzyme, with the consequence that blood flow (and thereby venom circulation) is not impeded by vasoconstriction. By imitating this action, captopril and its derivations find wide use in the treatment of hypertension. The use of an analogue of the active peptides, rather than the BPPs themselves, stems in part from the need to inject the natural toxins (as required for nearly all venom toxins). Captopril, by contrast, is orally active.

Venoms do not just present the intending drug designer with a bewildering variety of naturally bioactive peptides and proteins from which to choose a particularly appropriate activity to exploit. The study of the many toxins also provides clear lessons about the principles of design, targeting and strategy that will probably be needed to make proteinoid drugs a reality. The following sections highlight some of the lessons that can be drawn from the extensive studies that have been performed on certain venom toxins in recent years. Since many venom ingredients have yet to be fully characterized and understood, their promise to be many more useful lessons to be added in the future.

Common proteins can be engineered to produce potent pharmacological activities

Perhaps one of the major surprises of venom research has been the discovery that some of the most potent toxins are actually modifications of well-known proteins that perform benign roles in other contexts. Tiger snakes and sea snakes, for example, produce powerful toxins that specifically attack neuromuscular junctions in their prey. Termed b-neurotoxins, they damage the integrity of the presynaptic membranes at neuromuscular junctions and thereby interfere with the normal release of neurotransmitter. This disrupts muscular coordination and aids paralysis, of the prey. When these toxins were first isolated and sequenced, it was immediately apparent that they were representatives of the phospholipase A2 family, enzymes which hydrolyse 3-sn-phosphoglycerides. The previous context in which the enzyme was known was as a digestive enzyme produced in mammalian pancreas to hydrolyse dietary phospholipid. Phospholipase A2 is a component of many venoms and is an obvious choice as an ingredient since it can attack cell membrane lipids. However, what has been achieved in some venoms is the ability to target the enzyme so that it focuses attack on the membranes of critical cells (i.e. neuromuscular synapses and muscle cells). This remarkable targeting has been achieved without substantial alteration of the molecule. The toxins retain a very similar three-dimensional shape compared with digestive phospholipases A2 and differ only in details of sequence and a few insertions/deletions in the polypeptide chain (Fig. 1). Although membranes differ in the composition and distribution of phospholipid types, targeting of the b-neurotoxins seems to depend more on recognition of the integral protein/carbohydrate components than the lipid mix present. Hence,
sequence alterations outwith the catalytic site could be the key factor in transforming the enzyme into a neurotoxin. In some instances, b-neurotoxins show little or no enzyme activity when confronted with typical phospholipid substrates, so binding alone without follow-up hydrolysis may be sufficient. The lesson provided by the b-neurotoxins is that protein engineering to obtain new drugs does not have to commence with an obscure or rare bioactive peptide/protein. Many of the common benign proteins could serve just as well, as long as their specific targeting and containment mechanisms can be identified and altered. Moreover, whether an enzyme acts on proteins, carbohydrates, nucleic acids or lipids, the opportunity for targeting it via recognition of biomolecules other than those, which it can act upon, should not be overlooked. Much contemporary protein engineering concentrates on obtaining enzyme specificity by modifying the catalytic site and its associated binding subsites, but, since most in vivo environments are a blend of biomolecule types, this approach may be unnecessarily limited. Some enzymes are probably underestimated in terms of the accessory binding and control sites that are inherent in their superstructure as a direct result of concentrating too much on the relationship between enzyme and substrate.

Other notable examples of venom toxins that are closely related to peptides occurring elsewhere are the sarafotoxins and the dendrotoxins. Sarafotoxins are obtained from the venom of the burrowing asp (Atractaspis engaddensis) and they act as powerful modulators of the cardiovascular system. Vasoconstrictive effects are particularly marked. On analysis, the sequences of these 21-residue peptides turned out to be very similar to peptides with comparable activities in mammals, namely the endothelins. Dendrotoxins are presynaptically active neurotoxins from mamba venoms (Dendroaspis species), which block some types of potassium channels. When their amino acid sequences were solved, it was found that they were members of the Kunitz proteinase inhibitor family, which includes such well-characterized examples as bovine pancreatic trypsin inhibitor (BPTI or aprotinin).

One protein family can generate diverse pharmacological actions
On a general level, it is now understood that, despite the tremendous diversity of protein structure and action, most proteins can be reconciled into a limited number of families with characteristic sequences and chain folds. The same is true for venom toxins, where a common shape and sequence are often seen to underlie a range of seemingly unrelated toxic effects.

One of the best examples is the (a-neurotoxin-cytotoxin-cardiotoxin family characterized from the venoms of elapid snakes (i.e. cobras, kraits, sea snakes and tiger snakes). The a-neurotoxins are among the most outstanding individually lethal components in such venoms. They block nicotinic cholinoreceptors at neuromuscular junctions and thus prevent muscle stimulation by acetylcholine. Death of prey animals is caused by paralysis of the diaphragm and respiratory arrest. When examples of a-neurotoxins were purified and sequenced, it soon emerged that all the variants conformed to the same molecular archetype, namely a chain length of 60-75 residues and a threefinger loop structure cross-linked at one end by a minimum of four disulphide bridges (Fig. 2). As venom purifications and structural analyses proceeded, not only were many more a-neurotoxin variants discovered, but also variants with activities apparently unrelated to the theme of nicotinic cholinoreceptor binding at the neuromuscular synapse. Thus, the family was expanded to include cardiotoxins-cytotoxins fasciculins k-neurotoxins muscarinic toxins and, most recently, mambins. These examples target heart muscle/cell membranes, acetylcholinesterase, neuronal cholinoreceptors, muscarinic cholinoreceptors, and platelet aggregation mechanisms, respectively. In addition, a number of variants have been discovered that have no obvious pharmacological activity according to the assays that have been employed.

A central debate about this toxin family is whether the target tissues and receptors are themselves related in some way (i.e. do they contain common or related toxin binding moieties?), or whether independent and unprecedented recognition mechanisms have arisen from time to time on the same basic framework. It is interesting to speculate that the framework functions as an ideal general basis for targeting other
biomolecules because it is relatively small, conformationally adjustable and possibly evasive of defence mechanisms. Whatever the explanation, the lesson here for protein drug design is that some conformations and folds are extremely versatile and are targetable for effects against a diverse range of tissues and receptors. This natural toxin family is clearly an excellent starting point since so many sequences and activities are known. Should the activities prove to be related by way of common target features (e.g. a fundamental membrane component with individual characteristics according to cell differentiation), it could prove possible to target many cell types beyond those already focused on by the venom toxins.

Protein cocktails are more effective
The complexity and subtlety of venoms often comes as a surprise to those who expect them to be no more than lethal injections. The manner and speed of the death of a prey animal can be a major consideration. For the fish-hunting Conus snails, the prey needs to be paralyzed instantly, otherwise it is lost. With snakes, it is also important that the prey is subdued and immobilized quickly but, at the same time, a prompt circulatory arrest may not be in the interests of venom dispersal throughout the body of the prey. Moreover, the body of the prey needs to remain supple to allow ingestion, and the digestion processes that ensue need assistance from within the tissues of the victim by way of venom components that can initiate autodigestion. This "programmed death" requires a level of control over physiological events that is beyond the capability of a single ingredient a conspiracy is required between agents that synergize with, cooperate with, facilitate and complement the activities of other agents. There is often a tailoring of the toxin cocktail according to species and geographical location, reflecting the individual sensitivities of the various prey animals and prey selections. Significant conspiracies between venom toxins may pass unnoticed because the usual approach to venom research is to purify components to test individually for pharmacological activity. With so many components, there are numerous combinations to investigate. However, some interesting cases have come to light, which illustrate different cooperative strategies.

Integrated Approach to Development of New Drugs from Plants and Indigenous Remedies
Drugs, from whatever source they may be derived, are a means to relieve suffering and to achieve and maintain good health. In the past five decades the development and introduction of new drugs has indeed been remarkable, these have been mainly synthetics and antibiotics and have led to miraculous successes in the control of many diseases. But still drugs derived from plants form the mainstay of medical treatment in the developing countries. According to the June 1983 issue of World Health, it is estimated that more than one-half of the world’s population, most of them in the developing countries, relies mainly on traditional remedies. Even in industrialized countries a distinct trend is noticeable towards the use of plant drugs, perhaps as a sequel to the reports of occurrence of estrogenic diseases caused by some synthetic drugs and antibiotics.

There are several factors for the continued popularity of traditional remedies. One is their ready availability, compared to modern drugs, in rural areas and less accessible regions in developing countries; another is the shortage of practitioners of modern medicine in such areas. But more important is the sociocultural reason. In some countries where reverence for tradition is a way of life, there exists a long tradition of the use of such remedies. People, particularly in the rural areas, have more faith in the traditional physician, as he is a part of their community. The doctor speaks the same language as the village people, explains in the terms and concepts that are familiar to them and uses raw materials that are generally available in the neighbourhood for the preparation of his remedies. Both patient and physician are part of the same cultural milieu. Moreover, since modern medicine in these countries came in the wake of colonialism, it was
considered as an alien system imposed on the people. With independence, there has been a renewal of interest in the past of developing countries, including in their traditional systems of healing. This almost emotional attachment to traditional medicine is, thus, a part of the national resurgence. The traditional remedies and the traditional physicians cannot, therefore, be ignored and have to be integrated into one comprehensive system of health care, which also includes the best from traditional systems. Because their objectives are the same, a conscious effort has to be made to narrow down the divergence between the traditional systems and modern medicine.

In this context it is also necessary to appreciate that traditional materia medica as part of well-established systems of medicine, have evolved on a scientific basis. Thus, there is a need to differentiate between these remedies and folk medicines. The former are based on a corpus of organized knowledge, reduced to writing, while the latter consists merely of individual empirical observations without any theoretical basis. The major traditional systems of medicine in Asia are: the Chinese as practised in China and, with modifications, in Japan, Korea and Indo-China; the Ayurvedic, practised in India, Bangladesh, Nepal, Sri Lanka and to some extent Burma, and the Unani-Tibb (GraecoArab) limited also largely to the Indian subcontinent. These systems of medicine can by no means be considered empirical. They are based on a considerable amount of knowledge accumulated, by and large, through application of scientific method, individual observations, confirmation of these observations by others, formulation of hypotheses, followed by testing of the hypotheses by experimentation. Granted that some of the tools employed in ancient times when these systems were evolving were primitive by modern criteria, but the approach was undoubtedly scientific.

I shall try to illustrate my point by referring to Charak Samhita, one of the most important texts of the Ayurvedic system. According to Charak, knowledge is gained through: (i) "pratyaksha", that is direct observation, and (ii) "anumana", that is inference, induction, deduction and analogy, and the basis of "anumana" is "pratyaksha". He further states that although theoretical knowledge may be derived from authoritative instruction, this knowledge must then be investigated by observation and inference. While knowledge gained by direct visual perception is considered the most dependable, that gained by other senses is also valid. "anumana" based on "pratyaksha" enables one to draw conclusions in 3 ways having regard to the 3 aspects of time: past, present and future, a series of cause and effect. And "anumana" is again derived in 3 ways: a posteriori, a priori and commonly observed.

In addition to existing authoritative and valid knowledge, observation and inference. Charak also refers to "yukti" as a method for determining the truth. He has defined "yukti" as reasoning with a view to arrive at a correct judgement or conclusion. The faculty of reasoning is a function of "buddhi" or intellect, which evaluates the nature of several forces at work in a particular phenomenon having regard to the 3 aspects of time.

Thus, greater credence would have to be given to the materia medica of the traditional systems and a deeper study and understanding of the principles of these systems is required in order to make effective use of the knowledge enshrined in them. Only in this way can the full potential of traditional medicine be harnessed for the development of new drugs.

APPROACHES TO DRUG DEVELOPMENT

I would now like to discuss how best modern scientific methodology could be applied to investigate traditional remedies so that the results are acceptable to modern physicians and these drugs could be incorporated and integrated into one comprehensive armamentarium. Some of the approaches available are discussed below.

Standardization of Traditional Remedies
A serious limitation of traditional medicines is the absence of standardisation in regard to: (i) raw materials, (ii) method of production, and (iii) quality control of the finished product. Traditional physicians, who are generally their own pharmacists, rely on their individual experience or descriptions contained in ancient texts or verbally handed down for the identification of plants, which can sometimes be erroneous. Advancements made in recent years in pharmacognosy, phytochemistry and physicochemical instrumentation techniques can be of immense value in removing this major shortcoming of traditional medicine. These techniques could be utilized for: (i) correct botanical identification of the plants used, (ii) standardisation of processing and compounding methods, (iii) development of quality control methods.

It is often argued that as traditional remedies are made from powdered plants or their whole extracts, whose active constituents may not have been identified or only partly identified, what should be the criteria for quality control of such preparations. The fact that such remedies are frequently compounded of more than one plant only adds to the complexity of the problem. Of course, chemical assay would be almost impossible in the case of such complex mixtures containing unknown substances. A practical solution would be to develop bioassay methods, which are now possible with the advances that have taken place in techniques of bioassay in vitro and in vivo. But, with the improvements in methods of isolation using GLC and HPLC it would not be difficult to finger print even complex preparations and obtain at least qualitative idea of the chemical composition. Thus, the criteria of bioassay and composition fingerprint could help to control batch to batch variation.

**Safety criteria of largely used traditional drugs**

A number of traditional remedies, particularly general tonics and those recommended for chronic conditions are very popular and used for long periods in developing countries. As it is often difficult to clinically or experimentally evaluate the efficacy of such products in the absence of suitable parameters or animal models respectively, and as these are used by large numbers of people, at least the safety of these preparations should be ensured. This would be particularly necessary for those drugs, which, in the light of modern knowledge, are known to contain ingredients that may have harmful effects after prolonged use. It is, therefore, essential to scientifically evaluate the safety of such traditional drugs by the well-established preclinical toxicological studies.

**Evaluation of efficacy of traditional remedies**

We have also to consider how to evaluate scientifically traditional remedies so as to sift out drugs that are therapeutically effective from the useless ones, and to compare the effective ones with other drugs having similar action available today. As already mentioned, traditional remedies are generally compounded of a number of plants. The usual approach to investigating the efficacy of these remedies is to prepare alcoholic extracts of the individual plants separately and to subject each crude extract, semi-purified fractions prepared from it and in some cases even isolated pure product(s) to a biological test corresponding to the reputed therapeutic efficacy. It is not inconceivable that the processing of the plants according to traditional methods may modify the activity of the active ingredient(s), or that various chemical constituents present in the preparation may interact with each other resulting in alteration of the activity or toxicity of the individual constituents. Thus, good correlation between the results of biological testing of crude extracts, semi-purified fractions or pure constituents of individual plants and those of the compounded drug may be lacking. It would, therefore, be more meaningful to carry out the initial screening for biological activity of the drug in the very form it is used in clinical practice. If the activity is confirmed, then each individual plant could be tested to pinpoint the activity.

