CHEMICAL AND BIOLOGICAL STUDIES OF TERPENOID COMPOUNDS

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in the
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by

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Summary

The first section of this thesis reviews the literature dealing with the biological activities known to be present in the terpenoids, and in the main treats the subject in terms of the usual chemical classification of terpenoids as mono-, sesqui-, di- and tri-terpenoids. Selected examples are also given in order to illustrate how the group fits in with modern concepts of drug action, such as structural specificity, the supporting moiety theory and the theory of metabolite displacement. Certain representative members of each chemical sub group are discussed in detail but others are covered in a more general manner in order to lend perspective to the field.

The second section is concerned with the synthesis of certain terpenoid hemisuccinates as potential general anaesthetic agents. The preparation of these compounds is justified in terms of theories of general anaesthesia which are briefly discussed. Pharmacological testing showed that the compounds were inactive and this fact is compared with evidence previously reported which indicates that the steroidal general anaesthetics may be structurally specific agents unlike the simple gaseous anaesthetics which are believed to act by a "physical" or structurally non-specific mechanism.

The third section of the thesis describes the synthesis of structurally rigid acetylcholine-like bornane derivatives and the reasons for their preparation. Also included is a summary of present day knowledge of the role of acetylcholine in the animal body and of the different classes of drugs mimicking or antagonising this neurohormone at its various sites of action. A brief discussion of other biologically active bornane derivatives is also given.

Contents

INTRODUCTION

Despite the ready availability and widespread occurrence of the terpenoids very few members of the group are of any great pharmacological significance. In view of the detailed knowledge of their chemical properties and molecular structures now available it seemed a worthwhile endeavour to carry out investigations as to the suitability of various terpenoids as starting materials for the synthesis of new potentially biologically-active molecules and the results of these studies are reported in this thesis. Two lines of investigation were pursued. In the first, various representative terpenoids possessing an alcoholic function were converted to the corresponding hemisuccinates in order to assess such derivatives for general anaesthetic potency and in the second, the bornane skeleton was utilized as a rigid molecular framework with which to investigate activity in conformationally rigid acetylcholine-like molecules. A detailed discussion of the theoretical justifications for these studies is given in sections II and III of this thesis. In order to place these studies in clearer perspective in relationship to the biological

properties of the terpenoids as a whole, a review of biological activity in the terpenoids and their derivatives is included as section I of the thesis. This review is necessarily far from complete both on account of the extremely large number of studies reported in the literature and because a considerable number of studies have been reported in relatively inaccessible publications. Nevertheless a serious attempt has been made to ensure that the survey is as representative as possible.

Section I.

I. BIOLOGICAL ACTIVITY OF THE TERPENOIDS AND THEIR DERIVATIVES .

The terpenoids constitute one of the largest groups of naturally-occurring compounds being characterised by their extremely widespread distribu -tion in both the plant and animal kingdoms and by their great diversity of chemical structure (1). Extensive biological investigations have been carried out within the group and a number of terpenoids and their derivatives have seen medicinal application. Some are in use today, whereas others have disappeared from the pharmacopoeias and so the group affords many illustrations of the progress in clinical medicine. Other terpenoids are of considerable pharmacological interest, and still others are of value in horticulture and agriculture where they are employed as pesticides and fungicides. Many terpenoids and their derivatives are also of the greatest importance to the perfume and cosmetic industries.

THEORETICAL CONSIDERATIONS

Structural Specificity

In general, compounds showing biological activity are conveniently divided into two classes - the structurally specific and the structurally non-specific (2) - and one of the interesting features of the terpenoids as a class is the variation in the degree of structural specificity within the group. Although most members would appear to have little or no structural specificity (which serves to explain their relative unimportance in modern medicine with its emphasis on highly specific drugs), there are, nevertheless, some terpenoids of pronounced structural specificity. Examples are provided by the Erythrophleum alkaloids, mecamylamine and the vitamins A and K. As might be expected, terpenoids with a relatively simple chemical constitution tend to be non-specific whilst the more complex compounds tend to show greater specificity.

Compounds which are completely structurally non-specific exhibit biological activity solely by virtue of their favourable physical properties in accordance with the principle so elegantly established by Ferguson (3), and their biological properties

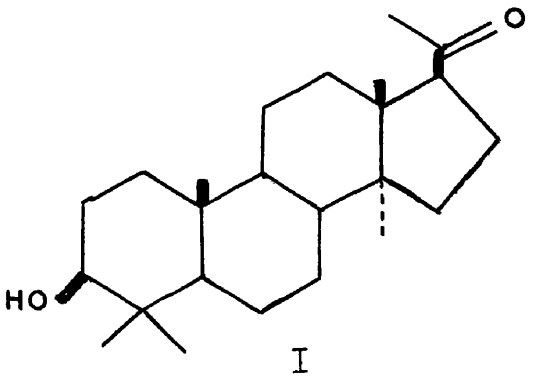
are entirely independent of their functional groups and stereochemistry. Such compounds may be expected to satisfy two criteria. Firstly they should exert an effect on a variety of biological phenomena and secondly their relative potencies should be independent of the test system employed. Although considerable work has been done in relating the potency of structurally non-specific agents of various chemical classes (which are active as general anaesthetics, insecticides, bactericides etc.) to thermodynamic activity, few quantitative studies have been carried out with the terpenoids. Most investigators have concentrated their efforts on such groups as the paraffins, simple ethers, ketones, inert gases and highly chlorinated compounds. In particular, much attention has been devoted to the homologous primary alcohols (4). Since one of the consequences of Ferguson's Principle is that optical antipodes should be equipotent, the neglect to include terpenoids in the quantitative study of non-specific agents is surprising in view of the fact that many of the simpler terpenoids are readily available from natural sources in both enantiomorphic forms. Di- and tri-terpenoids, however, appear to occur in nature in only one antipodal form - with the notable exception of the diterpene kaurene (5).

For compounds acting by a structurally specific mechanism, current theory postulates that biological activity is the result of interaction between the drug molecule and a specific receptor to form a drug-receptor complex. In the majority of cases the forces involved in the formation of the complex are of the nature of electrostatic and van der Waal's forces. Optimum conditions for complex formation will therefore be greatly dependent upon the functional groups and 3-dimensional geometrical properties of the drug molecule. The more dissimilar a given set of receptors are in their nature to all other receptors and the greater the restrictions imposed by these receptors on the achievement of adequate "fit", then the more structurally specific a drug which satisfies these restrictions will be.

Terpenoids as Metabolite Displacing Agents

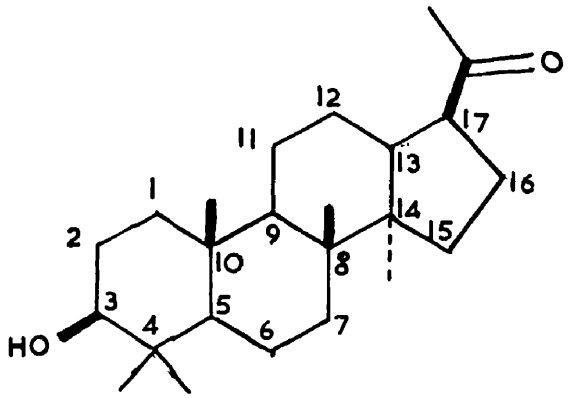
Often a structurally specific drug exerts its action by competing successfully with some metabolite of the organism, to which it is closely related in chemical and physical properties, for a receptor. It thus denies the natural agent access to the site where it

initiates the characteristic train of events which is observed as a biological response. In such cases of metabolite displacement the drug can be expected to intensify, mimic or oppose the action of the natural metabolite depending upon its affinity for the receptor and upon its intrinsic activity (6), a term introduced as a measure of the ability of the compound to evoke a positive physiological response once the receptor-complex has been formed. In favourable circumstances, where rigid molecules are involved, it should be possible to deduce something of the physical nature of the receptor-site from a consideration of the geometrical properties and electric charge distribution of those molecules capable of complexing with it and of those which lack this ability. Most of the work along these lines in the terpene field appears to have been carried out with triterpenoids, having been inspired by the similarity in chemical structure between these compounds and the steroids. For example compound I, prepared



I

from lanosterol (7), has been shown to exhibit progestational activity, but compound II, prepared from the triterpenoid dipterocarpol, is without such



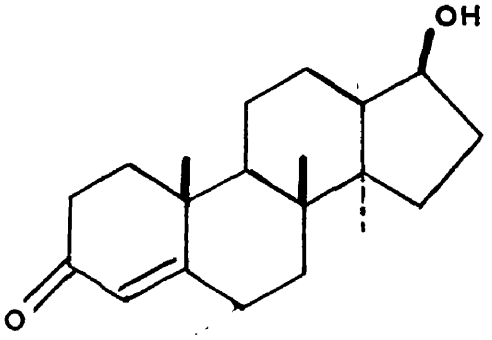
II

activity (8).

Unfortunately the case of compound I is complicated by the fact that the steroid hormones are formed in the body via lanosterol and it is conceivable that pathways exist for the <u>in vivo</u> conversion of this compound into progesterone. No similar ambiguity exists in the case of compound II however. As this substance does not antagonise the action of progesterone, or is not at least a powerful antagonist (9), it would seem that it owes its lack of activity to an inability to undergo complex-formation with the receptor (zero affinity) rather than to a low intrinsic activity. Thus the shape of the receptor may be such as to accommodate the flat steroid nucleus but not the deformed nucleus

of II with the strong non-bonded interaction between the methyl groups on C-8 and C-10.

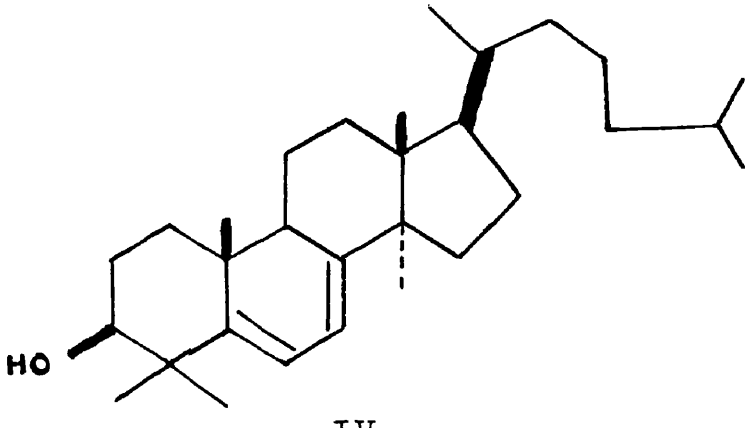
Similarly compound III, which was also prepared from



III

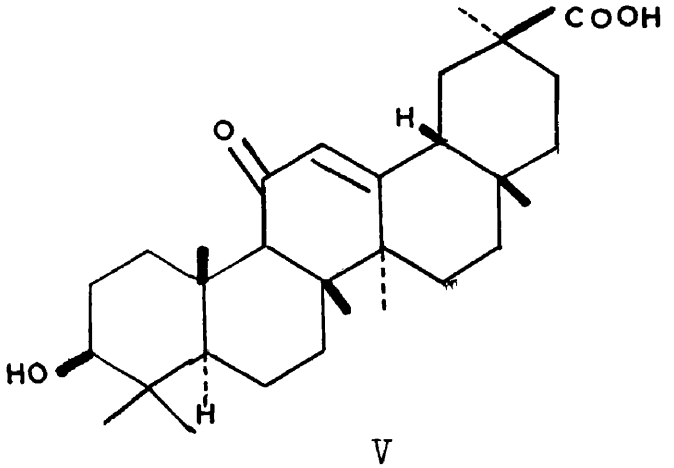
dipterocarpol (8), was found to be inactive as a male sex hormone and to lack appreciable antagonism to its analogue testosterone.

Another steroid analogue derived from a triterpenoid which is worthy of mention is the lanosterol analogue of provitamin D_3 (IV) (10), although it is without biological action as is the 8,14 seco 8[26],9[11],12-triene derived from methyl ursolate (11).



IV

The receptor site is, however, only one of three sites which must be taken into account when considering interference between a metabolite-displacing agent and the natural metabolite. Often the metabolite is held in an inactive or bound form in the cell and the displacing agent could conceivably effect release of the metabolite by a replacement reaction. Again the metabolite-displacing agent may compete for the site at which the normal metabolite undergoes inactivation with consequent prolongation of the action of the metabolite. Indeed there would seem to be good evidence that certain triterpenoids do act in this manner by preventing the inactivation of adrenocortical steroid hormones. The greatest attention has been paid to glycyrrhetinic acid (12), (<u>syn</u> glycyrrhetin) (V),



V

its synthetic derivatives (13), and glycyrrhizinic acid (<u>syn</u> glycyrrhizin), which is derived from glycyrrhetinic acid by combination with two

molecules of glucuronic acid. These triterpenoids are obtained from liquorice and the earlier investigators employed crude liquorice extracts. Other triterpenoids, including synthetic derivatives, have also been examined for adrenocorticoid activity (14).

The early work with the triterpenoids from liquorice led to many conflicting claims as to their mineralocorticoid, glucocorticoid and anti-inflammatory properties (15), and it was not until Atherden (16) demonstrated that glycyrrhetinic acid acted in vitro as a powerful inhibitor of the enzymes responsible for the inactivation of 11-deoxycorticosterone and progesterone that any clarification of the position was achieved. This evidence - taken in conjunction with the numerous clinical observations that glycyrrhetinic acid or glycyrrhizinic acid are active only in patients still retaining functional adrenal tissue (17), or are of value only as adjuncts to cortisone or hydrocortisone therapy (18) - would strongly indicate that the triterpenoids are themselves without adrenocortical activity but are able to interfere with the destruction of the steroidal hormones. Still further evidence is afforded by the demonstration that

ammonium glycyrrhizinate depresses the excretion of urinary 17-ketosteroids (19) and produces a rise in the plasma levels of hydroxycorticosteroids (20). The other actions of glycyrrhetinic and glycyrrhizinic acids, such as the ability to depress the secretion of adrenocorticotrophic hormone (21), the ability to produce hypertension in unilaterally nephrectomized rats (22) and the cortisone-like anti-leukaemic action (23), can also be explained by an inhibition of the biotransformation of adrenocortical steroids.