In the context of development of drugs from plants, the drug-control regulations of various countries have to be taken into consideration. In many developing countries which have a system of drug regulation, traditional drugs, if prepared strictly according to the methods laid down in the texts of their systems, are
allowed to be used without fulfilling the strict requirements of safety and efficacy evaluation, mandatory for new drugs. So, if the activity of a traditional drug is confirmed scientifically it could be promoted for use in modern medical practice without further evaluation. This would be particularly important in the areas of degenerative diseases and tropical parasitic diseases where there is a lack of effective drugs. In some cases, pure compounds isolated from these remedies may be more potent or have less side-effects than the remedies themselves. But prior to clinical use of such pure compounds, mandatory preclinical and clinical studies would have to be done, which normally take about 10 years. Thus, for diseases for which modern drugs are not available, but a traditional remedy is, it would be expedient to promote the use of the latter, even if it is not an ideal one according to modern criteria. Development of the ideal new drug from its pure constituent could continue side by side.

In this context, I would say that undue importance has been attached to the use of pure single ingredient from plants. In many cases this is not at all necessary from the standpoint of therapeutic efficacy and would certainly make the drug more expensive. This point is well illustrated by our work on the development of a hypolipidaemic product from Commiphora mukul resin, discussed later. Isolation of pure active constituents and elucidation of their structures is no doubt essential for study of structure-activity relationship and design of better synthetic drugs, but not necessarily for the use of these constituents in therapeutics.

**Broad spectrum biological screening of plants**

The number of plants used for preparing traditional remedies in various countries and those that have so far been investigated scientifically constitute only a small fraction of the plant resources of the world. A majority of the plants growing in developing countries, particularly of Africa and S. America, have not been investigated. Moreover, even in the case of plants that have been screened, one has to bear in mind the well known possibility of variation in the chemical composition of the same plant growing in different geographical and climatic regions. It is therefore, necessary for every country to undertake systematic biological screening of all the available plants from different climatic zones. It must be emphasized that the collection, storage and processing of plants must be done in such a manner that their chemical constituents are not affected. Bioassays in vitro and in vivo have to be developed which can measure even weak activities due to minor constituents. The biological test systems employed should be particularly directed to the therapeutic conditions for which satisfactory drugs are not so far available. Here it should be pointed out that the above approach is quite different from the classical investigation of medicinal plants. The latter concentrates primarily on isolation of pure chemical constituents. Their characterization and structural elucidation and the testing for biological activity of pure constituents come later. In contrast, the former approach involves monitoring for biological activity at every step of chemical fractionation, purification.

I would now like to discuss a few examples of these approaches from our own work on the development of new drugs from plants/traditional remedies.

**Target Site Delivery of Herbicidal Compounds**

The control of pests using pesticides has developed enormously during the last 50 years, and a vast range of insecticides, fungicides and herbicides is now available for the control of insect pests, disease pathogens and weeds. To be effective, these compounds must reach the target site in the specific organism in sufficient concentration to be lethal; many of the tissue barriers and pathways are common to all three pest types. In the case of foliage-applied systemic herbicides, formulation must be appropriate to achieve effective cuticle penetration and translocation to the target sites in the weed species. Selectivity must be sufficient to ensure that only the target species are affected, and residues of the compound must not remain in the harvested portion of the crop.

These features must be evident for registration and approval of novel compounds, irrespective of their source, be it the synthetic chemist or from natural products.
In this chapter, the principal features of target site delivery are discussed, with particular reference to the uptake, translocation, metabolism and biochemical action of herbicidal compounds. Consideration will also be given to aspects of formulation, which may influence the efficiency of these processes.

TARGET SITE DELIVERY

For effective weed control the appropriate choice of herbicide and timing of application has to be made to ensure maximum phytotoxicity. In turn, this depends on efficient target site delivery and effective inhibition of enzymes at the target site. Target functions and their organelles include photosynthesis, the mechanism per se or synthesis of the pigment systems (chloroplast), respiration and intermediary metabolism (Mitochondria) or nucleic, amino acid and protein synthesis (nucleus, ribosomes). The phytotoxicity or selectivity of a herbicide may hinge around the efficiency of target site delivery and, in turn, this may depend on the efficiency of uptake, translocation, detoxification or immobilization at non-target sites. In the case of foliage-applied compounds, a number of rate-limiting steps may influence uptake, including the efficiency of wetting and adhesion cuticle penetration, and tissue absorption. Appropriate formulation, droplet size and distribution of the herbicide normally will ensure that cuticle retention and penetration is sufficient to ensure that a potentially active dose is absorbed into the leaf tissues.

Aqueous formulations usually contain low concentrations of surfactants (e.g.- 0.05-0.5%), which are effective in reducing the contact angle, and improving surface spread of the droplets. Anionic or non-ionic class surfactants are more commonly used than cationic. Ammonium compounds such as ammonium thiocyanate are also used as adjuvants since they markedly enhance absorption into foliage, possibly by increasing cell membrane permeability. Mineral and vegetable oils, dimethyl sulphoxide (DMSO) and glycerol are also used as additives to enhance the rate of efficiency of cuticle penetration.

Normally, soil-applied herbicides are of relatively low water solubility and may be formulated as emulsifiable concentrates, or as wettable or flowable powders. After addition of water, these are applied as emulsions or fine suspensions respectively, and the herbicide should form a discrete band at the soil surface. Absorption of the active ingredient takes place via the roots or emerging shoots of germinating weeds and the selectivity between crop and weed may depend on differences in the rooting depths of crop and weed (â€œdepth Protectionâ€™). For many soil-applied herbicides the target site lies within the chloroplast and translocation to the leaf generally takes place in the transpiration stream (â€œxylemâ€™).

Uptake and translocation to target sites Foliage-applied herbicides

Leaves and stems are covered with a waxy coating called the cuticle, which prevents excessive loss of water and dehydration of the plant. The cuticle may form a barrier to the entry of herbicides, particularly polar compounds, which are repelled by the non-polar cuticle waxes. The cuticular membrane is composed of lipophilic cutins with embedded cuticular waxes and surface epicuticular waxes; the physicochemical properties of the latter are of particular importance in determining the wettability of the leaf. The morphology of these surface waxes is determined by the composition of the wax exudates, which are believed to be extruded through the cuticular membrane from the epidermis in volatile solvents.

Cuticle retention of herbicide is determined by a number of factors including

1. Shoot and leaf habit of growth,
2. Leaf surface of the herbicide droplets with particular regard to the physicochemical properties of the epicuticular waxes,
3. The molecular structure of the herbicide, with particular reference to its polarity,
4. Droplet formulation and size,
5. Spray volume, and
6. Environmental factors Prior to and at the time of spraying.

Polar herbicides are repelled by the non-polar waxes of the cuticle. To achieve effective retention, they are
normally formulated with a suitable surfactant (e.g. 0.05% Tween 20) to reduce surface tension and to improve spread and retention of the droplets.

The factors influencing retention and transfer of a pesticide have been reviewed by Tadros. Retention may be affected by spray impact and adhesion and, in turn, this may be influenced by droplet volume and velocity, together with the difference in surface energy between the droplets in flight and at the surface. Spray retention may be affected also by droplet sliding, and, on an inclined surface, this may depend on surface tension of the spray liquid and the difference between advancing and receding contact angles with the leaf surface. Wetting and spreading are influenced by surface roughness of the cuticle and the surface tension and contact angle of the droplets; in turn, these are affected by surfactant type and concentration. Surfactant micelles may enhance the flux of transport or herbicides facilitating diffusion and convection.

Cuticle penetration is affected also by the wax characteristics of the leaf and the physicochemical properties of the herbicide molecule. Lipophilic herbicides are believed to move via a lipophilic route, while hydrophilic compounds traverse an aqueous pathway. Any factor, which increases the lipophilicity of the herbicide, appears to increase the rate and efficiency of cuticle penetration. In general, the undissociated molecules of weak acids (e.g. 2,4-dichlorophenoxyacetic acid) penetrate more rapidly than dissociated molecules, and esters penetrate more rapidly than salt formulations.

Highly lipophilic compounds may not traverse the cuticle, since they accumulate in waxes and fail to partition into the aqueous phase. This is exemplified in a comparative study of the mode of action of foliage-applied MCPA and MCPB. When applied to beans (Vicia faba L.), the relatively lipophilic MCPB was not phytotoxic, largely because it was immobilized in the cuticle waxes; MCPA penetrated the cuticle more readily owing to its greater hydrophilicity.

Cuticle penetration of hydrophilic molecules is thought to take place by an aqueous route and the rate of movement is increased when the tissues are turgid. Overbeek believed that, under these conditions, wax platelets were forced apart and that an aqueous continuum extended to the leaf surface. Formulation of a polar herbicide with a surfactant of appropriate hydrophilic-lipophilic balance (HLB) can enhance cuticle penetration and, thus, tissue absorption. Increased rates of penetration of polar materials may be important in climatic conditions where heavy rainfall can remove the herbicide from leaves of target species.

Penetration of the leaf cuticle is believed to involve diffusion, the rate being a function of Fickâ€™s first law and the time of diffusion increasing with the square of the distance (Fickâ€™s second law). The rate of diffusion within the cuticle is influenced by a number of factors including molecular radius and structure, penetration coefficient, chemical formulation, cuticle thickness, wax viscosity, temperature, humidity and light conditions. Preferential penetration is believed to occur through the cuticle associated with guard cells, basal cells or trichomes, veins or the anteclinal walls of epidermal cells. These areas are reputed to be sites of high numbers of ectodesmata but the existence of these â€˜structuresâ€™ is doubted by some authors. There do appear, however, to be specific areas of the cuticle, which are relatively more permeable to polar compounds.

Within the leaf tissues, the mode of action of various herbicides differs according to certain factors. Compounds which are highly phytotoxic act as â€˜contactâ€™ herbicides, damaging cell and organelle membranes and inhibiting essential cellular functions such as oxidative and photophosphorylations. Compounds of the bipyridylium (e.g. diquat, paraquat) and hydroxybenzonitrile (e.g. ioxynil, bromoxynil) groups are examples of this type of action. Contact herbicides tend not to be systemic although movement will be influenced by the concentration applied.

Herbicides, which are not phytotoxic to the leaf tissues, may act at points remote from the point of application. There are two possible routes: one is via the cell wall continuum and the xylem (â€˜apoplasticâ€™), and the other is via the symplastic continuum and phloem (â€˜symplasticâ€™). Water solubility of the herbicide appears to be an important factor influencing the route, and uptake into the
Symplast may involve a saturable membrane carrier system or an energy-requiring proton pump system. Compounds, which inhibit ATP synthesis, may inhibit their own uptake into the symplast.

Phloem-translocated herbicides exhibit a pattern of movement, which is similar to that of sugars. They are translocated from "source" to "sink", i.e. from the leaves to any energy requiring region, such as the apical meristem, axillary buds, developing shoots and roots. The route of movement is the phloem system and the process is called long-distance transport; the mechanism involved is controversial, although it appears to be driven by osmotic and pressure gradients.

The efficiency of target site delivery for systemic herbicides is influenced by a number of factors including the efficiency of leaf absorption, the import/export of sugars by the treated leaf, the relative positions of source and sink, environmental conditions including light, temperature and humidity, binding to macromolecules at non-active sites, and conjugation to sugars or amino acids.

The characteristics of the herbicide molecule may be important in determining phloem translocation. In the case of weak acids such as 2,4-D it has been suggested that efficient translocation depends on the presence of a free carboxylic group or functional groups, which conjugate with sugars or amino acids; the HLB of the remaining portion of the molecule should not favour water or lipid.

Efficient absorption and phloem translocation of polar compounds requires a suitable HLB. The compound must be sufficiently lipophilic to penetrate cuticles and membranes, and also sufficiently hydrophilic to enable partitioning into the aqueous medium of the transport system. This balance of physicochemical properties has been considered by Tyree. They suggested that non-ionized compounds to which cell membranes have intermediate permeability should be phloem mobile. Membrane permeability should be sufficient to enable them to load into the phloem, but not so great that loss from the phloem sieve tubes during translocation would impair long-distance transport.

Supporting evidence for the hypothesis has been provided by the work of Bromilow. Using Ricinus communis, they determined the retention of a series of chemicals of varying octan-1-ol -H2O partition coefficients (log Kow) in the phloem, between the leaves and stem (10-15 cm). Retention declined from 70% for compounds having log Kow = 0.5 to 0% for those having log Kow < 1.0. The most phloem-mobile compounds, including the major classes of herbicides, are weak acids with log Kow values in the range from 1 to 3.

To conclude, photosynthetic inhibitors such as paraquat, linuron and prometryne rapidly increase membrane permeability and are not translocated in phloem. Slow-acting herbicides such as picloram and 2,4-D affect membrane permeability only slowly, or not at all and are systemically transported.

**Soil-applied herbicides**

Herbicides are applied to the soil before crops emerge in order to control germinating weeds, which would compete with the crop for space, light, water or nutrients. Such compounds normally remain as a relatively discrete toxic band at the surface. Their mobility in soil is determined by a number of factors including water solubility, adsorption, soil type, and rainfall.

The transfer of herbicide molecules from the soil surface to the absorption region may involve water and air phase diffusion, leaching and dynamic dispersion and may be influenced by the level of soil adsorption and the water solubility of the compound. The amount of active ingredient potentially available for weed absorption can be affected by photochemical degradation, volatilization, chemical and microbial degradation, and uptake by the crop plants.

Herbicide molecules which reach the rooting zone of weeds are rapidly absorbed and translocated to the shoot. Root absorption appears to take place through the root hairs, which are located, adjacent to the root apical meristem, although it can occur in older regions of the root the coleoptile and shoot may be a major absorption zone in some species. There is some evidence to suggest that uptake is biphasic, an initial rapid phase being followed by a continuing steady phase; there is some controversy as to whether uptake
is passive or active. The transport of water-soluble compounds across the cortex may involve apoplastic or symplastic routes, and the relative importance of either may be influenced by the physicochemical characteristics of the compound. Thereafter transport in the xylem occurs from the root to the shoots via the transpiration stream.

**Drug Research Based upon the Leads of the Chinese Traditional Medicine**

The bewildering varieties of molecular structures accomplished by the nature’s chemical architecture incite the chemists’ enthusiasm and curiosity to explore the kingdom of nature, especially in searching for biologically active constituents. Traditional medicine and folklore are based on long periods of practical experience in treating human disease. Taking them as the leads for drug search may save one from drowning in costly random screening. The written document of Chinese traditional medicine can be traced back to Shen Nong Ben Cao Jing (A.D. 22-250). Since then, it has been refined and enriched continually, and more than 300 classical books have been published. Ben Cao Gang Mu written by the greatest physician and naturalist Li Shizhen has been regarded as a more comprehensive pharmacopoeia containing total of 1894 entries published in 1596. Ben Cao Gang Mu, as well as the other classics, still serve as the valuable references for teaching, practicing and guides for drug research in China. About 80% of the Chinese traditional medicine comes from the plant kingdom. The animal and marine products and minerals cover the remaining 20%. A recent survey of Chinese traditional drugs and folklore has identified that about 2000 plant species are concerned. Majority of the plant species have been more or less investigated chemically throughout the world, but few pharmacological studies have been undertaken until recent years. Not all traditional medicines including the folkloric are as useful as described, but we do treasure them as an invaluable heritage and have confidence in most of their practical values. They have left medicinal chemists a vast, fertile field to be developed. To verify the clinical values of the traditional prescriptions and to bridge the gap between the traditional self-contained theories and the modern aspects, and then to find the active principles and take them as the leads for drug design, is not easy. The meagre experience obtained indicates that it is a difficult and complicated task, which demands not only great effort and endurance, but also more advanced scientific and medical knowledge and techniques. Some of which have yet to be devised. The study of the famous tonic ginseng started in 1905 in Japan. Since then, its chemical constituents and biological properties have attracted the interests of the scientists in various parts of the world. Nearly 80 years have elapsed. Many constituents have been identified among which over 10 glycosides of triterpenes have been shown to have the biological activities somewhat related to those described in the classics for ginseng. Such as antistress, cardiovascular, antiinflammatory, immunostimmulating and antitumor action. However it still demands more elaborate study to understand the polychrestic biological activities described and to assign each activity to the individual constituent to permit a basis for drug design. Therefore, it continues to be an active subject of investigation.