Glycyrrhetinic acid has been shown to produce an inhibition of oestrogen-induced uterine growth, but the inhibition is grossly non-competitive (24). It is not possible to deduce whether this is due to irreversible competitive antagonism or whether it is due to different receptors being involved.

Supporting Moiety Theory

Another interest in the terpenoids arises from the light which they cast on the "supporting moiety" theory. This theory, first formally stated by Cavallini (25), postulates that the molecules of substances possessing pharmacological activity consist of two portions, the supporting moiety which confers affinity for the site

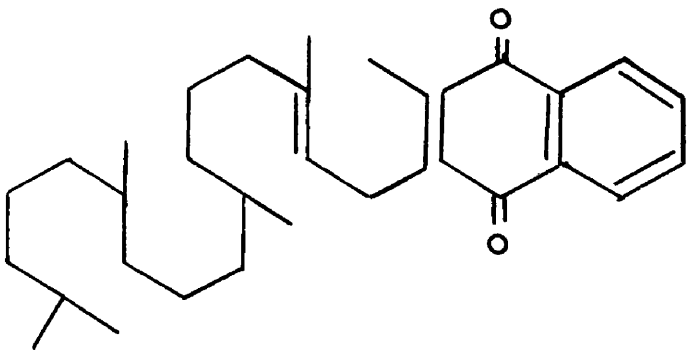
of action (and which could also be involved in transportation mechanisms etc.) and the radical moiety which determines the type of activity displayed. The broad scope of the theory is readily apparent from a consideration of the important role which groups such as the hydroxyl and amino functions play in determining the activity of so many diverse drugs. Strong support for the theory has been provided by studies in the terpenoid field, excellent examples being afforded by the Erythrophleum alkaloids and the vitamin K.

The Erythrophleum alkaloids which exhibit pronounced positive inotropic cardiac activity are esters of certain α,β- unsaturated tricyclic diterpene acids and β- dimethylaminoethanol or β- methylaminoethanol. The aminoalcohols themselves show the same qualitative action on the heart as do the derived alkaloids, but are some 800 times less potent on a molar basis (26). Thus the chemical combination of the diterpene acid moiety (the supporting moiety) with the alkanolamine (radical moiety) reinforces the biological property already characteristic of the radical moiety.

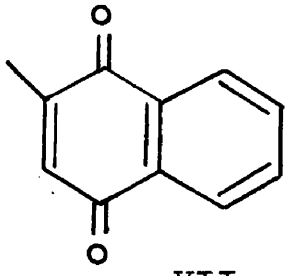
Replacement of the natural tricyclic diterpene acids by other acids, including the diterpenoid abietic

acid and the triterpenoid sarsasapogenoic acid has been shown in general to lead to reduction of activity (27). It is of interest that saturation of the double bonds in the supporting moieties of the natural alkaloids also leads to loss of activity (28). As reduction of the double bond of the unsaturated lactone ring of the steroidal cardiac glycosides is well known to lead to loss of potency there may be some biological correspondence between the α, β-unsaturated ester system of the Erythrophleum alkaloids and the α, β-unsaturated lactone ring of the cardiac glycosides, although the steric relationships of these unsaturated functions to the rest of their molecules are quite dissimilar.

Vitamin K_1 (VI) is 2-methyl-3-phytylnaphtho-1,4-quinone and therefore can be regarded as a diterpene derivative.



VI

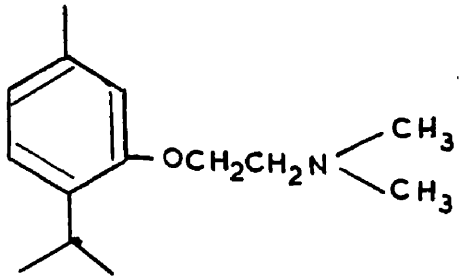


VII

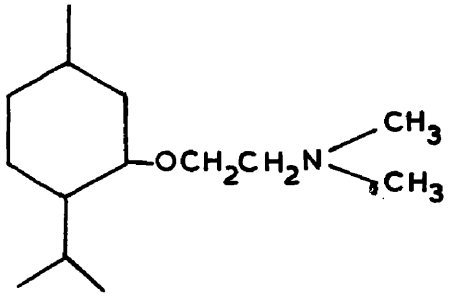
It was found, however, that on a molar basis the synthetic compound 2-methylnaphtho-1,4-quinone (menadione)

(VII) in the form of its soluble sodium bisulphite addition compound had greater antihaemorrhagic activity than the natural vitamin following oral administration to dogs or chicks (29). Thus it would appear at first glance that the quinonoid moiety is more active without the addition of a diterpenoid supporting moiety. However work with C^{14} labelled menadione in the rat has shown that menadione is converted by the addition of a diterpenoid side-chain into 3-(geranylgeranyl)-2-methylnaphtho-1, 4-quinone or vitamin K_2 [20] (30). The apparent higher activity of menadione as compared to that of vitamin K_1 is therefore probably due to the better absorption and penetration of the simpler compound prior to its biotransformation into vitamin K_2 [20]. Support for this assumption is provided by studies on the ability of menadione and various 3-polyisoprenoid-substituted menadiones to antagonise the action of anticoagulants, Isler et al. (31) having firmly established that a side chain of a certain minimum length is necessary for this activity. Later work by the same school (32) has shown that biological activity in the vitamin K-deficient chick is also dependent upon the length of the polyisoprenoid side chain attached to the quinonoid moiety.

Much other work having a bearing on the supporting moiety theory has been reported in the terpenoid field. For example Reinhard et al. (33) pointed out that the β -dimethylaminoethyl group formed an integral part of the molecular structure of many antispasmodic, local anaesthetic, sympathomimetic and antihistaminic drugs. Treating this function as the radical moiety, they investigated the effects of changing the supporting moiety. They discovered that thymyl-β -dimethylaminoethyl ether (VIII) and menthyl-β -dimethylaminoethyl ether (IX)

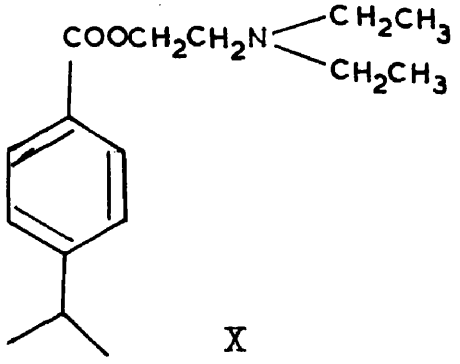


VIII



IX

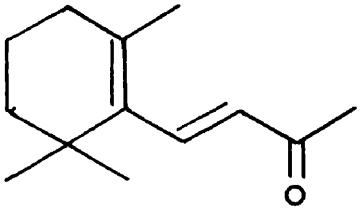
possessed pronounced antispasmodic activity. Similar considerations inspired the pharmacological investigation of the β -diethylaminoethyl ester of cumic acid (X)



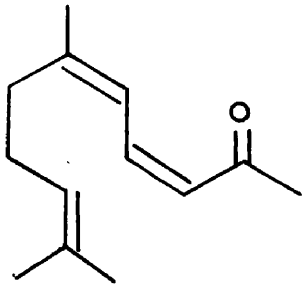
X

(34) which in the form of its hydrochloride is claimed to be equipotent with procaine hydrochloride as a local anaesthetic. A further example is afforded by two isomeric 2-hydroxy-3-methylaminocamphanes, prepared during an investigation of the pharmacological importance of the β-methylamino-∝-hydroxyethyl radical, which were shown to have pronounced action on the respiratory centre of rabbits (35).

With the discovery that certain thiosemicarbazones and acid hydrazones possess marked antituberculous properties a great deal of attention has been devoted to the preparation and screening of drugs belonging to these two classes. Included in these studies were the isonicotinyl hydrazone of cumaldehyde (36), the o-hydroxybenzoyl -hydrazone of perillaldehyde (37) and the thiosemicarba -zones of citronellal (38) and cumaldehyde (39). Thiosemicarbazones derived from β-ionone (XI) and the



XI



XII

isomeric ψ-ionones (e.g. XII) are claimed as anti-tuberculous agents in the patent literature (40).

Although strictly speaking the ionones are not true terpenoids they are customarily included in the group as they are simple condensation products of citral and cyclocitrals with acetone.

The ionones can be used to furnish other examples of the supporting moiety concept. For instance studies with various ionylamines (41) have shown that, in contrast to the β - ionylamines, the γ -ionylamines have a high spasmolytic activity in vivo, thus providing a good illustration of how minor changes in the supporting moiety can have a major effect on the activity displayed.

Further examples which may be quoted are the preparation of the geranyl, citronellyl and linaloyl esters of chaulmoogric acid (42) in attempts to increase the efficacy of chaulmoogra therapy for leprosy and the synthesis of terpenoid esters of piperonylic acid as potential insecticides (43).

In addition, certain chemical features possessed by various biologically active terpenoids have inspired the preparation and biological testing of other molecules possessing the same structural unit. This approach can be regarded as a special aspect of the supporting moiety theory in which attempts are made to isolate the

active radical moiety and combine it with other supporting moieties. An example of such work has been the preparation and testing of lactones (44) as potential anthelmintics since the sesquiterpenoid anthelmintic, santonin, possesses a lactone function.

Biotransformation

Terpenoids have played an important role in studies of biotransformation and metabolism of compounds foreign to the body. Because many terpenoids can be considered as normal constituents of animal food, the ability of the animal organism to metabolise them into compounds more easily excreted and eliminated has not had as great an impact on scientific thinking as has the ability of animals to effect biotransformation of the newer synthetic compounds to which life could never have been exposed in the whole course of evolution. Nevertheless, now that it is recognised that the liver microsomes are capable of adaptive enzyme-formation to metabolise to a greater or lesser degree innumerable compounds foreign to the body (45), the accumulated information on the biotransformations of the terpenoids achieves a new significance. An excellent summary of the

biotransformations undergone by monoterpenoids is to be found in the book by Williams (46).

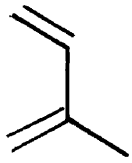
In general the main type of biotransformation suffered by monoterpenoids in the animal body is conjugation to glucuronides, with or without accompanying oxidations and reductions. The excretion of monoterpenoids as glucuronides has been proposed as the basis of a test of liver function (47), and another interesting application of the glucuronic detoxication mechanism was devised by Williams (48) who utilized it to resolve dl. menthol.

Other Interests in Terpenoids

Other interests in the terpenoids arise from the part that the quinonoid derivatives of some of the higher members, such as the ubiquinones and vitamin K, appear to play in the electron transferring system in respiratory chain phosphorylation (49,50) and from the role which the carotenoids and chlorophyll play in the energy transfer processes of photosynthesis in plants. These will not, however, be discussed here.

CLASSIFICATION

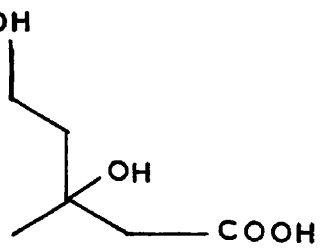
The terpenoids are customarily classified according to the number of carbon atoms present in the molecule, giving rise to four main groups. These are the monoterpenoids, which are derivatives of certain C_{10} hydrocarbons, the sesquiterpenoids, which are derivatives of certain C_{15} hydrocarbons, the diterpenoids, which are derivatives of certain C_{20} hydrocarbons, and the triterpenoids, which are derivatives of certain C_{30} hydrocarbons. In addition a fifth group, the carotenoids, which are derivatives of certain C_{40} hydrocarbons, are sometimes referred to as tetraterpenoids. Further subdivision within these groups is made according to the number of carbocyclic rings present in the molecule. Occasionally certain derivatives of isoprene (XIII) are



XIII

termed as hemiterpenoids.

All the terpenoids are elaborated in nature from acetate units via the key intermediate mevalonic acid (XIV) which can be considered to be the



XIV

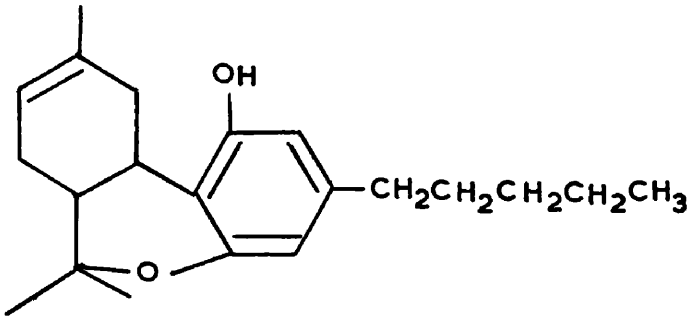
"active isoprene precursor" responsible for the validity of the isoprene rule (51), long recognised by organic chemists as applying to these compounds. In fact the criterion which determines the inclusion of a compound as a terpenoid is that its carbon skeleton obeys the isoprene rule, or is formally derived in a simple fashion from a carbon skeleton which does obey the isoprene rule by bond migration, loss or gain of carbon atoms, or through condensation with other simple molecules. Recent progress in the elucidation of the steps involved in the biogenesis of natural products has clearly shown that many compounds not formerly regarded as terpenoids are derived in nature from terpenoids by processes such as those just enumerated. One important group so related is the steroids (including vitamins D) which are formed in the plant or in the animal body by a route involving the triterpenoids squalene and lanosterol as intermediates (52,53). It is not proposed, however, to discuss the biological properties of the steroids in this

review. General accounts of the importance of steroids in pharmacology and medicine have appeared (54) and numerous specialised reviews dealing with specific classes of steroids are available in such publications as Vitamins and Hormones.

It is to be noted in passing that it is perhaps conceivable that the various terpenoid intermediates known to be involved in steroid biosynthesis may prove to be of some practical application in cases of steroid deficiency. In this connection it has been shown that administration of squalene maintained lactation in rats which otherwise lactated poorly on a squalene-free diet (55).