However, not all the studies on the traditional medicines are as perplexed and costly as the case of ginseng some of the projects are quite rewarding. The following 4 examples are selected to illustrate the more or less successful results obtained in the drug development based on the leads of the traditional medicines.

1. **Qinghao (Artemisia annua L.):** The Chinese drug Changshan (roots of Dichroa febrifuga Lour.) the oldest remedy for malaria which has been known since 200 B.C. Febrifugine 1, the active principle was isolated in 1948. The toxicity of the alkloid, especially the adverse action on gastrointestinal system, hindered it from developing into all useful remedy. Derivatives were prepared to lower its toxicity but with only limited success.

Qinghao was first described as the remedy for malaria in Zhou Hou Bei Ji Fang in 340 A.D. It also
has been collected in â€œBen Cao Gang Muâ€. But its value had been somewhat ignored until recent years. Ruziska first reported the study of Artemisia. Since then, the chemical studies of about 60 species of Artemisia including Artemisia annua have been carried out by other chemists, nothing pertaining to the antimalarial activity had been noticed until the isolation of artemisinine 2 (qinghaosu) which was reported by Chinese scientists. Along with artemisinine, 6 biologically inactive sesquiterpenes 3-8, the structures of which are closely related to artemisinine, have also been isolated. They all have the basic skeleton of amorphane 9. cis-fused decalin ring with an isopropyl group trans to the angular hydrogen. Compounds 2-4 biogenetically might be regarded as formed from amorphane through scission of C3-C4 bond by oxygen, then recyclization. Compounds 3 and 4 with all the structural features of artemisinine except the presence of an oxide ring in place of the peroxide bridge in the latter, are completely inactive. The importance of the presence of peroxide bridge for its biological activity is, thus, conceivable. In order to overcome the recrudescence after the treatment of artemisinine, preparation of derivatives has been undertaken. The hemilactol hydroartemisinine 10 obtained as a Mixture Of C12a and bOH in a ratio close to 1 : 1 by NaBH4 reduction is twice as active as the parent compound. Furthermore, the hemilactol provides a functional group OH which makes the preparation of derivatives possible without touching the basic structure particularly the peroxide bridge. Nearly 100 derivatives in the form of ethers, carboxylates and carbonates were prepared. Most of the derivatives are more active than artemisinine and the esters are more potent than the ethers. The carbonates are the most active class.

The quantitative structure-activity relationships were analyzed by Hanschâ€™s approach using various parameters. The following 2 parabolic equations for ethers and esters illustrated the close relationship between lipophilicity and antimalarial activity, The optimal values, logPo for ether and ester are 2.60 and 2.9 respectively. The electronic character of the substituents also plays its role in ethers as introduction of Taftâ€™s s* gives better correlation coefficient.

The therapeutic index against chloroquine-sensitive strain of P. berghei in mice of artemisinine (oil suspension) and artemether I I (oil solution) given by intramuscular injection (im), and sodium artesunate 12 (water solution) given by intravenous injection (iv) are 4987, 447 and 1733 respectively compared with the value of 95 for chloroquine (im). This indicates that artemisinines are much less toxic than chloroquine. The artemisinines are also effective against chloroquine-resistant P. berghei strain and the artemether and sodium artesunate are more active. After i.v. and oral administration of artemisinine and artemether, the drug concentrations in the blood were of short duration, while i.m. gave a prolonged blood level. As sodium artesunate (iv) was also eliminated very rapidly from the body, intravenous drip was recommended. The artemisinines can pass through the blood-brain and blood-placenta barrier and, hence, adverse effects on neuro and embryo systems should be taken into consideration. On the other hand, it may be useful in the treatment of cerebral malaria. In the past 7 years, clinically 3368 cases of plasmodium falciparum and Plasmodium vivax malaria were treated with artemisinine (2099 cases), artemether (1088 cases), and sodium artesunate. Artemisinine and artemether gave the radical cure rates of ca. 90% in falciparum cases and sodium artesunate showed a lower cure rate. They all are effective in the treatment of chloroquine-resistant P. falciparum, therefore, they may be the drug of choice for chloroquine-resistant falciparum malaria. No apparent toxic effect on heart, liver or kidneys has been observed in the clinical trails. From the urine of patients taking artemisinine, 3 inactive metabolites (3, 13, 14) have been identified. It was suggested that the metabolism is a reductive deactivating process. The preliminary studies suggested that the membrane system of the parasites may be the main site of action exerted by artemisinine and this mode of action may differ from the other known classical antimalarials. The importance of the discovery of artemisinine not only provides an useful antimalarial agent. but its greater significance lies more in the novel and unique structure with no precedent in the antimalarias. Recently. The total synthesis of artemisinine from isopulegal has been reported by Schmid.
In the early 70’s, another antimalarial herb Artabotrys uncinatus L. Merr was studied in our institute. The antimalarial action of yingzhaosu A 15 was verified, preliminarily both in experimental malaria and in clinic. The limited source of the plant interrupted further investigation. Yingzhaosu A, a sesquiterpene with the basic skeleton differs from artemisinine but it is most amazing that a peroxide bridge is also present in the molecule. The coincidence is indisputable evidence of the significance of the peroxide bridge. The structural differences between artemisinine and yingzhaosu A indicated the flexibility of the basic structure required for the biological activity. In retrospect, it seems more fruitful in researching parasiticides from traditional medicine, because the simple therapeutic action is identical to the direct screening method. Besides the antimalarias mentioned above, the ascaricides quisqualic acid 16 from the nuts of Quisqualis indica L. and the triterpene chuanliansu 17 from the bark of Melia toosendan S. et Z. The latter is especially welcomed by the pediatricians due to its efficacy and low toxicity. Agrimorphol 18 a taenialfuga isolated from the winter root-sprout of Agrimonia pilosa Ledeb. showed an efficacy as high as 98% (Anonymous 1974). Agrimorphol is a biphloroglucinol with an unusual linkage between the 2 phloroglucinol moieties.

Qingdai

Correlating the theory behind the Chinese traditional medicine to the symptoms of patients with chronic myelocytic leukemia (CML), of which the term is missing in the old medical classics, led to the treatment of CML with a commercially available honey pill. "Dang Gu Lu Hui Wan". 16 of the 22 treated cases showed positive reponse. The pill contains II ingredients. By subsequent combination and elimination of the ingredients based on the traditional medical theory and clinical results, "CoeQingdaiâ€ is found to be the active constituent. It is the floating solid formed after the indican-containing leaves of Baphicacanthus cusia have been soaked in water for 2 days and then treated with lime. The solid contains indigo as its major organic component with a small amount of indirubin, indigo brown, etc. Further study showed that indirubin 19 is responsible for the antitumor activity exhibited by qingdai. Indirubin is active against transplantable leukemia L7212, Lewis lung carcinoma in mice and Walker Ca 256 in rats. Of 314 CML patients treated with indirubin, 87% response rate was obtained. Abdominal pain and diarrhea are the side effects observed. Pharmacological studies revealed that indirubin has no effect on the hemopoietic system and immuno-system in normal rats and mice. The profiles of the content of uric acid (metabolite of purine), and of b-amino-isobutyric acid (metabolite of thymidine) eliminated in the urine of patients treated with indirubin, are different from those obtained with myleran, 6-MP and radiation therapy. Indirubin combined with DNA and inhibited the syntheses of DNA. RNA and protein in Walker 256 ascitic cells. Indirubin has been known for 100 years and can easily be synthesized by condensation of indoxyl with insatin. In 1946 Friedman reported the isolation of indirubin from urine of patients suffering from CML, and Landsheere found intramuscular administration of indirubin to guinea pigs caused the maximum reduction of the white blood cell count to 60%, eosinophils to 20%, granulocytes to 40% and no significant effect on lymphocytes and monocytes combined count compared with that of vehicle control. But no antitumor action was reported. However, their observations supported the therapeutic effect on CML found 2 decades later. Indirubin with its high m.p. is insoluble in water and its solubilities in most organic solvents are also exceedingly low. It was postulated that substitution on the nitrogen atom might increase the solubility and facilitate the absorption to improve the therapeutic effect. Among the derivatives prepared, compounds 20-23 with substitution on the amino nitrogen exhibited higher inhibitory action on experimental tumors than indirubin, while substitution on the other nitrogen atom abolished the activity.

Schizandra chinensis Baill
The dried kernal of Schizandra chinensis Baill (Wuweizi) is a herb described to have various physiological actions in the Chinese medical classics. It is a common ingredient in prescriptions and also can be used alone as a tonic, astringent, etc. Since the early 70’s, Chinese traditional doctors have used the honey pills of the kernal to treat hepatitis based upon the theory of Chinese traditional medicine. The therapeutic effects obtained soon induced an impetus to the chemical and pharmacological studies of this herb in China.

Chemical studies of Schizandra was first reported by the Russian chemists in 1952. Later, 5 dibenzocyclooctadiene lignans, schizandrin, r-schizandrin, deoxyschizandrin, schizandrol and pseudo-r-schizandrin were isolated by Kochetkov. In our institute, study under the pharmacological and clinical guidelines, ascertained that the ethanol extract possessed a lowering effect on the serum glutamic pyruvic transaminase (SGPT). On further studies 7 dibenzo cyclooctadienes 24-30 were isolated from the alcoholic extracts. The structures were assigned by chemical degradation and spectra analysis. During 1979-1982 a series of papers published by I keya reported the isolation of 34 dibenzocyclooctadienes and assignment of the absolute configurations based upon NOE of1HNMR. X-ray analysis and circular dichroism, but no pharmacological effects were mentioned. The 7 compounds 24-30 mentioned above, demonstrated various effects on the liver functions. They decreased the CC14 and thioacetamide-induced hepatotoxicity as indicated by the lowering of SGPT in the treated mice. The relative efficacies are in the order of Ser B>Sol B>Sin C>Sin B>Sin A³Ser A³Sol A. Sol B, Sol A, Sin B and Sin A increased the liver glycogenesis of the starved mice, but no significant activity was showed by the remaining 3. All 7 compounds affected the pentobarbitol sleeping time in mice. Some of their inhibitory actions pertinent to the liver microsomes might explain their protective activity against hepatotoxicity. Their biological activities varied with the slight change of structures. No structure-activity relationship can be drawn from the present data. However, the ability of increasing the liver glycogenesis seemed to be linked with R configuration of the biphenyl group. During the total synthesis of Wuweizisu C, one of the by-products of the intermediates, the substituted biphenyl ester 31 also showed protective action against hepatotoxicity caused by CCL4 and thioacetamide in mice as the schizandrin series and in addition, it lowered the elevated SGPT level induced by prednisolone while the schizandrins are inactive. Since 1977, 500 patients with a virus chronic hepatitis have been treated with DDB. About 85% of these patients showed a reduction of SGPT level and relief of other symptoms through a course of treatment. No side effect was observed. DDB is more effective than the flavone-lignon (silymarin) in a parallel comparative clinical study. The effectness of DDB sheds light on the therapeutic value of the simple diphenyl compound.

Pueraria lobala Ohwi (Wild)

In an attempt to relieve the stiff neck of hypertensive patients which usually persisted even after the blood pressure returned to normal, the study of the root of Pueraria lobata, a Chinese medicine described in the classics, as a remedy for fever, thirst, eye pain and stiffness of the neck, was undertaken in early 70’s. The decoction and also the ethanol extract of the root were introduced to the clinical trials and satisfactory results were obtained. From the alcoholic extract an isoflavone, daidzein 32 as well as its 3 glucosides, daidzin 33, puerarin 34 and daidzein 7,4'-diglucoside 35 were isolated.

The alcoholic extract, puerarin and daidzein are now used not only in the treatment of stiffness of the neck in hypertensive patients, but also as a useful treatment for angina pectoris and such other vascular diseases as migraine and sudden deafness. No noticable side effect has been observed in patients taking this drug orally for several years.

From the pharmacological studies of the total isoflavones or its major component puerarin, the following actions were observed (1) decreasing the activity of the sympathetic nervous system by lowering the elevated catecholamine level of the hypertensive patients and the patients with severe angina pectoris. (2) Increasing the cerebral and coronary blood flow and decreasing the coronary and cerebral vascular
resistance. (3) Inhibiting the platelet aggregation and the release of 5-HT from platelet, (4) improving myocardial ischemia through increasing oxygen content in the blood, decreasing myocardial oxygen consumption, and the lactate production in ischemic myocardium. These various physiological reactions may explain the therapeutic effects of these isoflavones obtained clinically. It seems that these isoflavones might act through a mechanism different from those of the other agents now used in the treatment of cardiac vascular disease though more elaborate studies will be required to substantiate this assumption.

Recent Work on Some Thai Medicinal Plants

Zingiberaceous floras are widely distributed in Southeast Asia. More than 200 species are found in Thailand. Many of these plants are used in Thai traditional medicine, e.g., Zingiber cassumunar Roxb, is used for relieving asthmatic symptom, Alpinia galanga Sw. and Curcuma longa Linn are used externally for the treatment of skin diseases, while Curcuma xanthorrhiza Roxb is an emmenagogue. A number of plants of this family are commonly used as flavouring agents, e.g., Amomum kervanh Pierre, Alpinia siamensis Schum., Alpinia galanga Sw. and Zingiber officinale.

Recent investigations of many zingiberaceous plants yielded novel compounds, which are of biological and chemical interest. Diarylheptanoids were isolated from Alpinia officinarum (compounds 1 to 9) Compounds 1, 5, 7 and 8 were shown to be inhibitors of prostaglandin biosynthesis. Compound 8, also isolated from Alpinia oxyphilla, was reported to be 125 times more pungent than zingerone (Itokawa et al. 1981b). Methanolic extract from the rhizome of Alpinia speciosa significantly possesses inhibitory activities against histamine and barium chloride by the Magnus method, using excised guinea-pig ileum. Chemical investigations of the extract led to the isolation of compounds 10 to 18.

The genus Costus has also been attracting a great deal of interest due to the presence of diosgenin, an important intermediate for the synthesis of steroidal drugs. More than 2% of diosgenin by dry weight has been isolated from the rhizomes of Costus speciosus.

Zingiber officinale, commonly known as ginger, has long been valued for its flavour and pungency, and its use in food and in medicine has been recorded in ancient Sanskrit, Hebrew and Chinese scriptures. The existence of (3)-, (4)-, (5)-, (6)-, (8), (10)- and (12)-gingerols 24 in this plant has been demonstrated. The synthesis of some of these compounds and the biosynthesis of (6)-gingerol has been studied. Kiuchi, in their recent investigations, isolated potent inhibitors of prostaglandin biosynthesis from zingiberaceous plants. These compounds were identified as (6)- and (10)- dehydrogingerdione 25 and 26, and (6)- and (10)-gingerdione 27 and 28. The isolation of compounds 25 and 27 from Z. officinale along with compounds 26 and 28 gives strong support to the biosynthetic pathway for (6)-gingerol as proposed by Denniff.

As part of our systematic investigation of Thai medicinal plants in the Zingiberaceae family, Boesenbergia pandurata (yellow and red forms) and Zingiber cassumunar have been chosen for detailed investigations due to their interesting biological activities.

B. pandurata (yellow form)

This plant is widely distributed in Thailand and the fresh rhizome is used in cooking, also in folk medicine for the treatment of colic and as an aphrodisiac. Previous investigations of this plant were concerned mainly with the constituents of the essential oil obtained from its rhizomes, Mongkolsuk have isolated (+)-pinostrobin 35 and (±)-alpinetin 18 from an ether extract of the dried rhizome. Our re-investigation of the rhizomes of this plant led to the isolation of pinocembrin 36, pinostrobin 35 together with 2α, 6α-dihydroxy-4α-methoxychalcone 37, cardamom 15 and two new chalcones. (±)-boesenbergin A 38 and B 39.

The structure of boesenbergin A was established on the basis of spectral data and x-ray diffraction analysis. A simple one-step synthesis of boesenbergin A has also been achieved. The reaction of citral with 2α, 6α-dihydroxy-4α-methoxychalcone 37 in the presence of pyridine gave a product, which
proved to be identical with the natural product. In view of the recent interest in the intramolecular \( \text{â€œcitranâ€œ} \) cyclization, the acid catalyzed cyclization of compound 38 was studied. The acid-catalyzed reaction of compound 38 gave a mixture of compounds 40 and 41. Compound 41 was presumably derived from the acid-catalyzed cyclization of compound 40 and in fact further reaction of compound 40 with dilute acid gave compound 41. Methylation of compound 40 gave compound 43, which was identical with the product obtained from the acid-catalysed cyclization of compound 42.

The nmr spectrum of boesenbergin B is very similar to that of boesenbergin A. The comparative nmr data are shown in Table I.

As a very small quantity of boesenbergin B was isolated, it was then decided to synthesize this compound using the same procedure described for the synthesis of boesenbergin A. When a mixture of equivalent amounts of 2\( \text{â€œ} \), 4\( \text{â€œ} \)-dihydroxy-6\( \text{â€œ} \)-methoxycalcone (cardamonin), distilled citral, pyridine and a few drops of dry dimethyl sulfoxide was heated at 90\( \text{o} \) for 24 h, boesenbergin B was obtained, upon purification, in 25% yield. The yield of the product could be improved up to 47% by carrying out the reaction in a sealed tube at 120\( \text{o} \) for 26 h. The NMR, IR spectra and TLC behavior of the synthetic sample are identical with those of the natural product.

Methylation of boesenbergin B with methyl iodide/potassium carbonate in boiling acetone gave a methyl ether derivative. The spectral data of this derivative differed from those of the methyl ether analogue obtained from the methylation of boesenbergin A.

On the basis of the evidence, structure 44 can be excluded. The structures 39 and 45 were differentiated by the following experiments. In the NOE experiment of boesenbergin B, there was a 32% increase in the integration of the aromatic signal at d5.9 upon irradiation of the methoxy signal. This result, together with the fact that the natural product gave a positive Gibbs test, clearly indicated that 39 (not 45) is the structure of boesenbergin B.

**B. pandurata (red form)**

The rhizome of this plant is used in Thailand for the treatment of colic disorders. Extraction of the rhizome with hexane followed by extensive chromatography of the extract led to the isolation of the new compound 46 (panduratin A), pinostrobin 35, pinocembrin 36 and 2 known chalcones, boesenbergin A 38 and rubranine 47.

The structure of compound 46 was established on the basis of spectral data. The stereochemistries at C-1\( \text{â€œ} \), C-2\( \text{â€œ} \) and C-6\( \text{â€œ} \) were established on the basis of NMR decoupling experiments. The coupling constants for J1, 6, and J1, 2, are 11 and 4.4 Hz respectively. These values are comparable to those reported for saggenon D and saggenon C (partial structures shown).

The structure of rubranine 47 was established by comparing its melting point and spectral data with those published. The synthesis of compound 47 was first reported by Bandaranayake by the reaction of citral with 5, 7-dihydroxyflavanone (pinocembrin) 36 in refluxing pyridine. The product (the yield of which was not reported) of this reaction was tentatively assigned structure 47. Subsequent work established the identity of compound 47, which was proved to be identical to a substance, named as rubranine, isolated from Aniba rosaeodora Ducke.

We have repeated the synthesis of rubranine using the reported reaction conditions and were able to isolated rubranine 47 in 2.6% yield. However, when the reaction was carried out at 140\( \text{o} \)C in a sealed tube for 12 h, 25% yield of rubranine could be isolated. The product thus obtained, was identical to the natural product.

**Z. cassumunar**

This plant has been used as anti-inflammatory and relieving brochial congestion in Thai traditional medicine. The chemical investigations led to the isolation of 13 compounds (compounds 48 to 60), 11 of
which were new compounds (48 to 58). The structures of all the compounds isolated were established on the basis of spectral data. The structures of compounds 48 and 53 were also confirmed by x-ray diffraction analysis. The syntheses of compounds 48 and 50 have been achieved. The dimerization of a mixture of compounds 54 and 55 gave compounds 48, 49 and 50. The Witting reaction between veratraldehyde and the ylide derived from 3-hydroxypropyl (triphenyl) phosphonium chloride gave compound 51. Acetylation of compound 51 gave the expected acetyl derivative 52.

Plant growth is coordinated and controlled by endogenous plant growth regulators. Many of these have been extracted, identified and synthesized, and the use of several has enabled the development of plant tissue culture techniques. While most plant growth regulators (e.g. auxins, gibberellins, cytokinins) are stimulatory, some are known to act as inhibitors, the classical example being abscisic acid (ABA). Recently the jasmonate group of compounds has aroused interest in view of their inhibitory properties, many of which are similar to those of ABA.

Abscisic acid, 1, is involved in the ageing processes of plants such as leaf senescence, fruit ripening (apparently by its ability to stimulate ethylene formation), and leaf and fruit abscission. Its other actions include induction of stomatal closure, which is responsible for its antitranspirant effect, and reduction of auxin-induced coleoptile growth. A more complete list of all its varied effects is given in Table 1. cis-Jasmone, 2, first isolated from Jasminum grandiflorum L. and the structurally related methyl jasmonate, 3a, which was first isolated from the same source and subsequently from Rosmarinus officinalis are widely used as perfume constituents, but the involvement in growth regulation of compounds of this family was first recognized when jasmonic acid, 3b, was identified as the active plant growth inhibitor of a fungus, Lasiodiplodia theobromae. Since then, jasmonic acid, 3a, and/or its methyl ester, 3b, have been found in a very wide range of plants of diverse families, recently including even a green alga, Euglena gracilis Z.

Related natural compounds which show similar activity include cucurbic acid, 4, and its methyl ester and a series of oxophytodienoic acids, e.g. 4-oxo-5-(2-pentenyl)-2cyclopentene-l-octanoic acid, 5 Vick have shown that the latter is synthesized in the plant from a polyunsaturated fatty acid, 9,12,15-linoleic acid, via the hydroperoxide 6 by a process reminiscent of prostaglandin biosynthesis, but showing significant differences. Such longer-chain acids are biodegraded by b-oxidation, ultimately leading to jasmonic acid. Present evidence is consistent with the view that such compounds only act as plant growth regulators by serving as precursors of jasmonic acid and they will therefore not be considered further in this account.

Tuberonic acid, its glucoside and its long-known lactone, 'Jasmine ketolactone', 7, have also been isolated from various plants. The glucoside (from Solanum tuberosum leaves) has powerful potato-tuber-inducing activity. Jasmonic and cucurbic acids also show strong activity as potato-tuber-inducing agents. The glucoside of dihydrotuberonic acid is one of the metabolites of dihydro jasmonic acid.

The effects of such plant growth inhibitors lead to a wide variety of potential uses, which may be exemplified by the following.

(a) The induction of stomatal closure leads to a substantial antitranspirant effect since most of the water loss from leaf surfaces occurs through the stomatal pores (~95%) Potentially, therefore, crop yields in arid regions could be improved by use of agents, which induce (temporary) stomatal closure.

(b) Induction of growth control which can complement genetic improvements, e.g. in growing grain on short, more wind-resistant stalks.

(c) Control of fruit ripening, allowing more convenient harvesting. The ripening process depends directly on the production of ethylene (and/or its precursor, L-amino cyclopropane-l-carboxylic acid) and methyl jasmonate is reported both to stimulate ethylene production (e.g. in preclimacteric apples), and to inhibit the same process in postclimacteric apples. Thus, such plant growth inhibitors may also improve fruit storage.
Senescence induction, which can be useful in facilitating harvesting of certain agricultural crops.

Inhibition of potato sprouting during storage, as shown by preliminary results of the present work.

Delay of flowering by inhibition of flower bud induction, which has potential value in reducing crop loss due to late frosts.

**STRUCTURE-ACTIVITY RELATIONS**

Although both abscisic acid, 1, and jasmonic acid, 3a, have carboxyl, keto and C=\(\text{C}\) functions, their gross structures differ widely. Moreover, whereas both frequently exert similar biological actions some tests reveal wide differences in their effects as well as their relative potency. This strongly suggests that interactions with several different receptors are involved. To date, very little is known about the actual mechanism of action.

In attempts to correlate structure with activity, many molecules related to jasmonic acid have been synthesized and their effects on plant growth reported. Unfortunately the results of different workers are difficult to correlate because of the use of different test methods. As early as 1975, Ravid tested a large number of mostly weakly active analogues of jasmonic acid for inhibition of lettuce seed germination and of radicle root growth, they reported good inhibitory activity for the cyclopentenone ester 8 and the corresponding saturated ester. Nevertheless, Yamane and coworkers on the basis of their own series of analogues on which they used a variety of biological test procedures, concluded that a ketone (or oxidizable hydroxyl), n-pentyl or Z-2-pentenyl adjacent to C=O and an acetic acid or ester group in the b-position were all essential for high activity. Their conclusions were based in part on tests of a series of synthetic analogues in which the 2-pentenyl side-chain was varied. Saturation of this side-chain to give dihydrojasmonic acid, 9b, or its methyl ester caused little drop in biological activity, whereas introduction of a triple bond in place of the double bond or changes in the length of the side-chain (even by one carbon atom) substantially reduced activity. Complete removal of the side-chain (compound 9a) or of the keto group gave inactive compounds. Some activity was retained in the open-chain analogue 10. Yamane and coworkers' above-mentioned conclusions seem to ignore the results of Ravid, e.g. the greater activity of compound 8 compared with that of 9b. The latter authors also found the cyclohexanone derivative 11 to be highly active in suppressing lettuce seed germination.

Apart from tests of various natural and synthetic derivatives of jasmonic and dihydrojasmonic acids (e.g. amino acid conjugates a glucosyl ester most of which had reduced activity, little new information on structure-activity relationships has appeared in the last ten years. We became interested because, despite the large number of known synthetic routes to methyl jasmonate, we had been able to demonstrate a more efficient and highly flexible route to dihydrojasmonate and analogues. Moreover, we believed that the above-mentioned results gave reasonable hope that molecules with more selective action than jasmonic acid and possibly with higher potency for specific effects might be found and might have useful practical applications in the control of for example plant growth, seed germination or water loss.

**CHEMICAL ASPECTS**

Our approach to jasmonate and dihydrojasmonate derivatives depends on the use of the Khand reaction to form cyclopentenones from alkynetheracarbonyldicobalt complexes and ethylene or other olefins. The acetate side-chain is then added by means of a Michael-type reaction. We have reported the use of this method to obtain methyl jasmonate, methyl dihydrojasmonate and various derivative, e.g. the bicyclic compounds 12a and 12b and analogues (13a, 13b) with phenyl in place of alkyl side-chains (Billington et al., 1988a), Thus, a typical example, compound 13a, results when phenylacetylene is treated with octacarbonyldicobalt to yield the complex 14, which need not be isolated before reacting with cyclopentene to give the bicyclic cyclopentenone derivative 15a, or with 2, 5-dihydrofuran to give compound 15b.

In at least one case, efficient catalysis of a Khand reaction has now been achieved by working under a high
pressure of ethylene and carbon monoxide. The frequently modest yields of the Khand reaction had seemed a barrier to its industrial use at the beginning of the present work, but considerable improvements in the method have been described in recent years. In our own laboratory, the beneficial effects of ultrasound on reaction rate and of phosphine oxides as promoters had been noted. Schore achieved excellent results by temporarily linking the alkyne component to a polymer support. Probably the most generally useful improvements have been achieved by the use of amine oxides and of dimethyl sulfoxide or other polar co-solvents. The present authors have contributed to these latest developments and have utilized the improved methods in obtaining the new compounds included in this chapter.

Since the Khand reaction is regioselective with unsymmetrical alkynes, but not with unsymmetrical alkenes, most of the compounds examined in the present work are derived from terminal alkynes, but symmetrical alkenes. Some selectivity is observed with terminal alkenes bearing electron-donating substituents and we have used, for example, allyl alcohol and but-3-en-l-ol in our work. Although the 5-substituted products 16c and 16d predominate in these cases over the 4-isomers 17c and 17d, tedious separations are still required and only very limited quantities of pure products have been obtained.

Early in our work, cis-jasmone, 2, was found to have significant effect as a plant growth inhibitor. This led us to test the cyclopentenones, initially prepared as intermediates. In most cases where comparison was made, these proved to be similar to the derived keto esters in biological activity. In some cases, especially for the less effective cyclopentenones, we therefore did not proceed with the addition of the b-side-chain. As reported, introduction of the acetic acid (or ester) side-chain was effected either by addition of malonate anion or of the TiCl4-activated ketene-acetal 18a. The latter method proved more effective with the less reactive cyclopentenones. Even this method failed when we attempted to add the side-chain to the bicyclic ketone 19. This compound, prepared by the efficient intramolecular Khand reaction of allyl-2octynyl ether, CH2=CHCH2 0CH2C= Câ€“(CH2)4CH3, thus shows the typical low reactivity of enones with a fully substituted double bond. However, following a somewhat similar example, addition of the less sterically demanding ketene acetal 18b proceeded in satisfactory yield to give the ester 20.

Immunoregulatory Compounds Derived from Hormones

There is a tendency to think of the immune system as an autonomous tissue, i.e. a tissue which reacts to an external challenge in a defined way and whose response is limited by that external challenge. For instance, once a bacterial or viral infection is eliminated, the response becomes redundant and is switched off by endogenous mechanisms involving communication between the various immunoeffector cells. However, examination of the general pattern of immunoreactivity in the population provides excellent evidence that the immune system is subject to the same neuroendocrine control as other tissues in the body. This chapter will examine the role of two hormone systems - the sex steroids and peptide hormones.

SEX HORMONES Background

A number of workers had suggested that sex steroids contributed to the immunological tolerance towards the foetus during pregnancy. Other workers had shown that there is a sex difference in the response to pathogenic organisms and also to experimental antigens. However, attempts to correlate sex steroid concentrations with the responses of isolated leucocytes in vitro were unsuccessful. These were the first indications that the effects of these steroids may proceed by indirect route(s) in the whole animal.