Many naturally-occurring compounds of biological interest are known which are elaborated by a mixed biogenesis where one portion of the molecule arises via the isoprenoid route while the remainder of the molecule is formed via linear polyacetate route or shikimic acid route. In some cases a complete terpenoid unit is involved but in the other cases single isoprenoid units are utilized. A case of incorporation of a monoterpenoid is afforded by the isomeric tetrahydrocannabinols which are the active principles of cannabis (marihuana),

the structure of one of which is shown in XV. It has



XV

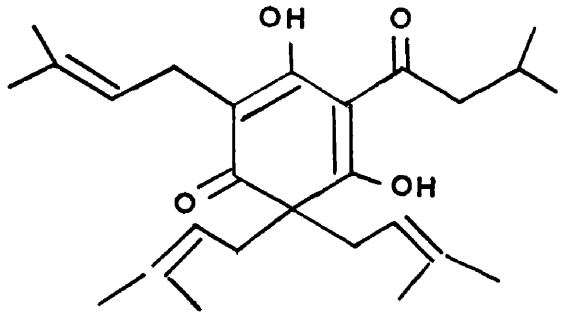
been postulated (56) that these compounds are formed by the condensation of a menthatriene with olivetol. Analogous reactions employing pulegone have been successfully performed under laboratory conditions (57).

Despite intensive research (58) the action of cannabis on the central nervous system in man is still not well understood. Several reviews dealing with cannabis addiction in the United States of America have been published (59) and leading references to the investigations of the biological properties of cannabis are given by Sollmann (60) and Goodman and Gilman (61). A considerable number of synthetic tetrahydrocannabinol-like compounds have been prepared for pharmacological study (62).

The vitamins K and E represent examples of mixed biogenesis incorporating diterpenoid units, and fumagillin can probably be regarded as the product of mixed biogenesis involving a sesquiterpenoid

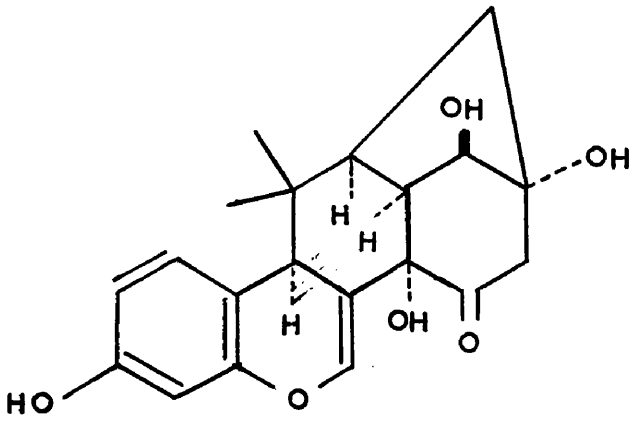
unit. These substances will be discussed more fully later in this review.

Examples of natural products in which seperate isoprene units have been incorporated are afforded by humulone and lupulone (XVI), which have been shown to be



XVI

antibacterial agents(63). Recently lysergic acid has been demonstrated to arise in nature by condensation of moieties derived from tryptophan and mevalonic acid (64), and a mixed biogenesis for the plant oestrogen miroestrol (XVII)



XVII

has been proposed in which an isoprene unit and

an isoflavenoid moiety are involved (65). Numerous other examples could be given (66) but it is not within the scope of this review to discuss these compounds further.

In reviewing such a broad field as the biological activities of the terpenoids, any treatment which is to be concise must necessarily be highly selective. No attempt has been made to cover the literature pertaining to galenical preparations which are either known or suspected to contain terpenoids, although isolated examples will be given. The coverage afforded to pure compounds is also far from exhaustive and many contributions to the literature must go unacknowledged. It is hoped, however, that the present review will serve to provide a perspective and that the references cited will serve as adequate keys to the voluminous literature.

MONOTERPENOIDS

Essential Oils

The majority of monoterpenoids are obtained from the essential oils of plants where they often occur in admixture with volatile compounds of other chemical groups (67). As a large number of investigations have been carried out with essential oils rather than

with their pure components, it is necessary to devote some attention to the biological properties of essential oils.

In nature the essential oils appear to play a considerable role in the economy of the vegetable kingdom. Their pronounced attraction for certain insects favours pollination whilst their repellant action towards other insects may serve to protect the plant against otherwise harmful species. Most essential oils and their constituent monoterpenoids are characterised by their agreeable odours and their palatable flavours and so monoterpenoids are of importance in the perfumery and cosmetic industries and are employed in pharmacy as flavouring agents to mask the disagreeable taste of certain medicines. Although odour and the closely related phenomenon of taste are properly regarded as manifestations of biological activity, it is not proposed to consider the use of the terpenoids as flavouring agents and perfumes in this review. A number of key references to the odoriferous properties of terpenoids and their derivatives are to be found in various reviews (68) and in the monograph published by the Society of Chemical Industry (69). The compositions of various

cosmetics and perfumes containing monoterpenoids are given in the book by Poucher (70).

It is to be noted, however, that substances possessing strong odours are able to produce reflex medullary stimulation resulting in increased respiration and a transient rise in blood pressure and so various essential oils have on occasion been used to revive the patient in cases of fainting, though more pungent substances such as ammonia are of greater value in this respect. Certain mixtures containing monoterpenoids have also been used as sedatives in cases of hysteria, their effect presumably being mediated by olfactory and psychic reflexes. One such preparation is valerian prepared from the dried roots and rhizomes of Valeriana officinalis and which contain bornyl valerate as well as other terpenoid derivatives.

At one time the essential oils saw considerable application in medicine, the Dispensatorium Valeri Cordi of 1592, listing some 60 different oils, as was pointed out by de Mayo (71). In recent times, however, the essential oils and monoterpenoids have become less and less important medicinally and a recent text book on therapy (72) gives no reference at all to essential oils as therapeutic agents and only one or two minor

references to monoterpenoids as constituents of lotions. Their main application today is as carminatives.

In general the essential oils and their constituent monoterpenoids are structurally non-specific agents acting on virtually all organs of the body and on a variety of biological systems. Thus when Eadie et al. (73) were seeking a drug conforming to Ferguson's principle with which to destroy infusoria in the sheep's rumen they turned to various monoterpenoids. Quite often the pharmacological action shown by monoterpenoids and essential oils is characterized by stimulation in low dosages and depression at higher dosages. For instance various monoterpenoids and essential oils have been shown to both stimulate and depress the gastro-intestinal tract (74). Similarly, although the principal central effects are reflex, in large enough doses the essential oils may exert a direct action on the central nervous system producing a depression which in some cases is preceded by stimulation.

Other actions shown by essential oils are at the neuromuscular junction where they produce a paralysis of the muscle (75), and on the heart, where there is again a stimulation and depression. Further

evidence of the non-specific nature of their actions is afforded by their anti-inflammatory properties (76), their haemolytic action (77), their ability to produce methaemoglobin (78) and their ability to inhibit serum cholinesterase (79). Recently work has been published which indicates that various essential oils may have the ability to stimulate the cellular defence mechanism of guinea pigs as evidenced by their beneficial influence on the outcome of experimental tuberculosis (80) and by the India ink and Trypan blue tests for phagocytosis (81).

Perhaps the most characteristic property of many essential oils is their irritant activity and to this may be traced many of their former clinical applications. For example the use of essential oils in stomachic mixture was due to the feeling of warmth produced on irritation of the mucous membranes of the mouth and digestive tract and to the increased salivation. Similarly the former use of such essential oils as oil of juniper as diuretics depended upon the irritation produced in the kidneys. It is of some interest that the active principle of oil of juniper has recently been identified as 4-terpineol (82). With some oils, e.g. oil of pennyroyal and oil of savin, the irritation of the kidneys and bladder can be so great as to set up reflex

uterine contractions and so these oils have on occasion been used as emmanogogues and abortifacients. There is, however, no direct action on the uterus (83).

Externally the essential oils can be used as counter-irritants and rubefacients in the form of embrocations and liniments. They produce an initial feeling of warmth and smarting which is often followed by a mild local anaesthesia. Such preparations have been used to relieve rheumatic pain, neuralgia and pleurisy and in the treatment of the common cold and bronchitis. External application may produce dermatitis especially in hypersensitive individuals (84). Oxidation products formed from various olefinic constituents appear to be the causative agents (85).

The use of certain essential oils in the relief of the symptoms of acute bronchitis stemmed from their mild expectorant action (86) resulting from irritation of the bronchial glands.

The essential oils and the monoterpenoids are mild antibacterial (87) and antifungal (88) agents and in nature these properties probably protect the plant against noxious bacteria and fungi. The utilization of their antiseptic potency by man is, however, limited by

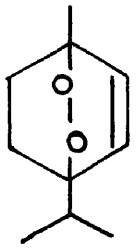
their sparing solubility in water although various techniques can be employed to increase their solubility (89) A claim has been made that the protisticidal potency of essential oils is related to their dehydrogenase arresting properties (90).

Several essential oils have seen use as urinary antiseptics (91) but they have long been regarded as being of little real clinical value (92) and are never used today with the availability of sulphonamides and antibiotics. The use of essential oils such as oil of eucalyptus in the treatment of pulmonary infections is likewise of no value. The mild antiseptic properties of monoterpenoids are perhaps of value in toothpastes where their rubefacient action on the gums is also of benefit. Reviews have been published on the antiseptic properties of essential oils (93) and there is also a review of the pine-oil disinfectants (94).

It has been reported that ultraviolet (95) and X-ray (96) irradiation of essential oils increase the anti-bacterial activity and it will be interesting to learn the exact nature of the chemical changes involved and whether or not terpenoids are implicated.

Oil of chenopodium formerly saw specialised use :

as an anthelmintic against hookworm (97) in both medical and veterinary practice. In this case the activity is due to the presence of one particular compound, ascaridole (XVIII). Unfortunately ascaridole is very toxic to the



XVIII

host as well as to Ascaris spp and its use in human medicine has been strongly opposed for a number of years (98). Attempts have been made to overcome its toxicity to the host by subsequent administration of physiological detoxifying agents such as ascorbic acid and glucuronic acid (99).

A detailed description of the pharmacological and toxicological properties of different essential oils are to be found in various text-books (100,101), and an account of their former use in the treatment of wounds has been published (102). The biological studies of essential oils have not been confined to animals. Their action on plants has also been studied (103).

Table I lists several essential oils which have been used therapeutically. The examples

TABLE I

ESSENTIAL OILS USED MEDICINALLY

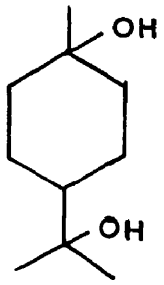
Oil	Source	Monoterpenoids present	Sesquiterpenoids present	Other Compounds	Application
Dill Oil	Ripe fruits of *Anethum graveolens*	Carvone (+) α-Phellandrene Limonene	-	Dillapoil	Carminative. In gripe water
Aniseed Oil	Ripe fruits of *Pimpinella anisum*	-	-	p-Cresol Isoanethole Anethole Homoguaiacol Hydroquinone Anisalcohol Anisaldehyde Anisyl ketone p-Methoxybenzoic acid Acetaldehyde	Carminative as anise water
Chinese Star Anise Oil	Ripe fruits of *Illicium verum*	p-Cymene (-)α-Phellandrene (+)β-Phellandrene (-)Limonene 3-Dipentene 3-Carene (+)α-Pinene (+)α-Terpineol 1:8-Cineole	Bisabolene Cadinene	Isoanethole Anethole Foeniculin Hydroquinone monomethyl ether Anisaldehyde Anisylketone Decanoic acid Tiglic acid Benzoic acid p-Methoxybenzoic acid 3,4-Dihydroxybenzoic acid	Carminative.
Oil of Cloves	Flower buds of *Eugenia caryophyllata*	-	Caryophyllene Humulene Caryophyllene oxide	2-Heptanol 2-Nonanol Eugenol Benzyl alcohol Vanillin Methyl amyl ketone Methyl heptyl ketone Diacetyl Benzoic esters α-Furfuryl alcohol Furfural 5-Methylfurfural 4,5-Dimethylfuran-3-aldehyde	Antispasmodic Carminative Antiseptic and relief of toothache
Nutmeg Oil	Seed kernels of *Myristica fragrans*	p-Cymene Dipentene (+)α-Pinene (+) Camphene (+) Linalool p-Menth-1-en-4-ol (+) Borneol	-	Isoeugenol Myristicin Octanoic acid Decanoic acid Dodecanoic acid Tetradecanoic acid	Carminative
Cinnamon Oil	Bark of *Cinnamonum zeylanicum*	p-Cymene (-)β-Phellandrene (-)α-Pinene (-) Linalool Cuminaldehyde	-	Eugenol Cinnamic alcohol n Nonanol Phenylpropionaldehyde Cinnamic aldehyde Methyl amyl ketone Benzoic acid Cinnamic acid Furfural	Carminative, also in treatment of catarrh and influenza
Sandalwood Oil	The wood of *Santalum album*	Santene Teresantalol Santenone Teresantalic acid	Santalene Santalol	Isovaleric aldehyde	Urinary disinfectant
Oil of Cajuput	Leaves and twigs of *Melaleuca leucadendron*	Dipentene (+)α-Pinene (±)α-Terpineol 1,8-Cineole	-	Eugenol methyl ether Butyraldehyde Valeraldehyde Benzaldehyde Propionic acid Isovaleric acid	Carminative. Also applied externally in relief of rheumatism.
Peppermint Oil	Flowering tops of *Mentha piperita*	(+)α-Phellandrene (-)Limonene (-)α-Pinene (-)Menthol (+)Neomenthol (-)Menthone (+)Isomenthone (+)Piperitone Piperitenone Isopiperitenone 1,8 Cineole Peperinic acid Menthofuran	Cadinene Caryophyllene	Isoamyl alcohol (+)Octanol *cis* Hex-3-en-1-ol Acetaldehyde Isovaleric aldehyde Jasmone Isovaleric acid Hex-2-en-1-oic acid Phenylacetic acid Dimethyl sulphide	Carminative.

have been selected at random to illustrate the great variation in their constituents (104) and to show the diverse sources from which they are obtained. Included in the table to emphasize the structurally non-specific nature of the monoterpenoids is aniseed oil which, although containing no monoterpenoids has been used interchangeably with Chinese star anise oil. It is to be emphasized that not all the components shown in the table were necessarily isolated from the same specimen of oil. It is well established that the nature and relative quantities of the constituents of essential oils vary with geographic location and climatic conditions (105). Also portions of the plant other than those used to produce the medicinal oil often elaborate essential oils of quite different nature.