Mechanism of action

As long ago as 1929 it had been shown that gonadectomy resulted in thymic hypertrophy, which was greater in female animals. This effect could even be seen in old animals. Previously, it had been thought
that adult thymus was essentially inactive, despite its crucial role in the embryonic and neonatal development of T-lymphocytes. Treatment with exogenous sex steroid can cause involution of the thymus. The fact that adult thymus is sensitive to variations in sex steroid level suggests that it remains active well beyond puberty - when its importance was previously thought to decline as it undergoes involution. In addition, there is considerable evidence of a two-way interdependence between lymphoid organs and gonads. This includes ovarian developmental abnormalities in nude mice and in normal animals subjected to early neonatal thymectomy.

Sex differences in immune responsiveness have been well documented. Circulating immunoglobulin levels are higher in females as are the primary and secondary responses to a number of antigens (Terres et al., 1968), including brucella, hepatitis B and rubella. Cell-mediated responses can be considered higher as measured by graft rejection, although some workers suggest that other indices of cell-mediated immunity (CMI) are lower in females. The increased reactivity may be related to the higher rates of autoimmune disease seen in women. These diseases include rheumatoid arthritis, systemic lupus erythematosus and autoimmune thyroiditis. Allergies are more common in males until puberty, after which the prevalence is greater in females.

**Pregnancy and immunity**

During pregnancy, immunological reactivity alters, not only in terms of the foetus but more generally. Cell-mediated immunity is depressed such that skin graft survival increases and immunoregulatory factors appear in the serum. This can at least partially be mimicked by oestrogen treatment of normal rats of either sex, but depends on the presence of an intact thymus. Thymic involution occurs during pregnancy. This involves a loss of thymocytes (immature T-lymphocytes). Shinomiya also showed that phagocytic function is depressed but antibody response is well maintained. Parasitic diseases which may previously have been held at a subclinical level can flare up during pregnancy. These include malaria, trypanosomiasis and toxoplasmosis. It is possible to suggest that, on balance, during pregnancy humoral immunity is enhanced and cell-mediated immunity depressed. The endocrinological changes during this time are complex, but there is evidence that the sex steroids are involved in these changes and may at least partially be responsible for the differences in disease occurrence between the sexes.

**Treatment with sex steroids**

Testosterone can increase susceptibility to infection, inhibit the development of autoimmune thyroiditis in male animals and ameliorate the effects of adjuvant arthritis. It has been shown to reduce cellular invasion of the lacrimal glands in a mouse model of Sjogren’s syndrome. It can cause changes in CD4+/CD8+ lymphocyte distribution in animals, in experiments involving castration and restoration with exogenous hormone. Oestrogens, on the other hand, generally appear to depress cell-mediated immunity something that occurs in pregnancy, as already described. Antibody responses are found to increase as are the responses to certain mitogens and lymphoblast transformation.

Progesterone is unusual in that it may bind to the glucocorticoid receptor directly but also through its own receptor it may bind to the same regulatory elements within DNA as glucocorticoid. The local concentration of progesterone in placental tissue ranges from 2000 to 6000 ng/g of tissue, at which concentration it can be shown to inhibit a number of in vitro lymphocyte assays. Thus, lymphocytes reaching the placenta may be inhibited directly by the locally high concentration of progesterone, possibly via occupation of glucocorticoid receptor. High doses of the progestogen medroxyprogesterone acetate used in breast cancer treatment have been shown to reduce the proportion of circulating CD4+ (helper phenotype) lymphocytes in the circulation. This could be expected to reduce immune responsiveness as would the increase in suppressor cell activity reported by Holdstock.

A profound effect of sex steroids on immunity in vivo, can be seen in the NZB/NZW F1 mouse, which is regarded as a good model for the human autoimmune disease systemic lupus erythematosus (SLE). This
occurs in a female:male ratio of 14 : 1, which is reflected in the increased incidence and severity in female NZB/NZW animals. Androgenic hormones such as testosterone or 5 a-dihydrotestosterone can be used as a prophylactic measure in young animals and, importantly, can give therapeutic benefit in older animals with established disease. Oestrogen has an opposite effect, giving rise to early and severe symptoms, even in male animals. These effects were at least partially dependent on the presence of the thymus. Huston demonstrated that the thymic epithelium was important to the development of the autoantibodies that are a characteristic feature of this disease. Steroid metabolism in SLE patients has been shown to be unusual, leading to the production of increased quantities of 16 a-hydroxyestrones, even in males. Excess oestrogen production is also found in Klinefelter’s syndrome, together with a higher incidence of SLE than in the general population. The menstrual cycle can influence disease severity in patients. During the luteal phase, women frequently experience worse symptoms. However, pregnancy seems to have little effect on the disease, although the number of reports of worsening exceed the number reporting improvement. Oral contraceptives containing oestrogen have been reported to potentiate SLE symptoms, but there is little evidence that Oral contraceptives have actually precipitated disease. Tamoxifen, an oestrogen receptor antagonist, has been used in SLE, but produced no effect. The antigonadotrophic cyproterone acetate, has been shown to have a beneficial effect. Its mechanism of action in this situation is difficult to determine, but it was shown that plasma testosterone concentration remained steady while oestrogen level fell during treatment. Androgen has been used clinically to treat SLE with some success, and oestrogen shown to worsen the condition.

Rheumatoid arthritis, on the other hand, although again more prevalent in women, responds favourably to pregnancy, the luteal phase of the menstrual cycle and in some cases to oral contraceptives. They may also help prevent onset of this disease. Holmdahl showed a reduction in incidence and severity of pathology in the collagen-induced arthritis model. These differences from SLE may be explained on the basis that humoral immune processes are more important in SLE pathology than cell-mediated processes, which in turn are more important in the pathology of rheumatoid arthritis. Unusual androgen metabolism in cases of rheumatoid arthritis has been reported, although the significance of this is not certain. Collectively, these data suggest a significant influence of the sex hormones on the immune system and the importance of thymic tissue, even in adults, for some of their effects.

Sex steroid receptors in immunological cells
The identification of sex steroid receptors in the thymus pointed to a mechanism for regulation by these hormones. Androgen oestrogen and progesteron receptors were reported. As well as these reports concerning rat and mouse, androgen and oestrogen receptors have been identified in human thymus. In each case, receptor was found only in the non-lymphocytic component of this tissue—that is to say the epithelial portion. This is summarized in Table 1. Tritium-labelled hormone has been used in a radioreceptor assay carried out on thymus tissue. The tissue was separated into two components: the thymocytes or T-lymphocytes which include immature cells through to comparatively mature immunocompetent cells, and the matrix including the epithelial cells, which produce thymopoietin and the various other thymic hormones. These cells also participate in the very important localized processing of T-lymphocytes. Only glucocorticoid receptor was identified in both fractions - this allows for both direct and indirect effects on lymphocytes. Sex hormone binding could be identified only in the epithelial portion, not on lymphocytes themselves. Anti-receptor antibodies have recently been used to confirm receptor distribution. These results provide an explanation as to why many of the immunomodulatory effects of these hormones are seen only in the whole animal and can be abolished by prior removal of the thymus.

Targeting of steroid
Table II presents the relative binding affinities of a number of ligands for the rat androgen receptor Data for the prostate, a classical androgen-responsive tissue, are also shown for comparison. The binding profile is
clearly highly androgenic. However, perhaps of greater interest from the point of view of pharmacological intervention is the fact that certain of the steroids showed a different pattern of binding in the two tissues. Nortestosterone, an anabolic steroid, had a relative binding affinity in the thymus of 71%, almost equivalent to 5α-dihydrotestosterone, but higher than that found in the prostate (43%). However, conversion of nortestosterone to 5α-dihyronortestosterone (a similar step to the normal metabolic conversion of testosterone to its 5α-dihydro form) yields an increase in binding to the thymus and a reduction in binding to prostate. These data suggest that the receptors in the two tissues are different and that a degree of tissue targeting may be possible. Other workers have also suggested that there may be differences in androgen receptor binding between immunological and "classical" androgen-sensitive tissues. These differences also apply in human thymus. Thus, it may be possible to obtain desirable immunological effects in SLE treatment for instance, without the virilization, which would follow from the use of testosterone itself. The 19-nor steroids are of lower androgenic potency than their parent molecules. This is of obvious importance for therapy in a disease, which occurs largely in women.

Circulating factors are produced by the thymus in response to steroid. Stimson showed that physiological concentrations of steroid could elicit the production of immunoregulatory factors from rat thymic epithelial cells in culture. Supernatants from these cultures were used in a variety of lymphocyte proliferation assays employing bone marrow, spleen and thymus derived cells. Over 30 days there was a steady accumulation of stimulatory factors in the thymus supernatant, independent of any steroid treatment. Interestingly, there were almost equal and opposite effects produced by oestradiol and testosterone: oestradiol inhibited the response in a concentration-dependent manner, and testosterone promoted the response. Progesterone had a slight suppressant effect at physiological concentration, and as expected there was a considerable inhibitory effect from glucocorticoid, both directly, and via thymus supernatant. Importantly, control lymphocyte assays using directly added sex steroid showed no effect over the physiological range. These results accord well with the data on receptor distribution.

In birds, processing of B-lymphocytes occurs in the Bursa of Fabricius in a manner analogous to thymic processing of T-lymphocytes. The sex hormones influence bursal size in much the same way as they do the thymus. Sullivan demonstrated androgen and oestrogen receptors in bursa, and progesterone binding was also identified. The functional equivalent of the bursa in mammals is believed to be the bone marrow, and it seems reasonable to assume that this could be another point of influence for steroids. There have been suggestions that testosterone can, among other things, reduce stem cell differentiation towards the B-cell lineage. Human spleen has also been shown to contain oestrogen receptor, although it was found in lymphoid cells. Since then, oestrogen receptor has been identified in circulating lymphocytes but only in a small proportion. These were the CD8+ cells, i.e. those of the suppressor-cytotoxic lineage. Androgen or progesterone binding could not be demonstrated. Similar work has been done with human spleen lymphocytes. Thus, direct effects of oestrogen on lymphocytes are possible. Suppressor cell assays have shown effects of oestrogen in vitro. Curiously, an effect from testosterone in vitro has also been reported. It may be that some as-yet unidentified subpopulation of cells is sensitive to this hormone. As cell isolation procedures become more sophisticated, then it may be possible to define the responding type.

We have examined the effect of sex steroids on the induction of rheumatoid factor (RF) in the mouse. Rheumatoid factor is an autoantibody of IgM class, whose target is in the Fc portion of self IgG and is found in many autoimmune diseases such as rheumatoid arthritis or SLE. In our studies, animals were injected with bacterial lipopolysaccharide (LPS), a B-cell mitogen. Among the wide spectrum of antibodies elicited is rheumatoid factor. Fig. 2 shows that there is a significantly higher production of RF in female CBA mice following an intra-peritoneal injection of 20 mg LPS derived from Salmonella typhosa, oestradiol inhibited RF production in both sexes, and testosterone apparently had little effect on initial RF concentration, but resulted in a slower decay with time (Fig. 3, females; Fig. 4, males). Although it is not clear which cell type
is the target for steroid action, it is attractive to suggest that the receptor-bearing suppressor-cytotoxic cells are involved in this female hormone effect. Other work has shown that the cells producing rheumatoid factor are greatly reduced by day 10 following injection of LPS, so the persistence of rheumatoid factor relates more to the rate of removal rather than rate of production. A sex difference has been noted in the half-life of plasma immunoglobulin, being greater in females than in males. An experimental system involving an antigen that is not itself directly mitogenic may be affected by steroid in a different way. Recent work has shown that the monocyte/macrophage is a target for oestrogen. Fig. 5 shows a Scatchard plot of oestradiol binding to the J111 rat cell line, which is monocyte derived. The data appear to describe two independent binding species or two binding sites with some sort of allosteric interaction. Their binding affinities, resolved by the method of Rosenthal, of 2.5 Å·10⁻⁹ M and 8.7 Å·10⁻¹¹ M, are similar to those found in chick oviduct (Raymoure et al., 1985). The picture is essentially the same for rat peritoneal macrophages. Table III shows the relative binding affinities of a number of ligands for the J111 macrophage cell line, compared with myometrium, a classical steroid target. The binding profile is highly oestrogenic, but again there are differences between the immunological and reference tissues. Diethylstilbestrol is a much more potent binder to J111, which raises the possibility of selective intervention directed towards the immune system, minimizing any potential feminizing effects.

Constituents of Indigenous Medicinal Plants

The bulk of the populations of the Afro-Asian countries, particularly those living in villages, rely on the indigenous medical systems to provide relief from disease. Systematic scientific investigations, particularly during the current century, have resulted in the identification of a growing number of active constituents many of which are now routinely used in modern medicine. These include reserpine for the treatment of cardiac arrhythmias, vincamine as a vasodilator, and vinblastine and vincristine as anti-tumour agents, etc. Isolation, structural and synthetic studies have, accordingly, been directed in many laboratories around the world, including ours, to isolating new natural products which could prove to be valuable chemotherapeutic agents. Some of the recent studies carried out by my group at Karachi are briefly presented here.

Isolation and Structural Studies on Berberis Aristata

Berberis aristata DC (Berberidaceae) is a shrub found in the northern mountainous regions of Pakistan and India as well as in the Nilgiri Hills of southern India. The extracts, made from the root bark are known as “rasaut” and are used in the traditional system of medicine for the treatment of jaundice and skin diseases. As a result of careful isolation studies, 2 new alkaloids, â€œKarachineâ€ (1) and â€œTaxilamineâ€ (2) have recently been isolated. Karachine is the first naturally occurring berbinoid of this skeletal system and is the most complex of more than 50 protoberberine alkaloids presently known. Its structure (1) has been elucidated largely on the basis of its high resolution mass and 360 MHz (FT) nmr spectra, and the positioning of groups confirmed by Nuclear Overhauser Effect studies.

The uv spectrum of karachine, lmaxEtOH 226 and 285 nm (log e 3.90 and 3.62), was suggestive of a tetrahydroprotoberberine. The mass spectrum shows the molecular ion at m/e 433, and the base peak at m/e 336. The latter peak fits exactly for the molecular ion of berberine or epiberberine and is formed by loss of 97 mass units from the molecular ion via cleavage alpha to the nitrogen atom (C-14 to C-e bond), followed by a retro-Diels-Alder process. The m/e 97 fragment corresponds to C6H9O, or, more specifically, to 2 moles of acetone minus the elements of water3. A sharp absorption band at 1710 cm⁻¹ in the ir spectrum (CHCl3) denoted the presence of a non-conjugated carbonyl. The 360 MHz (FT) nmr spectrum in CDC13 presented a complex pattern, but allowed for the tentative assignment of expression 1 to karachine.

In order to settle conclusively the nature of the substitution pattern in aromatic-rings A and D, an n.o.e. study was carried out. Irradiation of the C-10 methoxyl singlet at d 3.77 resulted in an overall 11.6%
increase in the area of the d 6.52 and d 6.55 ring D aromatic doublet of doublets. Alternatively, irradiation of the H-1 singlet at d 6.73 gave a 2.8% increase of the d 2.70 and 2.72 doublet of doublets assigned to the C-e protons, as well as to a 5.6% increase of the signal at d 3.07 due to H-13. Significantly, irradiation of either the H-1 or H-4 singlets at d 6.73 and d 6.17 respectively led to no observable n.o.e. for the methoxyl absorptions. Further support for the structure of karachine has come from its borohydride reduction, and analysis of the mass and nmr spectra of the corresponding alcohol.