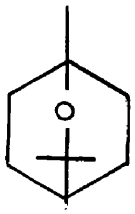
In addition to the oils listed in Table I countless others have been employed medicinally both in western medicine and the folk medicine of primitive peoples. Among these are Roman chamomile oil, fennel oil, cardamom oil, caraway oil, coriander oil, eucalyptus oil, oil of bay, oil of pimenta, oil of cubebs, oil of cascarilla, oil of lemon grass and oil of juniper.

Because of the non-specific nature of most of

the monoterpenoids many of the pure compounds have little biological interest apart from their ability to replace essential oils in the therapeutic applications already mentioned for the latter. The most commonly encountered are terpin hydrate (XIX), 1,8-cineole (syn eucalyptol (XX) which are occasionally employed in expectorant

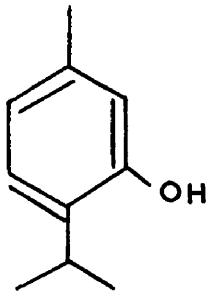


XIX

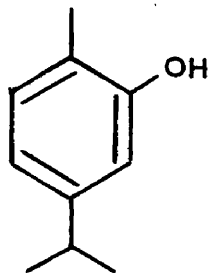


XX

mixtures; thymol (XXI) and carvacrol (XXII) which are



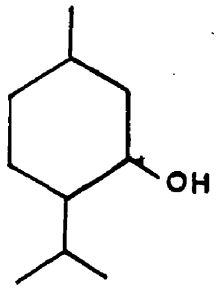
XXI



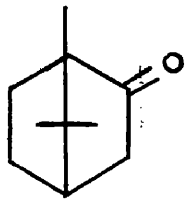
XXII

used as antiseptics; menthol (XXIII) which is sometimes used as a mild local anaesthetic and as an acongestant in nasal drops; and camphor (XXIV) which is still employed to some extent as a counter-irritant. It is

to be noted that thymol and carvacrol, in addition to



XXIII



XXIV

fulfilling the criteria for classification as monoterpenoids, are also phenols and so represent examples of the large class of phenolic disinfectants. Thymol at one time saw some use as an anthelmintic (106).

Camphor

In surveying the biological properties of the various monoterpenoids it is necessary to devote considerable space to camphor for this compound has been more intensively investigated than any single monoterpenoid, there being well over 400 publications concerned with its biological properties. Well documentated summaries of much of this work are available (107).

A considerable portion of the biological experiments conducted with camphor would appear to have been inspired by the desire to provide a rational basis for its former extensive use in clinical practice. Camphor had been introduced into Western medicine in mediaeval

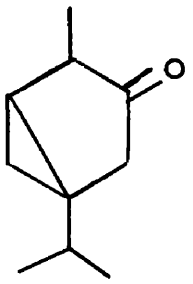
times after a long history of use in ancient Chinese remedies, and was at one time, without definite proof, thought to possess beneficial cardiovascular and respiratory stimulant properties. The results of the numerous animal experiments with camphor, although often conflicting, have however indicated that such properties, if they do indeed exist, are so unpronounced as to render the compound of little value as a therapeutic agent. It is now generally agreed that any observed clinical improvements produced by camphor were brought about as the result of reflex actions caused by the painful local irritation produced by the injection of the agent (108).

Besides its clinical use as a "stimulant" camphor also saw former medical application as a disinfectant of burns and wounds and was at one time used in the treatment of pulmonary tuberculosis and pneumococcal pneumonia, although again it would appear to have been of little value. Similarly animal experiments failed to support the contentions that camphor possessed antipyretic properties (109), or that it would prevent post-partum engorgement of the breast (110). The claim by Kawasaki (111) that camphor decreased the temperature of normal dogs only when given access to the liver and pancreas might indicate that a biotransformation product of camphor possesses antipyretic properties.

A survey of the literature pertaining to the biological properties of camphor is useful as it represents an almost complete course in pharmacology in miniature. Thus the investigations with camphor have clearly shown how the effect produced can vary with the physical state of the drug (112), the dose (113), the route of administration (114), the temperature (115) and the dietary history (116), age (117) and the state of health (118) of the experimental animal. The studies have also provided examples of tolerance (119), and idiosyncrasy (120), and species (121), racial (122) and individual (123) variations.

The biological actions of camphor have been studied in a wide range of species among the more unusual of which are the squid (124), the pike (125), the tench (126), the earthworm (127), the predaceous diving beetle (Dytiscus marginalis (128), the leech (129), the tortoise (130) and the crab (131).

Camphor possesses the irritant properties typical of so many monoterpenoids. The irritation which it produces on the cerebral cortex leads to central excitation (132). Several other monoterpenoid ketones (133) including thujone (XXV) (134), which is thought to be responsible for the convulsions sometimes seen in chronic absinthe



XXV

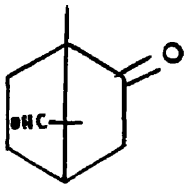
drinkers, also possess central nervous system stimulant properties but they are all too unreliable to be useful as clinical analeptics.

The studies on camphor have covered virtually every conceivable pharmacological action, but the major portion of the work dealt with its effects on the blood pressure, the heart and the central nervous system.

The effect of camphor on the blood pressure has been the subject of many conflicting reports, but resolution of the differences may be possible when its effects at several sites of action are taken into account. For instance, camphor is known to dilate certain blood vessels (135,136) and to procure the release of adrenaline (137) so that any observed change in blood pressure must be a summation of these effects together with those due to its actions, both reflex and direct, on the central nervous system.

Much of the conflicting evidence concerning the effect of camphor on the heart (138) may be explained

by the claim that d-trans π-oxocamphor (XXVI), a



XXVI

biotransformation product of camphor, stimulates the heart whilst camphor itself depresses it (139), although this contention has been denied (140).

Camphor has been shown to lower blood viscosity (141), to affect erythrocytes (142) and to act as an anti -haemorrhagic agent (143). It has also been shown to depress the isolated intestine (144), an action which has been classed as parasympathicolytic (145), and to produce an initial excitation followed by a depression of skeletal muscle (146). When applied to the rabbit eye camphor produces an initial mydriasis which is followed by miosis (147). Like picrotoxin camphor has been claimed to check insulin convulsions (148). Camphor has been claimed to possess weak androsterone-like activity as have borneol, menthol and menthone (149). Other studies include the effect of camphor on antitoxin production (150), on cilial movements (151) and on the secretion of gonadotropic hormones (152).

Camphor has seen considerable investigation as a potential bactericide (153) and insecticide (154), its former use as a moth-proofing agent being well-known. Another interesting application has been its incorpora-tion into poison dusts and sprays as a bee repellent, to minimise the destruction of these insects (155). It is claimed that camphorated oil increases the *in vitro* potency of the antibiotics streptomycin and the tetracyclines (156) and it has also been claimed that camphor promotes phagocytic activity (157).

A further property of camphor is its ability to produce polyploid forms in various microorganisms (158). It is also reported to induce the formation of structures resembling copulation tubes in yeasts, although nuclear fusion does not occur (159). It is noteworthy that other monoterpenoids, containing a carbonyl function, such as citral, citronellal and hydroxycitronellal have been shown to induce or select for mutations in *Drosophila melanogaster* (160).

Considerable attention has been paid to the biochemical effects of camphor. Thus its effect on amino acid excretion (161), phosphorus turnover (162), glycolysis (163) and acetylcholine metabolism (164) have been

determined as has its effect on the distribution of potassium and calcium ions in the central nervous system (165). Other studies have been concerned with its influence on the carbohydrate metabolism of the heart (166), on Vitamin C. metabolism (167), on the ammonia content of the central nervous system (168), and with its effects on the enzymes acetylcholinesterase (169), cytochrome oxidase (170) and oxolacetic and pyruvic carboxylases (171).

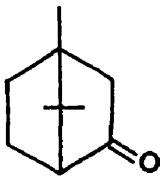
On the pharmaceutical side camphor has inspired studies on new solvent combinations on account of its relatively low solubility in water (172) and there is an extensive literature devoted to assay procedures for this compound (173).

Numerous detailed toxicological studies with camphor are on record (174) as are many detailed biotransforma -tion studies (175).

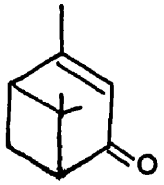
Camphor has also been utilized in admixture with other agents (176) and its ability to antagonise or support the action of various drugs has been well investigated (177).

Considerable attention has been paid to structure action investigations and numerous comparative studies

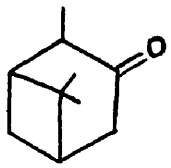
between camphor and closely related compounds have been reported. To cite a few specific instances the pharmacological actions of camphor have been compared with those of epicamphor (XXVII) (178), d-verbenone (XXVIII) (179), l-pinocamphone (XXIX) (179), fenchone (XXX) (180), thujone (181), menthol (182) and ketocineole (183). Other compounds which have seen



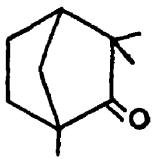
XXVII



XXVIII



XXIX



XXX

detailed comparison with camphor include 3-methyl-5-isopropylcyclohex-2-en-1-one (hexetone) (184), methylsantenone (185) and 6-oxofenchone (186). The pharmacology of various biotransformation products of camphor have also seen attention (187).

From the preceding account it is readily apparent that camphor is a structurally non-specific drug, and it is interesting to note that it will intensify urethane narcosis (188) and that its dose-response curve on the toad heart is very similar to that of ether (189).

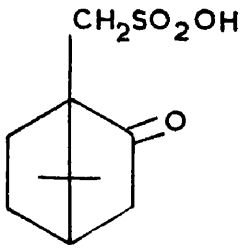
As was mentioned earlier, the optical antipodes of structurally non-specific drugs should show identical biological properties. In the case of camphor the evidence on this point is conflicting. Several workers (190) have claimed that the d and l isomers are indistinguishable pharmacologically and Joachimoglu (191) in careful studies employing several pharmacological preparations concluded that the d, l and dl isomers showed identical activity. Other workers (192), however, contended that the l form is more active than d form, whilst a third group (193) maintain that d-camphor is the more active pharmacologically. Toxicity studies have tended to show that the l isomer is more toxic than the d isomer (194). As the mammalian organism is known to effect biotransformation and elimination of optical antipodes at different rates (195), these results may merely reflect the length of time to which the animal is exposed to the particular enantiomorph or to its toxic transformation products rather than any difference in potency between the two optical isomers. In this connection it is to be noted that the urine of animals fed dl camphor has been reported to be richer in laevorotatory "campherol" (196), an observation which

was interpreted to mean that l camphor is less readily degraded in the body than the d isomer. As this work was conducted before the demonstration that "campherol" is a mixture, the subject would bear detailed re-investigation.

Derivatives of Camphor

Various synthetic derivatives of camphor have been prepared, both in attempts to improve its solubility characteristics and for the study of structure-action relationships. The extravagant claims sometimes accompanying the initial introduction of these agents followed by the inevitable re-evaluation of their worth, is unhappily even at the present day an all too familiar phenomenon linked with the marketing of new drugs.

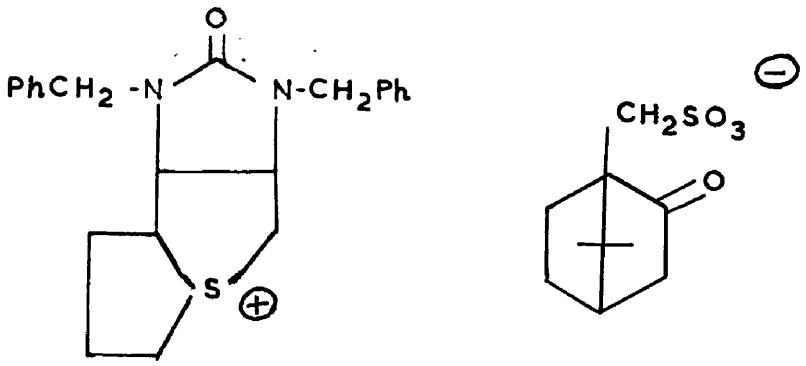
Among the derivatives prepared and tested were camphor-10-sulphonic acid (XXXI) and its derivatives.



XXXI

The biological properties of camphor-10-sulphonic acid have been well reviewed elsewhere (197).

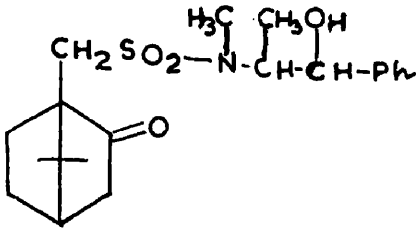
As the 10-camphorsulphonate anion is virtually devoid of any significant pharmacological activity, it has seen considerable utilization as a solubilising anion (198). Among the many salts which have been subjected to biological screening may be cited the 10-camphorsulphonates of p-hydroxyphenylaminoethanol (199), ephedrine (200,201), emetine (202), sparteine (203), quinine (204), antipyrine (205), pyrimadone (205), theophylline (206), dihydroxy -codeine (201), scopolamine (201), sulphanilamide (207) tetraethylammonium (208) and diethyl- (hydroxyethyl)- methylammonium (209). More recently a potent ganglion-blocking agent, trimetaphan camphorsulphonate (XXXII), has been introduced into clinical practice (210), and



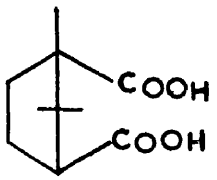
XXXII

N-(2′-hydroxy-1′-methyl-2′-phenylethyl), N-methyl-camphorsulphonamide (XXXIII) is reported to be a potent analeptic (211).

Camphoric acid (XXXIV) which is formed by oxidation of camphor has been particularly well investigated



XXXIII



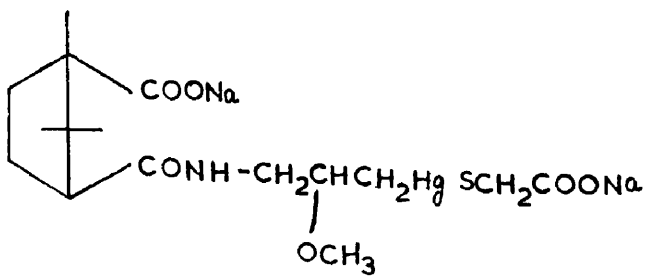
XXXIV

pharmacologically (212). It was at one time used against the night sweats of phthisis (213) but closer experimental investigation showed that it was of little value (214). Like isofenchonic acid, camphoric acid is said to stimulate the growth of haemolytic Streptococci in low concentrations (215).

Many derivatives of camphoric acid have been prepared and tested including various substituted camphorimides (216). Several substituted 4-chlorophenyl hydrogen-camphorates are claimed to be of value in the treatment of athlete's foot (217), and the salt formed between camphoric acid and hexamethylene: tetramine has been used as an alternative to hexamethylenetetramine mandelate in combatting urinary infections (218). Hexamethylenetetramine camphorate is also reported to be effective in the treatment of hard-pad in dogs (219).

The sodium salt of the monoester formed between p-tolylmethylcarbinol and camphoric acid is reported to be a cholagogue (220) and recently there has been considerable interest in the diethanolamine salt of the same monoester because of experiments showing that it inhibits serum and tissue cholesterol elevation in cholesterol-fed cockerels (221). It also hastens the regression of atherosclerotic lesions in birds reverting to a normal diet. In rats, however, the compound increases the plasma cholesterol level (222).

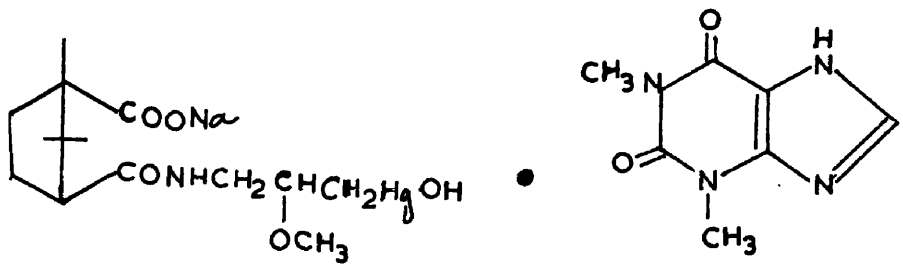
The derivatives of camphoric acid which are probably of the greatest interest are those in which the camphoric acid part of the molecule can be regarded as a supporting moiety, as for example in the bismuth therapy of syphilis. The mercurial diuretics, mercaptomerin (XXXV) and mercurophylline (XXXVI) are two other examples of the utilization of camphoric acid as a supporting moiety (223), and it is claimed that the positional



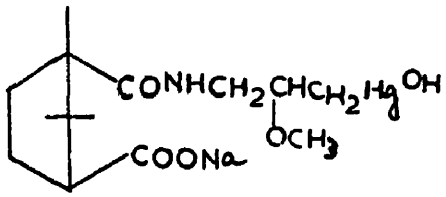
XXXV

isomer, (XXXVII) has greater diuretic potency than

mercurophylline (224). In these diuretics a definite



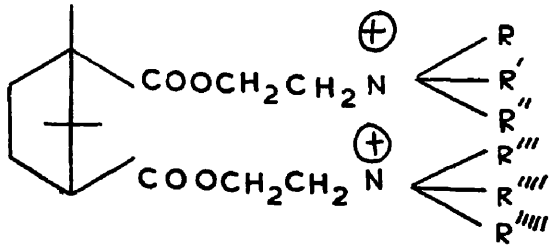
XXXVI



XXXVII

function can be assigned to the organic portion of the molecule in that it ensures slow production of ionic mercury which then inactivates the enzymes responsible for the tubular reabsorption of chloride ions by combination with the sulphydryl groups.

Compounds of type XXXVIII which can be regarded as analogues of succinylcholine, formed by replacement of

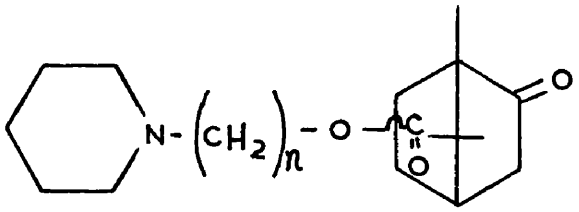


XXXVIII

the succinic acid moiety by camphoric acid, have been shown to be active as neuromuscular blocking agents (225). Other interesting camphoric acid derivatives are camphorylsulphathiazole which is said to be active *in vitro* against a strain of *Mycobacterium tuberculosis* (226), and the C-21 hemicamphorate of cortisol which was prepared in search for esters of cortisol possessing greater clinical potentiality than cortisol itself (227).

In the course of an investigation of the piperidine group as an active antitussive moiety, Kase and Yuizono (228) utilized esters of camphor-trans-π-carboxylic acid (*syn* isoketopinic acid) as the supporting moiety to give compounds of type XXXIX. Indeed these compounds turned out to be active antitussives. Similar derivatives

possessing a morpholine ring or a piperazine ring in place of the piperidine ring were investigated by Yoshimoto (229) as potential hypotensive agents. The



XXXIX

derived methiodides were studied as well.

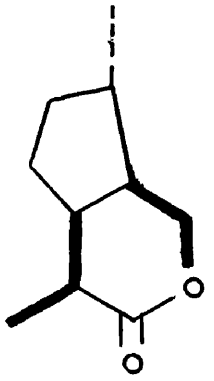
Camphor itself has been utilized as a supporting moiety in the form of its isonicotinyl hydrazone, which was prepared as a potential anti-tuberculous agent (230).

Of the other derivatives of camphor investigated for biological activity it will suffice to mention the hydroxycamphors (231), camphor oxime (232,233), isonitrosocamphor (233), camphorquinone (234), camphor-3-carboxylic acid (235), basic bismuth camphocarboxylate (236), and 3-carbamoyloxy-2-hydroxy -camphane which was recently been shown to possess potent anticonvulsant activity (237).

Biologically-active monoterpenoids from insects

As yet this group is relatively small, but it is nevertheless of considerable interest.

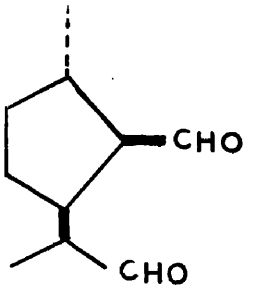
Various species of ants are reported to use odoriferous secretions in attack and defence against other insects and in the laying of odour trails from food to the nest, and an interesting monoterpenoid, iridomyrmecin (XL) possessing antibacterial and



XL

insecticidal activity (238) has been isolated from the workers of the Argentine ant (Iridomyrmex humilis) in which it occurs to the extent of 1% of the body weight. The pharmacology of this compound has been investigated by Pavan (239) and it would appear to be without significant activity. Two other compounds, iridodial (XLI) and isoiridomyrmecin (syn iridolactone) which are closely related chemically to iridomyrmecin have been

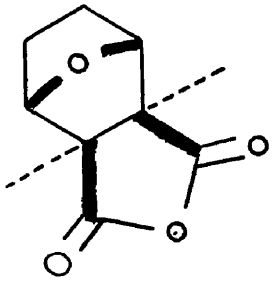
isolated from other species of ant (240), and an



XLI

unidentified compound which may be closely akin chemically occurs in fire ant venom (241).

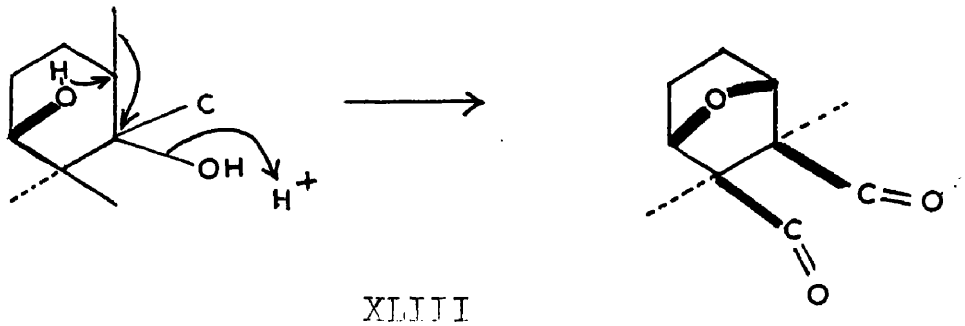
Another active principle which is obtained from insects, and which can probably be regarded as a monoterpenoid is cantharidin (XLII) the vesicant principle occurring in various beetles. At first sight this



XLII

optically-inactive compound would appear to result from the tail-to-tail coupling of two isoprene units, but, theoretically at least an alternative biosynthetic pathway exists by which

it could arise through rearrangement of a normal monoterpenoid skeleton of the β-cyclocitral type bearing suitable substituents as in XLIII. Like



XLIII

iridomyrmecin cantharidin exhibits insecticidal properties (242) towards some insects. Towards others, it exhibits a pronounced attraction. Its action on plants is also reported to parallel that of iridomyrmecin (243).

Cantharidin possesses the irritant properties so characterstic of monoterpenoids in general, but to a very much more pronounced degree. Thus it causes inflammation (244) and inhibits the growth of fibroblasts in tissue culture (245) in a similar way to turpentine. It has been shown to produce vessication in the skin of corpses (246). Cantharidin has in fact been termed a "cell poison" (247).

At one time cantharidin was used medicinally to produce prolonged counterirritation in the treatment of neuralgic and rheumatic pains, but this use was dangerous because of the risk of toxic effects arising from absorption of the drug. Cantharidin has a marked toxicity for epithelial tissue and the kidneys are especially sensitive to it (248). Small doses produce dilation of of the glomeruli with albuminuria (249), and larger doses affect the epithelium of the convoluted tubules producing severe nephritis.

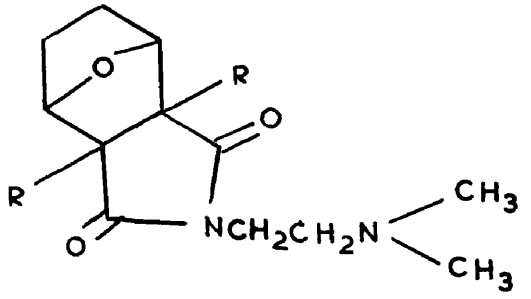
There are reports in the literature that cantharidin can successfully inhibit tumour induction by carcinogenic tar (250). This action may well be a result of its toxicity to the epithelial cells with consequent sloughing of the carcinogen and embryonic tumour. There is also a report of a successful destruction of a cancer in man (251).

Cantharidin has been used as an aphrodisiac in cattle breeding, as it produces a reflex erection through its irritation of the urethral mucous membrane (252), but its use is not without risk as effective doses may produce nephritis. It is of some interest that various essential oils and monoterpenoids have quantitatively

similar but less intense action.

Claims have been advanced that cantharidin will initiate oestrus in castrated female rats and infantile mice (253) and produce an increase in the urinary excretion of oestrogens and progesterone in female rabbits (254), but the results of other workers (255) indicate that cantharidin is without oestrogenic action. Detailed pharmacological studies have been performed on cantharidin (256) and these have shown remarkable species differences in sensitivity to the drug (257).

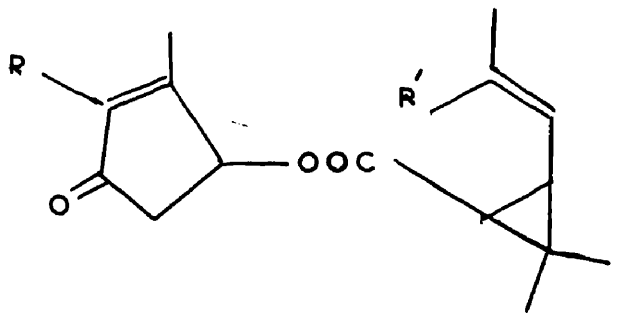
Various analogues and derivatives of cantharidin have been prepared synthetically. Thus, 1,2-dimethyl-1, 2-dihydrophthalic anhydride is reported to have cantharidin-like activity (258) whilst various heavy metal salts of the condensation product of cantharidin and ethylene diamine are claimed to possess bactericidal properties (259). More recently compounds of type XLIV have been claimed to be anti-hypertensive agents (260).



XLIV

The Pyrethrins

Various naturally-occurring monoterpenoids or their derivatives possess insecticidal properties (261) and of these the most potent are the pyrethrins I and II and the cinerins I and II which occur in the flower heads of Chrysanthemum cineriifolium. These four compounds, which are conveniently represented by the general formula XLV, are not always classified as monoterpenoid derivatives but they can be so regarded because of the



Pyrethrin I : R = $-CH_2CH=CHCH=CH_2$; R' = $-CH_3$

Pyrethrin II : R = $-CH_2CH=CHCH=CH_2$; R' = $-COOCH_3$

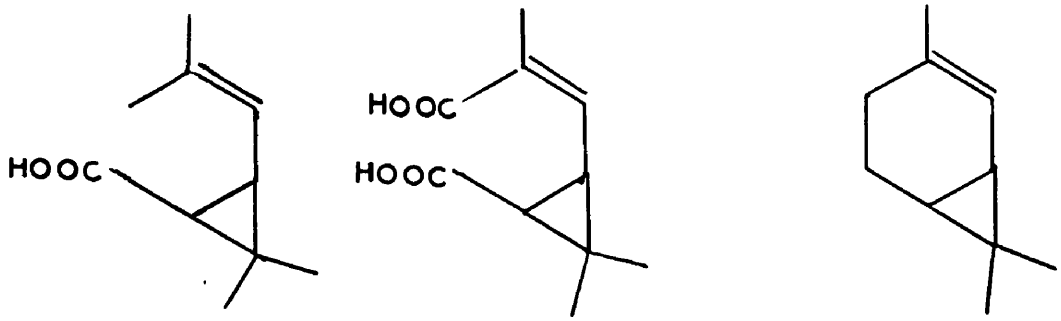
Cinerin I : R = $-CH_2CH=CHCH_3$; R' = CH_3

Cinerin II : R = $-CH_2CH=CHCH_3$; R' = $COOCH_3$

XLV

obvious formal relationship of chrysanthemic acid (XLVI) and chrysanthemum dicarboxylic acid (XLVII) to car-4-ene (XLVIII) which is a true monoterpenoid.