Karachine must arise by the condensation of berberine (3) with 2 moles of acetone and accompanying loss of water, as suggested in the Scheme (1). It is the first naturally occurring berberinoid incorporating acetone units. It is a true alkaloid and not an artefact of isolation since (a) optically active, as well as inactive, naturally occurring adducts of the related benzo-phenanthridine alkaloids with acetone are known no acetone was used during the isolation process, and (c) various attempts on our part to obtain karachine by condensation of berberine with acetone at varying pH’s were to no avail.

Taxilamine (2) was isolated by chromatography of the alkaloidal fraction (8g) using neutral alumina. Besides a consistent u.v. spectrum, the 360 MHz (FT, CDCl3) nmr spectrum of taxilamine shows H-5 and H-8 as singlets at d 7.15 and d 7.40, respectively; H-3 and H-4 as a doublet of doublets at d 7.46 (Jvic = 5.5 Hz); and H-5 and H-6 as another doublet of doublets at d 6.44 and d 7.28 (Jvic = 9.1 Hz). The 4 methoxyl signals appear as singlets at d 3.92, 3.96, 3.97, and 4.06. This spectrum bears a distinct resemblance to that reported for rugosinone (Wu et al. 1980). The mass spectrum of taxilamine confirmed the molecular formulation C20H19O6N and the structure (2) assigned.

Taxilamine (2) is the fourth member of its class of pseudobenzyl isoquinoline alkaloids and must probably have been formed in nature through oxidative rearrangement of palmatine to supply initially polycarpine. Hydrolytic N-deformylation followed by further oxidation would then afford taxilamine.

**ISOLATION AND STRUCTURAL STUDIES ON THE CHEMICAL CONSTITUENTS OF FAGONIA INDICA**

Fagonia indica Linn. is a small spiny undershrub which is widely distributed in Pakistan. An aqueous decoction of the leaves and young twigs is a popular remedy for cancer in its early stages. A new sapogenin â€œNahageninâ€ (5) has been isolated from the hydrolysed extracts of the aerial parts of the plant, and its structure has been elucidated on the basis of a 400 MHz NMR spectrum, a 100 MHz CMR spectrum and high resolution mass spectrum.

The substance analyzed for C30H48O4 (confirmed by high resolution mass spectrometry, m/z = 472.3740 mass, 472.3552 for C30H48O4). Major peaks in the MS occurred at m/z 454, 436, 424, 409, 395 and 261. The IR spectrum (CHCl3) showed peak at 1740 cmâ€“1 and 3460 cmâ€“1 suggesting a d-lactone and hydroxy groups. The substance readily afforded a diacetate (m/e-556), but was found to be remarkably inert to attempted hydrolysis of the lactone. The 1H NMR showed no olefinic protons. The 13C NMR recorded on a 400 MHz instrument confirmed the presence of 30 carbons. The carbon atoms in the A and B rings were readily recognised by comparison with corresponding signals of known pentacyclic triterpenoids. Eight quaternary centres and 6 methyl groups were also identified. The 13C NMR displayed a resonance at d 177.29 for the carbonyl carbon, and 3 resonances at d 84.72 (s), 76.54 (dd) and 71.92 (d) for the oxygen-bearing carbons C(20), C(3) and C(23) respectively. On the basis of these spectral data, structure (5) was assigned to nahagenin which has been confirmed by an unambiguous structure determination by a single crystal X-ray diffraction analysis.

**ISOLATION AND STRUCTURAL STUDIES ON BUXUS PAPILOSA**

*Buxus papilosa* (Buxaceae) is a shrub, which occurs abundantly in the northern regions of Pakistan. Extracts of *Buxus* species have been used since ancient times for the treatment of a wide variety of diseases including malaria and venereal disease. *Buxus papilosa* has found use in the indigenous system of medicine as a febrifuge for relief of rheumatism and for the treatment of a number of other ailments. Four
new alkaloids, papilamine (6) Papilicine (7) moenjodaramine (8) and harappamine (9), have recently been isolated by us from the leaves of this plant and their structures elucidated on the basis of the spectral data of the alkaloids as well as their derivatives. The spectral data obtained for each alkaloid are given against each structure.

The UV spectrum of moenjodaramine showed absorption maxima at 207, 237, 245 and 254 nm, characteristic of the presence of a 9(10â€“19) abeo-diene system. An identical u.v. spectrum is encountered in buxamine E, buxaminol E and papilamine. The proton NMR spectrum (CDCl3) showed 3 singlets, corresponding to the 3 tertiary methyl groups at δ 0.71, δ 0.75 and δ 1.03. The secondary (C-21) methyl group resonated as a doublet at δ 0.88 (J = 6 Hz). A 3-proton singlet resonating at δ 2.1, was assigned to the â€“ NCH3 group, while another peak resonating at δ 2.2 and integrating for 6 protons was assigned to the â€“ N(CH3)2 group attached to C-20. A set of AB doublets resonating at δ 3.24 and δ 3.82 was assigned to C-29 methylene protons α- to the C-3 nitrogen. A singlet at δ 5.98 was ascribed to the isolated olefinic proton at C-19 while a multiplet centred at δ 5.55 was assigned to the C-11 olefinic proton. The mass spectrum of the compound afforded the molecular ion at m/z = 426.3609 which corresponded to the formula C28H46N2O (calcd. 426.3609). The substance showed a base peak at m/z 58.0650 corresponding to the composition C3H8N+ which suggested the loss of CH2N+ (CH3)2 characteristically encountered in alkaloids bearing a â€“ N(CH3)2 grouping on ring A, and which may be formed in moenjodaramine by intramolecular proton transfer and cleavage. Another peak at m/z 57.0625 corresponded to the fragment CH2 = +N(CH2)CH3. A peak at m/z 85.0883 was in accordance with the composition C5H11N (calc. 85.089) which was attributed to (CH2)2CH = N+ (CH3)2 formed by the cleavage of ring A along with the side chain. A peak at m/z 72.0810 having the composition C4H10N+ corresponded to the loss of CH3.CH = N+(CH3)2 commonly encountered in alkaloids bearing a â€“ CH(CH3) â€“ N(CH3)2 grouping on ring D. Another peak at m/z = 71.0734 having formula C4H9N+ was assigned to the fragment CH2â€“CH = N+(CH3)2 formed by cleavage of ring A along with the side chain.

In the light of the above studies, structure (8) has been assigned to moenjodaramine. This substance has previously been reported as a synthetic product prepared from desoxy-16-buxidienine C, but it has not been isolated. A second alkaloid, harappamine was similarly established to have structure (9).

Moenjodaramine (8) and harappamine (9) are the first representative numbers of a new class of pentacyclic natural products bearing both a tetrahydrooxazine ring and a 9(10â€“19) abeo-diene system.

(a) ISOLATION AND STRUCTURAL STUDIES ON THE CHEMICAL CONSTITUENTS OF CATHARANTHUS ROSEUS

Studies on the alkaloids of Catharanthus roseus have resulted in the isolation of a new alkaloid, to which structure (10) has been assigned. The substance afforded a u.v. spectrum which was typical of a dihydroindole system, showing absorption maxima at 212, 246 and 303 nm and minima at 276, 226 nm. The i.r. spectrum showed the presence of an ester carbonyl absorption at 1730 cmâ€“1. The mass spectrum was very similar to that reported for vindoline and 19-epi-vindolinine. A high resolution mass measurement on the molecular ion afforded the exact mass to be m/z 336.1837 in agreement with the formula C21H24N2O2 (calcd. 336.1836). The C-13 NMR spectrum of the alkaloid (10) (broad-band and off-resonance) showed interesting similarities to the C-13 NMR spectra reported for 19-R-vindoline, 19-S-vindolinine, and 16-epi-19-R-vindolinine. The ester carbonyl carbon resonated at δ 173.47, whereas the methyl of the ester group resonated at δ 52.6 (quartet). The substance afforded 4 doublets for the tertiary aromatic carbons, and 2 singlets for the 2 quaternary aromatic carbon atoms. A characteristic singlet appeared at δ 81.36 corresponding to the quaternary carbon atom α to the indoline nitrogen.

The H-NMR spectrum of (10) recorded on a 200 MHz instrument showed the presence of a doublet at δ 0.62 (J = 7.4 Hz) which is assigned to the C-18 methyl protons. The proton adjacent to the carbomethoxyl function resonated as a double doublet at δ 3.18 (J1 = 12.2 Hz, J2 = 5.8 Hz). A double-doublet at δ 6.41
was assigned to the olefinic proton at C-15, showing coupling with the vicinal olefinic proton and an allylic coupling with the C-3 proton (J1 = 10 Hz, J2 = 2.8 Hz). The other olefinic proton at C-14 resonated as a doublet of doublets at d 5.84 (J1 = 10 Hz, J2 = 5.2 Hz, J3 = 1.8 Hz). The chemical shift of d 0.62 for the methyl group is consistent with a 19-S-configuration as the methyl group of 19-vindolinine resonates at d 0.57 while the methyl group in 19-R-vindolinine resonates at d 0.95.

Direct t.l.c. comparison with authentic samples of vindolinine and epi- vindolinene showed that the substance could be just separated from these 2 materials in 25% ethanol in ethylacetate on a silica gel plate. In order to confirm the structure, the alkaloid (10) was subjected to an oxidative cleavage reaction with iodine/THF/H2O/Na2CO3 when it was found to be smoothly converted to the iodo compound (11). On hydrogenolysis with Raney Ni at 300C for 2 h, the iodo compound was found to be transformed to (â€”)-vincadifformine (12). When the same hydrogenolysis experiment was repeated at 00C for 5 min. quantitative conversion to tabersonine (13) was observed (Scheme 2). The identity of the synthetic hydrogenolysis products was established by direct chromatographic and spectroscopic comparison with authentic samples of tabersonine and vincadifformine.

16-Epi-19-S-vindolinine, when refluxed in benzene for 3 h in the presence of an equimolar amount of lead tetraacetate, was found to be smoothly transformed to 2 faster running products. The major product formed in 70% yield afforded a normal indolic u.v. spectrum. The i.r. spectrum (KBr) showed bands at 1655 cm-1 and 1730 cmâ€”1, which were assigned to Nb-CHO and -CO2CH3 groups respectively. The mass spectrum showed M+ at 352.1783 (calc. for C21H24N2O3, 352.1786), and other major peaks at 320, 293, 214, 169 and 154. The PMR spectrum (CDCl3) showed resonance at d 1.23, (3H, d, J = 5.6 Hz C = CH-CH3), d 3.67) (3H, s, OCH3), d 5.46 (IH,q, J = 5.6Hz, C = CH-CH3), d 5.7-6.1 (2H,m, HC=CH), d 7.6-6.9 (4H, m, aromatic), d 8.00(IH, s, Nb-CHO) and d 8.35 (1 H. s, NH). Irradiation at d 5.46 resulted in the collapse of the methyl group at d 1.23 to a singlet.

The above spectroscopic data were identical with those for (14), a product previously reported to be formed from 19-iodo-tabersonine on heating with sodium acetate in DMF. In order to confirm the structure of the oxidation product, 16-epi-19-S-vindolinine (10) was oxidized with iodine under conditions previously described for the oxidation of its diastereo isomer. This afforded the corresponding 19-iodo-tabersonine in quantitative yields. Treatment of the latter with sodium acetate in hot DMF afforded (14). A direct spectroscopic and chromatographic comparison of the product formed by lead tetraacetate oxidation with that prepared from 19 iodotabersonine unambiguously established its structure. A plausible mechanism for the formation of (14) is presented in (Scheme 3). The second minor product formed in the lead tetraacetate oxidation possessed a u.v. characteristic for the dihydroindole system. Further work on the structure of this material is under progress.

Marine Sterols
The isolation and structure elucidation of novel plant sterols from terrestrial sources peaked in the 1950â€™s and within another decade had declined in terms of academic interest, since the majority of unusual structures had been encountered by then. Economically, sterols have played a major role. Cholesterol from animal sources was the first economically viable starting material for the synthesis of the medicinally important steroid hormones, to be displaced by the plant steroidal sapogenins. In the 1970â€™s partly for political reasons, their importance declined at the expense of the conventional plant sterols, sitosterol and stigmasterol, which have proved to be excellent starting materials for microbiological conversions to intermediates in corticosteroid synthesis as well as for the preparation of other, medicinally useful, steroids.

Given the decline of activity in the plant sterol field and the greatly reduced number of new plant sterols reported in the literature, it was particularly surprising to encounter a veritable explosion of new sterol
structure, from marine sources in the 1970s and early 1980s; probably prompted by the structure elucidation of the marine sterol gorgosterol (14) with its totally unexpected features: the presence of a cyclopropane ring and the fact that every carbon atom of the cholesterol side chain bore a carbon substituent. If we recall that plant and animal sterols possess either unsubstituted side chains (I R=H) or a carbon substituent at C-24 (1, R=Me or Et) and that the biosynthesis of such side chains had been studied in exquisite detail (Lederer 1969), it was indeed surprising to encounter a sterol side chain substitution pattern based on 2, especially since the pioneer of marine sterol chemistry, Werner Bergmann in his extensive studies of marine sterols had only encountered variants of 1. As the Field of marine sterols has grown so rapidly since 1970. I shall focus in the present paper on unusual variations in the sterol side chain and in ring A. with primary emphasis on results from our own laboratory.

UNUSUAL STRUCTURAL FEATURES OF MARINE STEROLS

Marine sterols are distinguished from their terrestrial counterparts by 3 unique features: (a) unprecedented bioalkylation patterns in the side chain; (b) existence of three-membered rings in the side chain: (c) nuclear variations in ring A. which had previously not been found in nature. Each of these has interesting biosynthetic implications, not encountered previously in terrestrial counterparts, and some may also be of potential economic utility.

Examples of unusual bioalkylation

Work performed since 1970, notably in Italy, Japan and the U.S., has produced such a plethora of unusual side chain substitution patterns that even recent reviews are already outdated. As well over 100 new sterols have been isolated from marine sources, notably sponges, it will suffice to illustrate the range of bioalkylation possibilities by giving the structures (Fig. 1) of a few marine sterols isolated in our laboratory which arise by triple (e.g., 5-7) and quadruple (e.g., 9-11) biomethylation sequences. With one exception such multiple biomethylations have not been encountered in terrestrial sterols. Except for pulchrasterol (5 in Fig. 1), which possesses a different nucleus, it is likely that all of the other sterols described in Fig. 1 arise, directly or indirectly, from an epicocisterol (3) or epiclerosterol (4) precursor as discussed in more detail below.