Indeed it is tempting to think that chrysanthemum

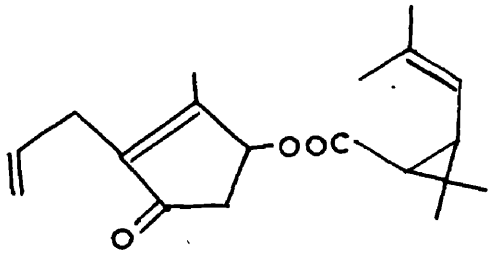


XLVI XLVII XLVIII

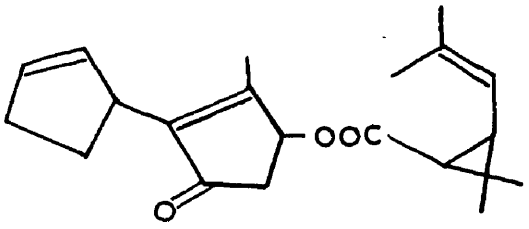
dicarboxylic acid arises by oxidative cleavage of the 2,3 double bond in 2,4-caradiene with subsequent epimerisation at original carbon atom 1 to give the trans relationship of the substituents on the cyclopropane ring.

The pyrethrins and cinerins find wide application in fly sprays and as horticultural insecticides on account of their low toxicity to warm-blooded animals and their ability to achieve quick "knockdown", a term used to describe the rapidity with which the insects are paralysed. In addition to their insecticidal properties they also possess anthelmintic activity (262). The early work on the physiological action of the pyrethrins is described in the book by Gaudin (263) and their toxicology and pharmacology are discussed by Feinstein and Jacobsen (264) who also refer to several synthetic

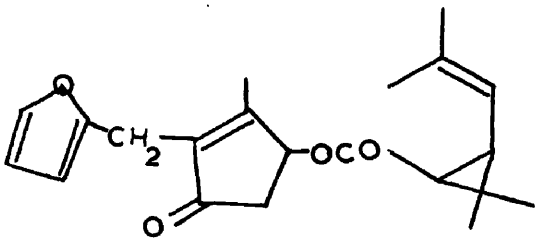
rethrins. Examples of such synthetic rethrins which are of commercial importance are allethrin (XLIX) (265) which is the allyl homologue of cinerin I, cyclethrin (L) (266) and furethrin (LI) (267). Besides these commercially



XLIX



L



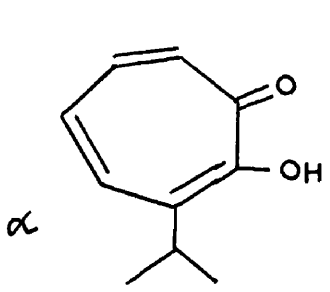
LI

important synthetic compounds many others have been prepared (268), among which are several terpenoid esters of chrysanthemic acid (269).

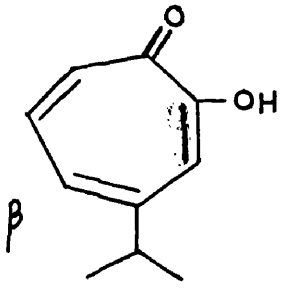
The precise mechanism of action of the rethrins on insects is still obscure, and they are usually vaguely described as neuromuscular or nerve poisons producing degenerative changes in the cells of the nerve ganglia. Structure action relationships in the rethrin series are discussed by Sexton (270) and in greater detail in the excellent review by Elliot (271).

Thujaplicins

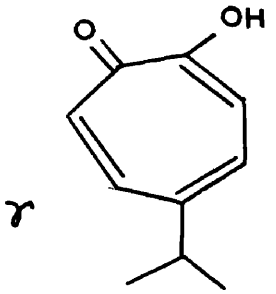
The resistance of certain heartwoods to attack by fungi and insects is often at least partly due to the presence of toxic compounds. One such group are the α-, β- and γ- thujaplicins (LII-LIV), which - although not obeying isoprene rule - are mentioned in this survey



LII



LIII



LIV

because they are generally considered to arise in nature from terpenoid precursors (272). Erdtman (273) has proposed a detailed scheme whereby a monoterpenoid precursor undergoes ring expansion to generate the tropolone ring of these compounds.

The thujaplicins have been shown to possess strong antifungal activity (274) and beta-thujaplicin, also known as hinokitiol, has been shown to have potent antibacterial properties (275), in some cases exhibiting a higher potency than the common antibiotics (276). The interesting claim has been made that bacteria

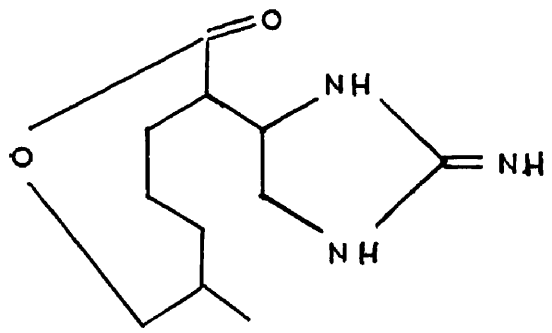
do not readily acquire resistance to hinokitiol (277). In the form of its sodium salt, hinokitiol shows leptospiricidal action (278).

The pharmacological properties of β-thujaplicin (279) and γ-thujaplicin (280) have seen detailed investigation. These compounds would appear to produce mixed depression and stimulation of the central nervous system and so have been compared to camphor with respect to their biological properties. The sodium salt of β-thujaplicin shows pronounced hyperglycaemic and diuretic properties, but these actions are accompanied by considerable tissue damage (281). The effects of hinokitiol on respiratory enzymes have been studied in detail (282), and gamma-thujaplicin has been reported to possess tumour-damaging properties (283).

Synthetic derivatives prepared from hinokitiol have been subjected to biological studies. These include certain derivatives of hinokitiol-7-sulphonic acid which have been shown to stimulate the guinea pig uterus (284). The thujaplicins can be readily converted into azulenes, azaazulenes and oxaazulenes (285) and several such compounds have been claimed to suppress plant growth (286) or to be of value in the treatment of infant asthma (287).

Amino Derivatives of Monoterpenes

Relatively few monoterpenoid alkaloids appear to exist in nature. Apart from actinidine (288) (see p. 72) only one member of the group appears to have seen biological investigation and this is chaksine which occurs in the seed kernels of Cassia absus, Linn., long used in India in the treatment of skin infections and as a cathartic, and whose structure has recently been established as LV (289).

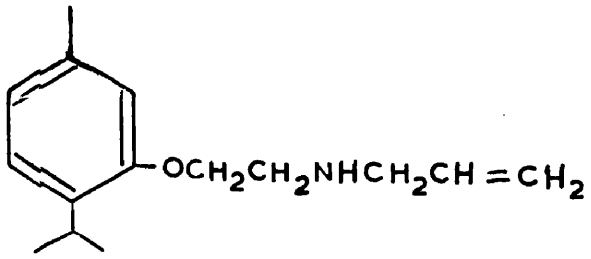


LV

Chaksine has been the subject of several pharmacological investigations but the results do not appear to be completely concordant. One group employing chaksine iodide claim that this salt produces a fall in blood pressure by capillary dilation, stimulates smooth muscle and exhibits atropinic and weak curariform properties (290). Other workers employing the sulphate or the free base state that the alkaloid is a general depressant of the central nervous system and of smooth

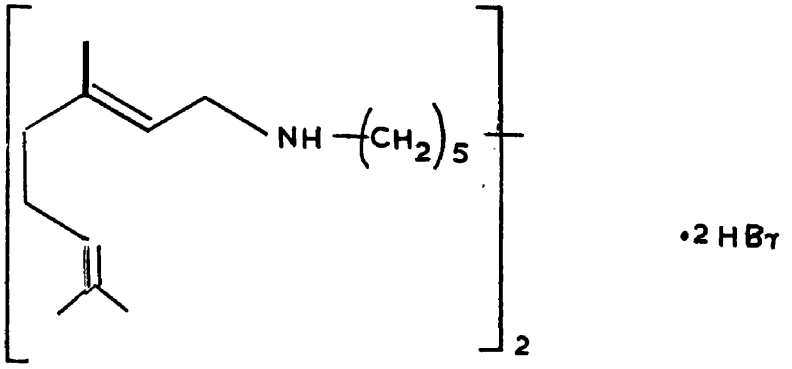
muscle (291). Chaksine has further been reported to possess antibacterial properties (292).

Numerous synthetic amino derivatives of monoterpenes are known, but it is only in recent years that any great interest has been shown in the biological properties of such compounds. The earlier work had, however, shown that ω -aminopinane possessed germicidal properties (293) and that thymoxyethylallylamine (LVI) possessed



LVI

adrenergic blocking properties (294). The pharmacology of camphenilylamine was also investigated (295) and 1:10-bis-(geranylamino)-decane dihydrobromide (LVII)

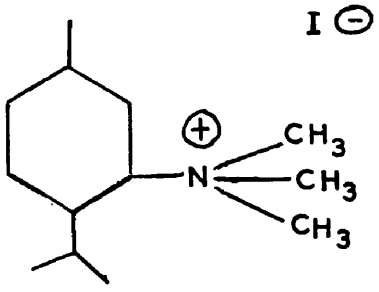


LVII

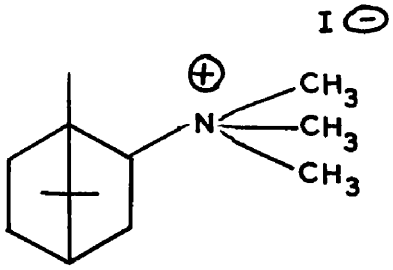
was shown to possess a high potency as an amoebicide

in vivo (296) whilst menthyl aminoacetate was claimed to possess anaesthetic properties (297). More recently experiments with various 3-dialkylaminoacylaminocamphors and diethylaminoethylbornylether have shown that these compounds possess central and smooth muscle depressant properties (298). Certain bis quaternary diamines derived from β-ionone are bacteriostatic (299), and certain derivatives of 2-amino-1-p-menthanol which were synthesised from d-limonene possess fungicidal properties (300), although they are without physiological activity.

The recent interest in amino-terpenoids has resulted from the demonstration that several such compounds possess ganglion-blocking properties and so have application as anti-hypertensive agents. For instance trimethyl-l-menthylammonium iodide (LVIII) and trimethyl-d- bornylammonium iodide (LIX) have been shown to markedly lower blood pressure (301), a property which



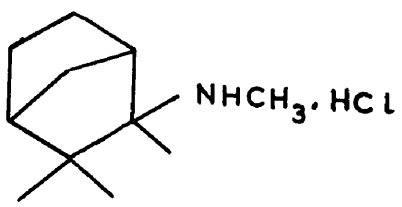
LVIII



LIX

is shared to some extent by various simple terpenoid primary, secondary and tertiary amines (302,303). Unfortunately, however, these compounds also possess a degree of curarimimetic activity and so are not as specific in their action as is to be desired. As ganglion-blocking activity is usually associated with quaternary nitrogen atoms, especially where the molecule is relatively simple in its chemical structure, the quaternary terpenoid derivatives are of interest from the supporting moiety concept.

In general quaternary ammonium salts are poorly and irregularly absorbed on oral administration, so the introduction into clinical practice of the orally-active competitive ganglion-blocking agent 3-methylaminoisocamphane hydrochloride (LX) (<u>syn</u> mecamylamine) marked a distinct advance in the treatment of hypertension. This terpenoid derivative is characterised by its high potency and prolonged duration of action (304), but like all ganglion-blocking agents so far tested it does not show



LX

complete specificity for sympathetic ganglia and consequently it produces the usual undesirable side effects resulting from blockade of the parasympathetic ganglia. Mecamylamine also exhibits a direct depressant action on smooth muscle.

The encouraging results obtained with these simple amino-terpenoids has led to the search for other similar compounds. Besides the various analogues of mecamylamine which have been prepared and tested (305), amino-terpenoids which have been investigated include N-allyl-2-aminoisocamphane (306), N-methylcamphidine (303) and quaternary salts derived from camphidine (307).

Synthetic Insecticides Derived from Monoterpenoids

In addition to monoterpenoid insecticides of natural occurrence such as the pyrethrins mentioned previously, various synthetic monoterpenoid derivatives have seen widespread application as insecticides and pesticides. Because the number of publications describing the efficacy of these agents against various species of insects or describing their toxicity to man and livestock is so great, no attempt can be made to give a comprehensive account here. Certain key references to commercially available terpenoid insecticides are to be found in the

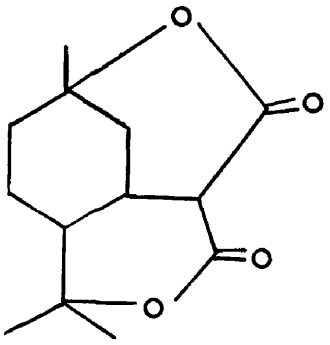
handbook published by the Canada Department of Agricul -ture (308), as well as in other specialized monographs (309). With the introduction of the phosphate insecticides the importance of the terpenoid insecticides would appear to be on the decline.

The synthetic terpenoid derivatives which have been shown to possess insecticidal properties cover a wide range of chemical diversity and include simple haloderivatives (310), ethers (311), esters (312) and various sulphur compounds (313).

Chlorinated camphene (314) was one of the most important of the commercially used insecticides, which also included other chlorinated monoterpene hydrocarbons (315) and certain thiocyanoacetic ester derivatives (316).