Sterol side chains with cyclopropane and cyclopropene rings

This sub-group of marine sterols contains not only entirely unprecedented examples of sterol side-chain substitution, but they also raise biosynthetic questions for which there is little precedent except for studies in the fatty acid field. With the exception of 23 demethylgorgosterol, petrosterol and calysterol, all other sterols summarized in Fig. 2 were isolated and structurally characterized in our laboratory. Even cursory inspection of Fig. 2 makes evident the wide range of cyclopropyl substitution, which can be found among marine sterols, but 2 special points merit emphasis. The first refers to the cyclopropanes: with the exception of 24, 26-cyclocholesterol and papakusterol, the three-membered ring invariably includes an extra carbon atom introduced into the cholesterol side chain by S-adenosylmethionine (SAM) biomethylation. The 2 cyclopropanes 20 and 21 are biosynthetically distinct in that no additional carbon atom is involved in the generation of the three-membered ring. The second comment refers to the three cyclopropenes calysterol (16), (23R)-23H-isocalysterol, and (24S)-24-H-isocalysterol. The cyclopropene functionality is one of the rarest among natural products, the best known example being sterculic acid and its congeners. Biosynthetically, the origin of the steroidal and the fatty-acid cyclopropene is similar. Thus, Yano have shown that the double bond in sterculic acid is introduced as the last step by biochemical dehydrogenation of the corresponding cyclopropane. Similarly, we have recently been able to show (Catalan to be published) by radioactive tracer studies that the recently isolated 23,24-dihydrocalysterol (19) is the biosynthetic precursor of the steroidal cyclopropanes 16-18. The possible biosynthetic role of steroidal cyclopropanes has already been speculated upon and will be discussed below.
Nuclear variations in ring A

Three types of nuclear alterations have been observed, which are unique to the marine sterol field. The first are 9, 11-seco sterols of Type C, which were first encountered in the gorgosterol series (cf. 14 in Fig. 2). Recent work in our laboratory has shown that such 9, 11-seco sterols can also have a variety of other and more conventional side chains, but that both the 8a- and 8b-isomers are naturally occurring, an observation that may be of significance when the hitherto unknown biosynthetic origin of these seco sterols is eventually unravelled.

The other 2 types of nuclear variants are both of potential practical use and have first been discovered by Minale’s group in Italy, namely the A-nor sterols of Type D and 19-nor stanols of type E. In each case, the associated side chains were of the conventional type, but subsequent work in our laboratory has shown that almost every conceivable side chain, including the most unusual ones including cyclopropane rings, can be associated with the A-nor skeleton D. Both direct and indirect, evidence has been presented to show that sterols with a variety of side chains but the conventional cholesterol nucleus (A) are acquired by the sponge through dietary intake and then transformed efficiently into the A-nor skeleton D.

Similar generalizations can be made about the 19-nor stanols of type E, where both direct, i.e., radioactive incorporation studies and indirect, i.e., observation of wide variety of different side chains, evidence has been provided to show that sponges can efficiently transform dietary sterols into 19-nor steranes (E).

BIOSYNTHETIC ASPECTS

In contrast to the enormous amount of work that has been performed during the past quarter century on the biosynthesis of terrestrial animal and plant steroids, relatively little has been accomplished among marine species and especially lower marine animals like sponges. The work of Minale has shown that sponges can efficiently carry out ring A nuclear modifications (A® D; A® E) and Minale have also noted the incorporation (albeit in poor yield) of fucosterol into calysterol (16 in Fig. 2). The reasons for the paucity of relevant experiments are of a technical nature, how to keep a sponge alive while feeding precursors, as well as lack of availability of many potentially relevant precursors. A considerable effort has been expended in our laboratory along these lines and as an example of an unambiguous experiment, we can cite the high yield incorporation of radioactive epicodisterol (3) into 25-dehydroaplysterol (22) and, thence, into aplysterol (23), whereas codisterol (3a) was not utilized by the sponge Low, though definite, incorporation into verongulasterol (24) in the same sponge (Aplysina fistularis) was also demonstrated. The results are, thus, consistent with the expected sequence summarized in Fig. 3 and, as earlier work by Minale had shown that de novo sterol synthesis does not occur in this sponge genus, the efficient side chain modification of dietary sterols by the sponge is established. Feeding experiments with a variety of other precursors and other sponges are now under way in our laboratory and the preliminary results indicate that the course of the multiple bioalkylation of the sterol side chain will soon be completely clarified. Recently, we have also succeeded be in incorporating deuterated methionine into cultured unicellular organisms which produce gorgosterol (14 in Fig. 2) thus opening the way to the elucidation of the biosynthetic pathway to such cyclopropyl-containing sterols.

This brings us to the question of the role of such cyclopropyl-containing sterols. Are these metabolic end-products or are they themselves active intermediates to some other sterol structures? We have speculated earlier that such cyclopropanes may be enzymatically isomerized to allylic methyl functionalities and, thus, provide an alternative to conventional SAM methylation of olefins. While no direct evidence has so far been provided to settle this question, 2 biomimetic experiments (Fig. 4) from our laboratory are relevant. Lang were able to show that mild acid treatment of petrosterol (15) led to 26-dehydro-25-epiaplysterol (25) which had been isolated earlier by us from the same sponge. Even more intriguing is the observation by Catalan that acid treatment of 24,26-cyclocholesterol (20) yields as the major product 24-methyl-27-nor-5,25cholestadien-3b ol(26). The latter has the 27-norergostene side chain, which has been encountered in
various marine organisms and whose biosynthetic origin is as yet unknown.

**BIOLOGICAL FUNCTION OF MARINE STEROLS WITH UNUSUAL STRUCTURES**

While nothing can be said at the present time about the possible biological role of unusual trace sterols in marine animals, speculation is much more justified in those instances where practically no "conventional" sterols such as cholesterol are present and where the latter is replaced by one or more marine sterol(s) of unusual structure. We have suggested that such sterols replace cholesterol in cell membranes and that they, thus, play a functional role. As most of the unusual structural features reside in the side chain, which would be situated deep within the lipophilic portion of the phospholipid bilayer of cell membranes, we have examined several such sponges for unusual phospholipid fatty acids. In point of fact, every one of the sponges examined by us contained unusual fatty acids, never encountered before in terrestrial organisms. Current work in our laboratory deals with the synthesis of phospholipids containing these usual fatty acids, preparation of model membranes and examination of membrane properties by comparing the appropriate marine sterols with cholesterol. These experiments should shed light on the question of the biological role of these unusual sterols.

**POTENTIAL PRACTICAL SIGNIFICANCE OF MARINE STEROLS**

The sterols with unusual side chains or ring A substitution cited in this paper are unlikely to possess intrinsic medicinal properties that would make them of economic significance. Antagonism to cholesterol deposition may be a theoretical possibility, but none of them have been examined in this regard. However at least theoretically, some of them may be of economic significance as starting materials for the synthesis of medicinally important drugs, similar to the present utilization of terrestrial plant sterols. Two possible applications are cited below.

**A New Look at Muscle Relaxants Alternatives to Curare**

**MUSCLE RELAXANTS**

Muscle relaxants, or neuromuscular blocking agents, are drugs that are used as adjuncts during general anaesthesia. They are not general anaesthetics: in balanced anaesthesia, loss of consciousness is produced by the general anaesthetic itself, pain is limited by an analgesic, and skeletal muscle relaxation is produced by the muscle relaxant drug. Muscle relaxants are primarily used during surgery. They facilitate endotracheal intubation by relaxing the muscles of the larynx, the neck and some of the lower jaw muscles. They also improve the surgical operating conditions so that the skeletal muscles are relaxed, allowing the surgeon a wider field in which to operate. In addition, they are used in intensive care units to permit mechanical ventilation over prolonged periods. The prototype muscle relaxant, tubocurarine, derives from plant origins and it has been in use since the 1940s. Almost all the developments in the field have been based on substances from natural origins, but, although newer analogues have brought improvements, there are still unmet needs and research remains active in the area.

**NEUROMUSCULAR TRANSMISSION AND BLOCK**

The pharmacology of clinically useful muscle relaxants involves primarily, but not entirely, interference with the postjunctional action of acetylcholine on nicotinic receptors at the skeletal neuromuscular junction. The process of chemical transmission involving acetylcholine acts as a mechanism for amplifying the electrical signal in the tiny nerve terminal so that it is able to activate the very much larger muscle fibre. The basic physiology of neuromuscular transmission involves the chemical transmitter acetylcholine. Acetylcholine is synthesized in the motor nerve terminal cytoplasm from choline and acetyl coenzyme A, catalysed by choline acetyltransferase. Choline derives from extraterminal sources: plasma choline, plus
choline produced as the breakdown product of enzymatic hydrolysis of acetylcholine, its uptake being mediated by a high affinity sodium-dependent mechanism. Synthesized acetylcholine is taken up from the cytoplasm into synaptic vesicles by an ATP-dependent uptake process. None of the clinically used muscle relaxants has significant effects on presynaptic acetylcholine metabolism.

Not all the stored acetylcholine has an equal chance of being released by nerve impulses; there are probably two readily or immediately releasable stores of acetylcholine, plus a depot or back-up store. The mobilization of acetylcholine, or vesicles, into the immediately releasable stores is susceptible to neuromuscular blocking agents, resulting in the phenomena of train-of-four or tetanic fade, i.e. the diminution of tension responses during medium and high frequency stimulation.

The release process itself is highly dependent on calcium entry into the terminal subsequent to terminal depolarisation. At rest, individual quanta are released at random, but depolarization of the terminals by a nerve action potential results in the simultaneous release of many quanta. After release, the acetylcholine diffuses across the junctional cleft and activates the postjunctional acetylcholine receptors. The nicotinic receptor-ion channel complex consists of five membrane spanning protein subunits, each of which crosses the membrane four or five times. Two of the five subunits are identical (a-subunits): also, there are one b, one d, and one e-subunit in adult mammalian muscle. The recognition sites for acetylcholine are situated on the a-subunits and activation of the receptor-channel complex is much more likely when both recognition sites are occupied. A conformational change in the subunits of the receptor leads to the opening of the ionic channel. This cation-selective channel conducts mainly Na+ and K+ down their concentration and electrical gradients. The ion flux reduces the membrane potential of the endplate region towards zero potential. The fall in membrane potential in the chemically excitable region of the receptors leads to a disparity in potential with the adjacent electrically excitable non-receptor-rich area. Local circuit currents flow in the area between the chemically and electrically excitable regions, depolarizing the electrically excitable membrane. At a critical threshold potential, sodium channels open in the muscle membrane and initiate an all-or-nothing action potential, which results in muscle contraction. During the period of the muscle action potential and the muscle contraction, acetylcholine is hydrolysed by acetylcholinesterase; hence depolarization is limited in duration, resulting in one nerve action potential producing one muscle contraction.

The major actions of muscle relaxant drugs are on the postjunctional acetylcholine receptors. At this site, the relaxants can be divided into two major classes: depolarizing and non-depolarizing agents. Suxamethonium, a synthetic compound, is the only example of a depolarizing agent in widespread clinical use, although decamethonium and carbolonium were used in the past. The non-depolarizing class of the relaxants includes the currently used agents tubocurarine, pancuronium, atracurium and vecuronium. The action of both classes involves binding to the acetylcholine receptor recognition site. With the depolarizers this results in activation of the receptor, i.e. an agonist action, with nondepolarizers no activation is seen, but rather. by occupying the receptor, the nondepolarizers prevent activation by acetylcholine, i.e. an antagonist action. The antagonism of the action of acetylcholine prevents the level of postjunctional depolarization produced by released acetylcholine from reaching the threshold required to fire a muscle action potential. Thus, muscle contraction ceases. Depolarizing agents such as suxamethonium produce receptor activation and initial muscle contractions, but, unlike acetylcholine, suxamethonium is not hydrolysed by acetylcholinesterase. Thus, suxamethonium produces a persistent endplate depolarization. This leads to inactivation of the sodium channels in the electrically excitable membrane surrounding the endplate, producing a zone of electrical inexcitability. Again, muscle contraction fails.

As mentioned above, in addition to blockade of postjunctional receptors, non-depolarizing relaxants possess a prejunctional action which leads to a decrease of transmitter release during a high frequency burst of nerve activity. As normal voluntary muscle movement results from rapid nerve stimulation, this would result in a decrease of voluntary muscle movement. When the nerve stimulation is reduced, the muscle relaxation increases and this is referred to as the effect of an indirect action.
mechanism has potential clinical relevance, being manifest as tetanic or train-of-four fade. It has been postulated that the prejunctional action of nicotinic antagonists indicates the presence of a prejunctional nicotinic acetylcholine receptor that is involved in a positive feedback control mechanism for transmitter mobilization. Although neuromuscular blocking drugs primarily block nicotinic-type acetylcholine receptors at the skeletal muscle neuromuscular junction, acetylcholine receptors are present at other synapses in the body, and actions of muscle relaxants on synapses in the autonomic nervous system are responsible for their major side effects. Thus tubocurarine blocks nicotinic receptors in autonomic ganglia. This results in a fall in general arterial blood pressure due to loss of sympathetic tone because of blockade of the excitatory ganglionic nicotinic receptors at sympathetic ganglia. In addition, tubocurarine releases histamine, which also results in hypotension. Another autonomic blocking action of quaternary muscle relaxants is seen at the muscarinic receptor of the sino-atrial node, resulting in certain drugs (most noticeably the discontinued drug, gallamine, but also to a lesser extent pancuronium and alcuronium) producing a selective atropine-like action on the heart, which leads to tachycardia.

ALKALOIDS AND THEIR SEMISYNTHETIC DERIVATIVES

History
Almost all of the chemicals that are in use at the moment as muscle relaxants derive directly or indirectly from chemicals from plant sources. In the sixteenth and seventeenth centuries, European explorers returning from South America reported many poisonous plants, including ourariâ€™ which was used as an arrow poison to paralyse wild animals. Later samples of arrow poisons were known as curares, one of which was used in Claude Bernardâ€™s classical experiments in which he showed that the site of action of curare was the neuromuscular junction. Various attempts were made to use curare clinically for the treatment of rabies, tetanus, chorea and epilepsy, but these studies were frustrated by the variable potency and quality of the extracts used. However, the 1930s and 1940s saw developments on many fronts: (1) acetylcholine was shown to be the chemical transmitter at the neuromuscular junction, and curare antagonized its actions, (2) highly purified fractions of curare were used in tetanus and spastic disorders, (3) curare was found to prevent bone fractures during electroconvulsive therapy, (4) the initial structure of tubocurarine was proposed from a sample of unknown botanical origin, (5) the same compound was isolated from an authenticated sample of Chondrodendron tomentosum and (6) perhaps most importantly, tubocurarine was shown to relax skeletal musculature during general anaesthesia, reducing the need for large dangerous concentrations of general anaesthetics.

The botanical sources of the South American curares were the Strychnos species, found in the eastern Amazon region and the Chondrodendron species, found mainly in western Amazonia. The original samples were classified according to the containers in which they arrived in the civilized world: tube curare (hence tubocurarine) in bamboo tubes, calabash curare in gourds, and pot curare in earthenware jars.

Tubocurarine and derivatives
The structure of tubocurarine was originally proposed to be that shown in Fig. 1. i.e. a bisquaternary compound, with an approximate interonium distance of 10nm. This basic bisquaternary structure was used as a template for the synthesis and testing of many thousand of synthetic and semisynthetic compounds in the 1940s.

Several direct analogues of tubocurarine were synthesized and tested, the most successful being O,ON-trimethyltubocurarine, originally known as dimethyl-tubocurarine or metocurine. This compound was more potent than tubocurarine was longer acting, and possessed fewer autonomic side effects. It had a resurgence in use in the United States in the 1970s because of its lack of side effects.