Besides the insecticidal compounds within the group, the monoterpenoids embrace compounds which possess insect-repellent properties. The best known of these is citronellal, long used as a mosquito repellent. Various synthetic derivatives have also been claimed to possess such activity including 2-amino-3-isobornyloxy-2-methyl-1-propanol (317) and "citral-malonic acid condensate" (318) although pure citrylidene malonic acid (LXI) was found to be virtually inactive (319). Tests for insect-

repellancy on other monoterpenoids have been reported



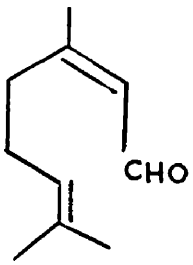
LXI

by Roadhouse (320) and Morton et al. (321).

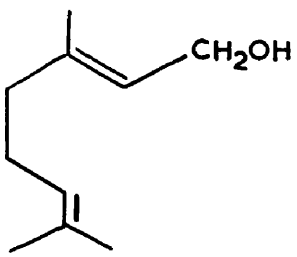
Some monoterpenoids possess attractive properties for insects and geraniol for instance has been employed to bait traps for the Japanese beetle, Papilla japonica (322).

Antihistaminic Monoterpenoids

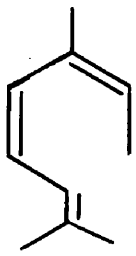
Several monoterpenoids have recently aroused a certain degree of interest, especially in Russia, on account of their reputed antihistaminic properties. Many of the claims pertain to citral (LXII) (323), but geraniol (LXIII), β-ionone, ψ-ionone and alloöcimene (LXIV) are also claimed to possess antihistaminic activity (324), although there is a



LXII



LXIII



LXIV

report that ionone produces allergic skin reactions resulting in eczema (325). Because of the structural relationship of these compounds to vitamin A, the Russian workers have advanced the hypothesis that one of the functions of vitamin A in the body is to give rise to antihistaminic compounds (326). They further claim that allo$\ddot{o}$cimene, ψ-ionone, ionylidenecrotonic acid and carotene act biologically by activating oxygen and peroxide oxygen (327). Because of its antiallergic anti-inflammatory and analgesic properties, citral is apparently widely used by Russian opthalmologists (328).

Furthermore citral has been reported to possess carcinostatic properties (329), as has the closely related compound citronellal (330), but the thiosemicarbazone, oxime, semicarbazone and bisulphite addition product of citral are without carcinostatic activity (331).

There is a claim in the literature that citral is of benefit in the prevention of alimentary atherosclerosis in rabbits (332), but, as it has been shown to produce fatty liver infiltrations in rats (333), more work is needed before a complete evaluation of its potential usefulness can be made. The condensation product between

citral and sulphanilamide is reported to be a potent antimicrobial agent (334).

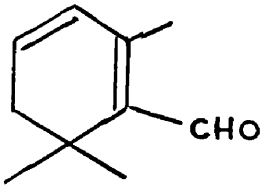
In addition to its antihistaminic activity β-ionone is claimed to possess potent anti-acetylcholine (335), and anti-thyroid (336) activity, to weakly inhibit co-carboxylase (337), and to slightly prolong the life span of mice with Ehrlich ascites tumour (338).

Allo"cimene has been shown to be active in promoting the formation of coagulation factors in the chick (339).

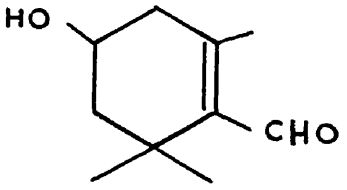
Other Individual Monoterpenoids and Their Derivatives

The investigations carried out on other individual monoterpenoids and their derivatives are many and varied, but in this summary an endeavour will be made to emphasize those properties of unusual interest, many of which may indicate a degree of structural specificity.

Safranal (LXV) became the centre of considerable attention when the startling claim was advanced (340) that this compound, together with 4-hydroxy-β-cyclocitral (LXVI) and its glucoside, picrocrocin, functioned as sex-determining factors or termones in Chlamydomonas eugametos.



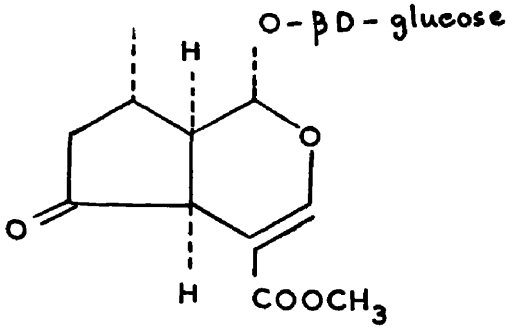
LXV



LXVI

These claims have since been completely refuted (341) as have the claims that the carotenoid derivatives, crocin and the cis and trans crocetin dimethylesters, acted as hormones controlling motility, gamete formation and copulation in the same organism. Picrocrocin is of interest in another respect, however, in that it is the bitter principle of saffron, a drug formerly used medicinally(342).

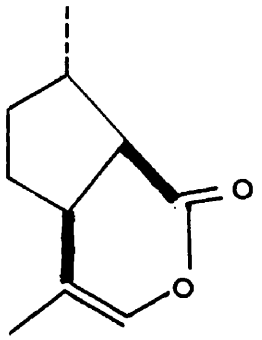
Another monoterpenoid glycoside which has been the subject of biological investigations is verbenalin, which has been shown to possess structure LXVII (343). This



LXVII

compound is claimed to be a weak parasympathomimetic agent and to have a pronounced action on the uterus (344). The closely related compound aucubin (345) possesses antibacterial activity (346). Studies of the general pharmacology of aucubin have been reported by Ogata and Nishioji (347), whilst its effect on uric acid excretion has been studied by Kato (348).

Nepetalactone (LXVIII) is an extremely interesting

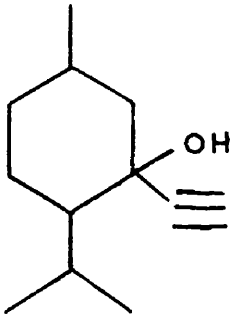


LXVIII

monoterpenoid, being the agent responsible for the marked attraction which catmint (Nepeta cateria) holds for the felids. A dramatic experiment clearly demonstrating that this compound was indeed the active principle was performed upon the lions at the Vilas Zoo, Madison (349). Nepetalactone arouses felids from a state of lethargy to one of intense excitement in which they try to transfer the compound to their fur. The close similarity in chemical structure between nepetalactone and iridomyrmecin could explain the intense interest in ants commonly displayed by cats. Indeed matatabilactone which is identical with isoiridomyrmecin (350) and the closely related monoterpene alkaloid actinidine, both of which occur in Actinidia polygama have been shown to attract felids (350,351).

Because the unsaturated lactone ring is believed to play an important role in the activity of the steroidal cardiac glycosides, nepetalactone was tested for possible inotropic action on the frog heart, but it was found to be inactive (352).

Menthol is of some physiological interest as it has been reported to selectively stimulate the cold receptors of the lingual nerve (353). It is stated to be effective as a topical dental anaesthetic but it suffers from the great disadvantage that it produces sloughing and irritation (354). It is also reported to potentiate the anaesthetic potency of nupercaine (355). Biochemical investigations have shown that menthol inhibits the activity of liver and kidney dehydrogenases (356). Considerable attention has been paid to the comparision of the pharmacological properties of the stereoisomers of menthol (357) and several synthetic derivatives of potential pharmacological interest have been prepared, including the menthyl ester of cinchophen (358), the hypnotic 2-isopropyl-5-methyl-1-ethynylcyclohexane (LXIX) (359), the bacteriostat menthylthiocyanoacetate (360) and the anti-rheumatic menthyl ether of methyl salicylate (361). The bornyl ester of salicylic acid



LXIX

has also been prepared as an anti-rheumatic agent (362), and menthyl salicylate has seen use as a constituent of suntan cosmetics (363).

Several synthetic derivatives of thymol and carvacrol have been the subject of biological investigations. Among these may be mentioned various simple ethers (364), halogenated derivatives (365) chloromercuri derivatives (366), sulphonamide derivatives (367) and substituted carbamic esters. These carbamic ester derivatives are cholinesterase inhibitors and possess insecticidal activity (368).

The naturally-occurring p-methoxythymol, like thymoquinone (369) is a potent fungicide and is thought to be at least partially responsible for the decay-resistance of Lebocedrus decurrens (370).

A number of studies have been made on the effect of various monoterpenoids on the secretion of bile (371), and certain mixtures containing monoterpenoids are claimed

to be therapeutically useful in dissolving concretions in the biliary and urinary tracts (372). Considerable attention has been given to the anthelmintic (373) and antibacterial (374) properties of such monoterpenoids as geraniol, menthol,1:8-cineole, pinene and p-cymene and certain monoterpenoids have been utilized in studies of the speed of penetration of compounds through the skin (375). Recently studies on the speed of penetration of inhaled vapours have been conducted using tritium labelled menthol and camphor (376).

Various ozonized monoterpenoids have been investigated for antibacterial activity (377), whilst the ozonide of pinene was at one time used clinically in the treatment of pulmonary tuberculosis (378). Other oxidation products of monoterpenoids have also been shown to be toxic to bacteria (379) and helminths (380), and the mono and bis epoxides of dipentene have been investigated for possible tumour-inhibiting and cytotoxic activities (381).

Other individual monoterpenoids which have been subjected to detailed pharmacological investigation include piperitone (382,383), piperitenone (383), carvone (383), carvomenthone (383), sabinol (384),

cineole (385,386), phellandrene (386), ketocineole (387), α-pinene (388), β-pinene (389) and hydroxypinocamphone (390). Citral and citronellal were included in a study of the effect of aldehydes on blood pressure and found to have a depressor action (391). Linalool has been claimed to inhibit experimental gas gangrene in rabbits (392).

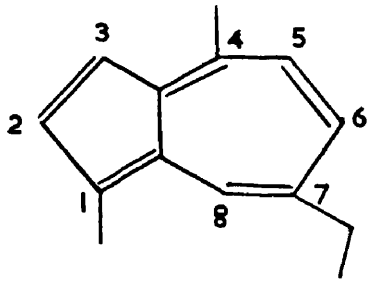
Other monoterpenoid derivatives of interest include some terpenyl substituted fatty acids which were prepared as potential antibacterial agents (393), 2-diethylamino -ethyl fencholate (394) which is a fungicide and various monoterpenoid esters (395). Dibornyl sulphide has recently been investigated and is stated to have pharmacological properties similar to camphor (396).

SESQUITERPENOIDS

Like the monoterpenoids the sesquiterpenoids are of widespread occurrence throughout the plant kingdom and show considerable variation in their chemical complexity (397,398).

Azulenes

Among the sesquiterpenoids with the simplest chemical structure are the sesquiterpene azulene hydrocarbons (399) and interest in these compounds from a biological viewpoint was first aroused when it was claimed that chamazulene (LXX) possessed anti-inflammatory properties

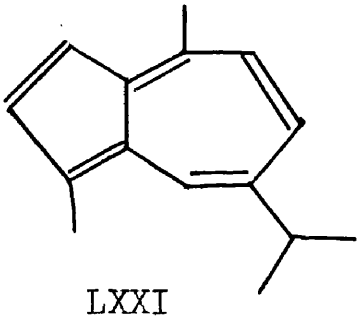


LXX

(400). The earlier work dealing with the biological properties of this compound has been reviewed by Janistyn (401) and by Thomas (402).

Chamazulene which occurs in camomile oil obtained from Matricaria chamomila (a plant long used in Folk medicine and mentioned in the writings of Dioscorides

and Pliny) is not strictly a true sesquiterpene but it can be regarded as being formally related to S-guaiazulene (LXXI) by loss of a carbon atom. Moreover it is believed



LXXI

to arise in nature from a guaianolide precursor by oxidative degradation (403).

Both chamazulene and S-guaiazulene as well as other sesquiterpenoid azulenes have been subjected to numerous clinical and pharmacological trials (404), especially in Europe, but the results are conflicting. Whilst some workers have concluded that these compounds are without any demonstrable antiphlogistic action (405), other workers have claimed that in addition to possessing anti-inflammatory properties, the azulenes are of value for treating various allergic disorders (406), including asthma. It is possible that some of the discrepancies are attributable to variations in dose or the route and timing of administration. Others may be explained by the

claim that, whilst the azulenes are themselves without effect, certain decomposition products are active (407).

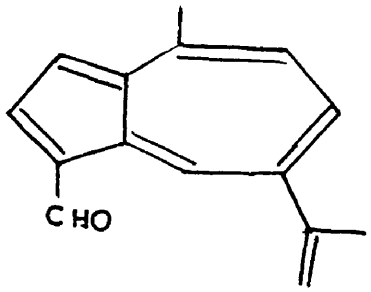
The azulenes have seen clinical trial in the treatment of migraine (408), radiological dermatitis (409), eczema (410), ultraviolet erythema of the skin (411), burns (412), ulcerative colitis (413) and gastritis (414). S-guaiazulene has been employed in depilatory preparations with the object of rendering them non-irritant (415). Various azulenes are also claimed to possess bacteriostatic (416), cancerostatic (417) and antiarthritic properties (418), although 1-isopropyl-5-methylazulene has been shown to be devoid of anti-cancer activity (419). The tumour inhibiting properties of S-guaiazulene are thought to result from inhibition of succinic dehydrogenase (420).

Considerable work has been devoted to studying the effect of azulenes on anaphylactic shock induced in guinea pigs. Various authors (421) state that these compounds are without effect, but others maintain that although the compounds are without influence on histamine shock they prevent anaphylactic shock (422) or both anaphylactic shock and serotonin-shock (423).