Laudexium (Fig. 3) was essentially the simplest combination of readily available oxygen-bearing benzylisoquinoline units, mimicking tubocurarine, with the decamethylene chain of decamethonium. Although laudexium is obviously structurally related to tubocurarine, its solution structure is very different:
the two benzylisoquinoline units are unable to adopt the head-to-tail arrangement found in tubocurarine. It is probably an indication of receptor flexibility that laudexium is more potent than tubocurarine. An effort to reduce the duration of action by incorporating ester functions in the central chain was successful in oxalolaudonium (Fig. 3), but the compound proved too unstable for exploitation. The incorporation of ester links is a theme that has been revisited many times, ultimately with success (vide infra).

Efforts to shorten the action of atracurium by the incorporation of further labile groups served to demonstrate the difficulties involved in combining a non-depolarizing mode of action with ultrashort duration.

Toxiferines and derivatives.

The toxiferine alkaloids, derived from Strychnos toxifera and the calabash curares, are the most potent of the naturally occurring curares. Much work on their structural elucidation was performed by Karrer, and their pharmacology was extensively studied by Waser, who subsequently used the high potency, shape and size of the C-toxiferine I molecule to propose that non-depolarizing relaxants occluded the pore or channel opened by the chemical transmitter acetylcholine. A semisynthetic analogue of toxiferine 1, diallyl-bisnortoxiferine or alcuronium (Fig. 6), is as long acting as tubocurarine, but causes a relatively low incidence of side effects.

Quaternary steroids

Lainé observed that a steroidal molecule isolated from Malouétia species, malouétine (Fig. 7) possessed neuromuscular blocking properties. Several groups followed this lead, and the compounds dipyrandium, dimethylconessine and stercuronium a conessine derivative, were tested but not developed further (Fig. 7), because of either inappropriate duration of action or unacceptably high autonomic effects.

A successful synthetic approach based on the androstane nucleus, the chemical starting material of which is derived from Mexican yams, has been aminosteroids containing acetylcholine-like, or similar, fragments. The first of these was pancuronium (Fig. 8), a bisquaternary molecule containing two acetylcholine fragments; pancuronium is highly potent, around 10 times as potent as tubocurarine, and when introduced in the late 1960s was thought to cause minimal autonomic side effects. However, in the 1970s it came to be appreciated that cardiac stimulation brought about by cardiac muscarinic receptor block was an undesirable side effect in patients undergoing surgery while in marginal cardiovascular condition. As pancuronium produces mild tachycardia, a search was instituted for ‘cleaner’ derivatives; the result was vecuronium (Fig. 8), a monoquaternary analogue of pancuronium, with a time course profile almost identical to that of atracurium. Subsequent developments have produced rocuronium (Fig- 8), which has a faster onset and pipercuronium (Fig. 8), which has a very high potency and long duration of action somewhat similar to that of doxacurium. Fig. 9 shows a representative record of the comparative effects of vecuronium and atracurium, the two most widely used non-depolarizing relaxants, in an anaesthetized animal model.

A further approach using homosteroids led to chandonium iodide, which was very short acting in cats, but proved long lasting in primates and man, and also produced tachycardia.

The Search for New Antimicrobial Agents: Unusual Sources

Despite what one might imagine to be the case after forty years of highly organized and intensive industrial research on antibiotics from soil micro-organisms, new chemical entities are being discovered at a higher annual rate than ever before. In early years, the bacteria, Fungi Imperfecti and soil streptomycetes accounted for the vast majority of reports, whereas in recent years newer and less usual sources are being explored increasingly. These conclusions can be supported by an examination of Fig. 1 and Tables I and
One notes that thirty years ago, 57 antibiotics were described belonging to 11 different structural types. Most (35, 61%) of these were isolated from streptomycetes. By 1982, 230 new antibiotics were being described but only 46 % (106) of these were from streptomycetes. Tables I and 11 contrast the relationship between type of antibiotic and producing organism for these same two years. The differences are dramatic. In 1952, antibiotics were isolated only from bacteria, streptomycetes, nocardiae, fungi and micromonosporae. By 1982, antibiotics were also being isolated from streptosporangiae, actinomadurae, kitasatoae, actinoplanetes, dactylosporangia, saccharopolysporae, streptoalloteichi and streptoverticilliae as well. On the other hand, the structural classes were essentially the same (15 structural families). From Fig. 1 one sees that the move toward exploitation of rare micro-organisms became quite discernable by 1977 and is now quite pronounced. It is also apparent that the rarer organisms are mostly producing new antibiotics classifiable into the same well established structural types.

As gratifying as this picture appears at first glance, unfortunately, we cannot say that mankind’s needs for anti-infective agents are largely satisfied. The combination of the genetic versatility of microbes and widespread overuse of antibiotics has led to increasing clinical resistance of previously sensitive microorganisms and the emergence of previously uncommon infections. One can mention in this regard, anaerobes, amoebae, fungi, legionellae, toxogenic staphylococci, the unknown agent responsible for the acquired immune deficiency syndrome (AIDS), and so on. Infectious diseases still are estimated to rank fourth on the list of causes of death in the US. Despite the level of effort described, infectious diseases still account for 7% of all deaths and a very substantial Morbidity.

There are various ways of trying to deal with this problem. One of these involves the search for antibiotics of a chemical and biological type not previously explored. This, in the hope that novel activity spectra, novel modes of action and less cross-resistance will be seen than with presently utilized antibiotics. Ideally such novel agents would go to the clinic directly, less ideally, but still usefully, they would serve as leads for medicinal chemists to modify to enhance potency, alter spectrum, sharpen selectivity, reduce toxicity or enhance pharmacodynamic character.

In industrial laboratories a conservative approach, which makes maximal use of existing equipment and human resources, emphasizes fermentation of less commonly explored genera and use of unusual habitats (soils from sea bed, mine shafts, deserts, etc.). This approach became popular in the last decade and has been fruitful. Most of the antibiotics so discovered represent structural variations on themes familiar to antibiotic chemists.

The discovery of the antibacterial and antitumor agent maytansine (2) from the Ethiopian shrub Maytenus ovatus has awakened considerable interest in the properties of this subfamily of ansamacrolides, distantly related to rifamycin (l). The ansamitocins (4), including 3-deacetylmaytanbutacine (3) are amazingly, not only isolated from several streptomycetes (S. sclerotialus, S. castaneus, S. flavochromogenes, S. olivaceisceroticus, S. flavoscleroticus, and S. luridus) but also from Chainianigra, an unspeciated Nocardia, and Streptosporangium roseum as well. Considering that they are also related to a compound from a higher plant, these are remarkable examples of the point being made.

Cationomycin (5), isolated from Actinomadura azurea, is a rather typical example of the ionophorous polyether antibiotics more commonly associated with the streptomycetes. Aminocyclitol antibiotics such as streptomycin, neomycin and kanamycin are quite common among the streptomycetes and the gentamicins are common among the micromonosporae, however, the aminocyclitols are not limited by any means to these genera. For example, 2'-N-formylsisomycin (6) is produced by Dactylosporangium thailandense and sporaricin E (7) is produced by Saccharopolyspora hirsula. The latter is the C-2 deoxy analogue of the micromonospora antibiotic fortimicin B (8).

Differinol A (9) represents another example of an apparently widespread antibiotic type. Isoflavones are
widely distributed amongst higher plants extracts. Differinol A is isolable from Micromonospora halophytica as well as from the fungus Aspergillus niger. It has been suggested that differinol A may occur as a glucoside (genistin) in soya beans. Soya bean meal is a medium constituent in these fermentations so the possibility exists that differenol A is an artifact produced by those microbes, which have the appropriate glycosidase.

Antitumor and antibacterial anthracycline antibiotics such as doxorubicin are produced by streptomycetes. Close relatives are produced by rarer soil microorganisms. One may cite the dihydrosteflimycins (10) from Actinoplanes utahensis and 4-hydroxybaumycinols (11) from an Actinomadura species.

The reader should not be led to the impression that all antibiotics from rare organisms fall into well precedented classes. For example kijanimycin (12), from Actinomadura kijaniata, and siderochelin A (13), from a Nocardia species, have no obvious streptomycete-derived counterparts as yet. Still, the basic point remains that in most instances published recently, the structures produced by rare organisms are related to previously explored substances.

From this brief analysis, one concludes that if one is to discover truly unusual Structural types, one must look more closely at fermentation residues, make use of novel screening methodologies, or make some more fundamental alteration in approach. Amongst the alternate approaches being examined at present is the search for antimicrobial agents from marine organisms where such agents appear to be common. In our case, we have been examining the relatively neglected and surprisingly plentiful antimicrobial agents from terrestrial higher plants. Plants are subject to infections by bacteria, fungi and viruses. The specific organisms involved are rarely the same as those infecting animals, but the overall phenomenon has many similarities. While plants lack an immune system and an efficient circulatory system, they possess several means of defense. Amongst these is the pre-infection elaboration of antimicrobial agents and the ability to produce phytoalexins. (A number of apparently innocuous substances are present in plants. Enzymes brought in by plant pathogens can effect structural changes on these compounds. Occasionally, these changes result in amplification of the quantity of a previously minor antimicrobial constituent or in formation of a new antimicrobial agent. Post-infectional antibiotics are known as phytoalexins and many are known). For practical reasons, phytoalexins are hard to deal with in broad screening programs and, despite the many fascinating co-evolutionary relationships they represent, there is less opportunity for man to exploit these for his own protection than there is for use of preformed agents. Fortunately the latter are plentiful. The literature dealing with antimicrobial agents from higher plants is somewhat hard to evaluate because it is widely scattered, is often found in relatively obscure journals, and has emerged with no uniformity in the choice of test organism or in the means used to report potency. In our work, we have developed a useful screening system, which uses indicator organisms predictive of possible utility against human bacterial diseases. By this approach, as many as 1 in 5 extracts of higher plants shows reproducible antimicrobial activity. On the other hand following up literature reports and folkloric accounts results in detection of activity in 1 in 4 extracts. A wide variety of plant genera are involved.

Using a standardized fractionation scheme devised for the purpose. bioassay directed methods usually produced the active agent(s). The structures of pre-infectional higher-plant antimicrobial agents are usually quite different than those from fermentation of soil microorganisms. They are not very different from the structures of the phytoalexins suggesting that the distinction is not a particularly useful one for our purposes. For example the active agent from the Bolivian coral tree, Erythrina crista-galli is the pterocarpene erycristagallin (14). A very similar antimicrobial agent was discovered from the leaves of E. abyssinica and named erythrabyssin 11 (15) Pterocarpans are quite often identified as phytoalexins and many of the structures are very similar to that of erycristagallin. This underlines another important point. The structures of antibiotics from higher plants are types usually familiar to natural products chemists. Such well-studied classes as flavanoids, quinones, coumarins, aliphatics, terpenes, phenolics, and the like, are
involved. Among specific examples from our recent work are the indole alkaloid harmine (16) from Peganum harmala seeds the phenanthroquinolizidine alkaloid cryptopleurine (17) from Boehmeria cylindrica var. drummondiana, the piperidine alkaloid 3,4-dimethoxy-\(w\)-(2'-piperidyl) acetophe- none (18) from the same plant, the flavan derivatives dracorhodin (19) and dracorubin (20) from Daemonorops draco resin the diterpene acids trachyloban- 19-oic (21) and (-)-kaur-16-enoic (22) acids from the stems of the prairie sunflower, Helianthus annuus (Mitscher et al. 1983b), and the novel bibenzyls amorfrutin A (23) and B (24) from the fruits of the false indigo plant, Amorpha fruticosa.

While disappointing from the standpoint of structural excitement, this indicates that many compounds whose structures are known to the literature have unsuspected antimicrobial activity and that testing of congeners be a worthwhile effort. It is also clear that these molecules should be readily accessible to synthesis. The structures are relatively simple and one rarely has significant optical isomerism to worry about. Also, as the compounds have been coevolutionarily optimized against different pathogens than those infecting humans, it seems quite likely that synthetic analogues would possess usefully altered antimicrobial spectra and, perhaps, enhanced activity. These thoughts are supported by studies on the antifungal antibiotic tryptanthrin (25). Strobilanthes cusia has a folkloric reputation as a topical antidermatophytic agent in Taiwan. The active constituent is tryptanthrin. This compound, remarkably, has also been isolated from the unrelated species Polygonum tinctorium, Isatis tinctoria and Candida lipolytica. Synthetic studies and directed biosyntheses have allowed the preparation of a number of analogues. The flexibility of these processes and the alterations in spectra and potency of the unnatural analogues produced, support the optimistic view that persistence might produce a clinically useful agent.

To illustrate how far a field such studies can take one, pteleatinium chloride (26), a quaternary quinolinium alkaloid, is the constituent responsible for the ancient use of the leaves of the false hop tree, Ptelea trifoliata, in brewing beer. We developed a new synthesis as a laboratory exercise, but the products were usually devoid of significant antibacterial activity. This work did, however, draw our attention to the chemically related synthetic urinary tract antimicrobial agents typified by oxoilinic acid (27, \(R = H\)). Our synthesis (route b) was more powerful in scope than that classically used (route a) so we were able to undertake a systematic study of some previously unexplored structure-activity relationships. We quickly showed that the previously unknown C-2-substituted analogues were all less active than the lead substance and that the methylenedioxy moiety was optimally placed at C-6,7. At about this time it was discovered that the molecular target of the action of this antimicrobial agent class was the newly discovered enzyme DNA-gyrase. This fascinating enzyme presents an important new target for chemotherapy. As it seems to have no counterpart in human biochemistry, an outstanding opportunity for selective toxicity is presented. Thus, we were encouraged to continue this effort. In order to investigate the putative mode of action of oxolinic acid, we undertook a synthetic evaluation of the importance of the N-1 atom and its pendent groups. For this it was necessary to develop different synthetic routes. The following schemes lead to the bio-inactive 1-carba and 1-oxa analogues. From the properties of these compounds both in bacteria and against the purified enzyme, it is apparent that the N-1 linkage plays a vital role in upsetting the workings of DNA-gyrase.

As one of the advantages of bioassay-directed fractionation, even quite thoroughly studied plants can produce interesting new findings. While the active principle of the Jamaican plant Zanthoxylum elephantiasis turned out to be the previously known alkaloid canthin-6-one (28), it had not been known to have antibiotic activity. Ptelea trifoliata had been the subject of at least 5 previous investigations, but the major alkaloid, pteleatinium chloride, had been missed. Few plants have been as thoroughly studied as licorice, Glycyrrhiza glabra var. Spanish. Licorice has been used as a sweetening and flavoring agent since prehistoric times. A study of the roots resulted in the isolation of numerous active antimicrobial agents, several of which were new to the literature. These were hispaglabridin A (29), B (30), 4'-O-methyl-glabridin...
(31) and 3-hydroxyglabrol (32). The most significant agent, glabrol (33), was previously known to be present in Glycyrrhiza species, but was not known to be an antibiotic. Interestingly, the only American Glycyrrhiza species, G. lepidota, grows wild in Kansas. It is not usefully sweet. Extracts were found by us to be antibiotically active and fractionation showed that these agents, except for glabranin (35. R = prenyl), were different from those of G. glabra. While 3,5-dihydroxy-4-(3methyl-2-butenyl)-bibenzyl (34) and pinocembrin (36) were known from other plants, glepidotin A (37) and glepidotin B (38) were new.