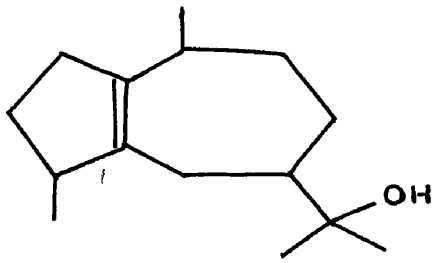
Evidence has been advanced that the azulenes affect phagocytosis (424), and at one time it was suggested that this action was mediated through the release of histamine (425) in accordance with the theory advanced by Jancso (426). More recently considerable doubt has been cast on this assumption (427). In fact some schools (414,428) have claimed that the azulenes act by preventing the release of histamine, one group going so far as to postulate that the histamine-release is prevented by activation of the pituitary-adrenal system with consequent release of adrenocorticotrophin or cortisone which in turn prevents fibrolysin from causing histamine discharge (429).

The pharmacology and toxicology of S-guaiazulene have been studied by Caujolle and Stanislas (430) who also investigated the effect of this compound on plants (431). The interest in the biological properties of azulenes has inspired the synthesis and biological screening of several synthetic compounds. These include non-isoprenoid azulene hydrocarbons (432) as well as simple derivatives such as sulphonated isoprenoid azulenes (433). The azulene aldehyde lactaroviolin (LXXII) has been reported to show anti-tubercular activity <u>in vitro</u> (434), and the

perhydroazulenic alcohol guaiol (LXXIII) is claimed to possess insecticidal properties (435).



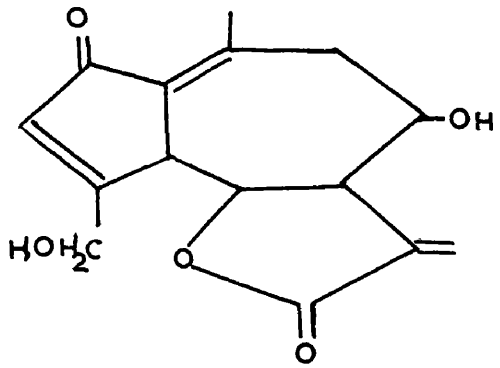
LXXII



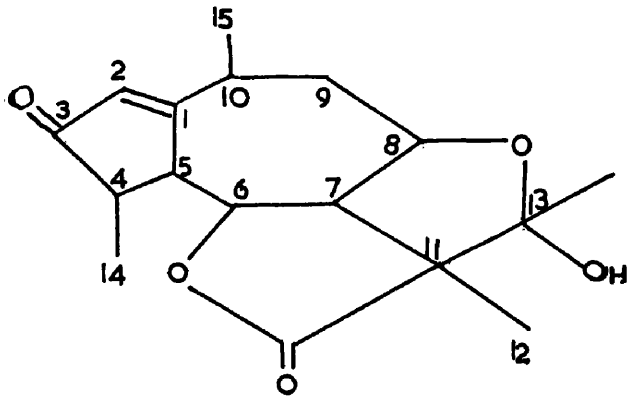
LXXIII

Guaianolides

Apart from their role as azulene precursors, certain guaianolides are of biological interest in their own right. Several sesquiterpenoids of the guaianolide type have been identified as the bitter principles of various plants. Examples are lactucin (LXXIV), geigerin, helenalin and tenulin (LXXV). Lactucin is the main bitter



LXXIV



LXXV

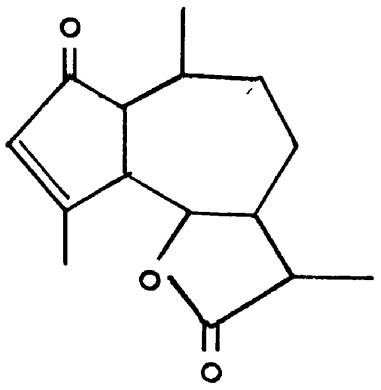
principle of the latex of Lactuca virosa which was formerly used in pharmacy under the name of lettuce opium, and which enjoyed a reputation as hypnotic.

Geigerin is obtained from the vermeerbos (Geigeria aspera) and is believed to be closely related chemically to the active emetic principle vermeeric acid (436). Helenalin and tenulin, which possesses two carbon atoms more than the basic sesquiterpenoid skeleton in the form of a modified acetate unit, occur in Helenium spp.

The guaianolides are postulated to arise in nature from a 10-membered ring precursor (437) and in this connection it is interesting to find that another sesquiterpenoid bitter principle cnicin, which occurs in Cnicus benedictus L., possesses such a 10-membered ring system (438).

Tenulin and helenalin were tested as potential insecticides but found to be inactive (439). Helenalin is, however, reported to be an effective fish poison (440). The pharmacology of helenalin has been studied in detail by Lamson (441) who also reviewed the earlier knowledge of the biological properties of this compound. Helenalin has a severe irritant action on mucous membrane and produces violent sneezing by reflex action.

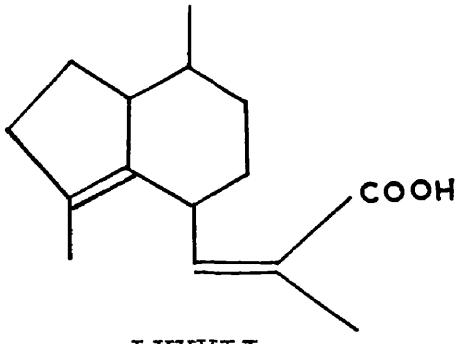
Another guaianolide, carpesialactone (LXXVI), has been shown to be active as an anthelmintic (442) and to possess muscle-relaxant and central nervous system



LXXVI

depressant properties in mammals (443). Two further guaianolides which have been investigated pharmacologically are matricin and artabasin (444), which are stated to be similar in their actions on intestinal muscle to chamazulene.

An interesting sesquiterpenoid acid which is postulated to arise in nature by rearrangement of the guaianolide skeleton (445) is valerenic acid (LXXVII),

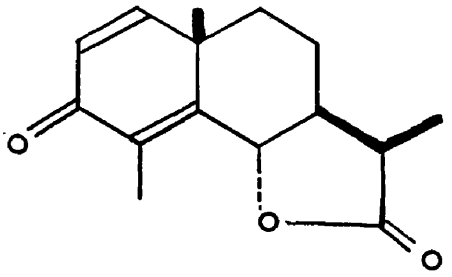


LXXVII

which shows marked spasmolytic activity (446).

Santonin

Perhaps the best known sesquiterpenoid from the biological point of view is l-α-santonin (LXXVIII), more commonly known simply as santonin. As well



LXXVIII

documented reviews of the biological properties of this drug have been published (447) it is not necessary to consider it in great detail in this review.

Santonin until recently was widely used in the treatment of human ascariasis (448), having been employed for this purpose since Dioscorides. Although effective against Ascaris spp. santonin appears to be ineffective against other parasitic worms. It has, however, been reported to be highly toxic to the leech (449). It has been claimed that santonin is only effective as an anthelmintic in the presence of base, e.g. in the presence of bile salts (450), when it exists

as an alkali-metal santoninate, and this would support the report that sodium santoninate is as effective as santonin itself against Ascaris (451).

The anthelmintic action of santonin has been ascribed to its ability to cause pronounced contraction of the musculature of the worm (452), but Baldwin (453) has concluded that santonin acts on the central nervous system, a conclusion also reached by other workers (454). It has been further postulated that this stimulation of the nervous system of the worm results in chemotropism towards the drug (455). Another somewhat intriguing explanation of the mode of action of santonin is that the drug deprives Ascaris of its resistance to hydrogen sulphide in the intestine (456), although in vitro studies showing that hydrogen sulphide in the presence of santonin is no more toxic to the worms than is hydrogen sulphide alone (457) would not support this contention.

Whatever the ultimate mechanism of action of santonin proves to be, the demonstration that d-santonin is inactive as an anthelmintic and that dl-santonin is one-half as active as the naturally occurring l-form (458) conclusively shows that santonin is a structurally specific drug.

Apart from its action in expelling Ascaris spp. perhaps the most interesting biological property of santonin is its ability to cause visual disturbances in human beings resulting in yellow and violet vision (xanthopsia). Taste, smell and hearing may also be affected. A detailed study of the effects of santonin on vision was made by Marshall (459), who gives a summary of the earlier work in this field. The changes in the electrical phenomena of the optic nerves in animals, which presumably accompany visual disturbances similar to those in man, have also been studied (460).

Santonin has been shown to exert a mild diuretic effect (461) and to increase the concentration of uric acid in the urine (462). Unlike alantolactone (463), another sesquiterpenoid lactone whose chemical structure is still incompletely established (464)- santonin is reported to have no effect on the urinary excretion of sulphate (465).

Several investigations have been concerned with the effect of santonin on the concentration of sugar in the blood. At one time santonin saw clinical trial in the treatment of diabetes (466) but it has been shown that the hypoglucaemia produced by prolonged treatment with

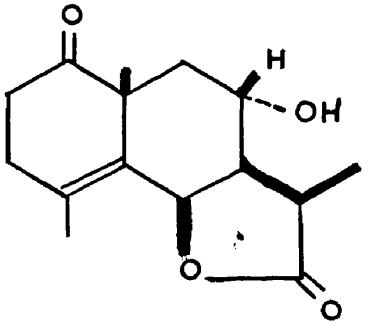
santonin is due to liver damage (467). Single doses of santonin have been reported to produce a hyperglycaemia (468,469) or to increase the hyperglycaemia due to the administration of glucose (470), although other workers (471) have concluded that, whilst there is no direct action on the blood sugar level, phlorhizin glycosuria is decreased, presumably by a rise in the renal threshold for glucose.

In high doses santonin produces convulsions in various species (469,472). The symptoms of severe santonin intoxication have been described by Binz (473) and Hernach (474).

Other biological studies with santonin have shown that it inhibits the serum cholinesterase of the guinea pig (475), that it exerts a slight vasodilator action on the frog (476), that it relaxes the guinea pig uterus (477) and the rabbit intestine (478), that it prevents the gross oedema induced by p-phenylenediamine (479) and that it antagonises the action of veratrine (480).

The biotransformation of santonin has been well studied (481) and has been proposed as a test of hepatic function (482).

The isomeric β-santonin has been compared in its pharmacological properties to santonin (483) and synthetic derivatives of santonin have been tested biologically (484). Various naturally occurring sesquiterpenoid lactones closely related in chemical structure to santonin have also been studied. One such compound is ψ santonin (LXXIX) (485) which was claimed



LXXIX

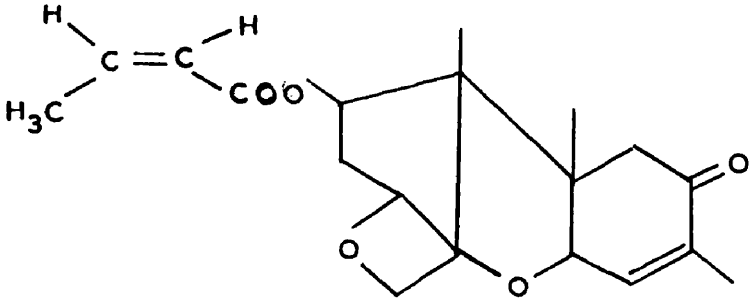
to exert an action on the frog heart resembling that of the steroidal cardiac glycosides (486) although later work employing dogs and rabbits showed negative results (487). Other studies have shown that isoartemisin produces no immediate effect on the blood sugar level of fasting rabbits (488).

Alantolactone (syn helenin) has been investigated pharmacologically by Kuribayashi and Hara (489). Its efficacy in ascariasis has been in dispute (490), but it is claimed to be toxic to the Schistosome carrier,

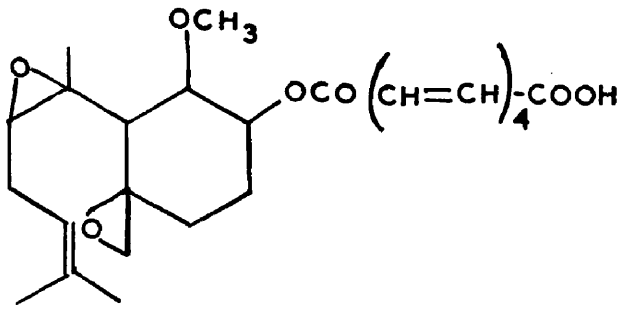
Australorbis glabratus (491). Alantolactone has been shown to have a choleretic effect, being some 20 per cent as potent as dehydrocholic acid on a molar basis (492).

Sesquiterpenoid Antibiotics

Our knowledge of this group of sesquiterpenoids is of very recent origin and so far two representative members would appear to be well established. These are trichothecin (LXXX) (493) and fumagillin (LXXXI) (494). The alcoholic moiety of trichothecin has been shown to



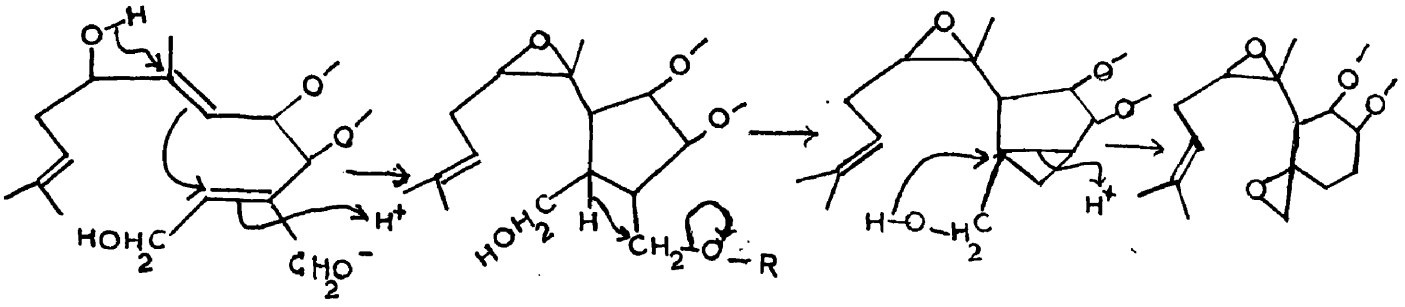
LXXX



LXXXI

arise in nature from a true sesquiterpenoid precursor by a double 1,2-methyl group migration (495) but little is known of the biogenesis of fumagillin. Several possibilities exist. The alcoholic moiety of fumagillin can perhaps be regarded as a substituted isoprenalogue of carquejol (496), or as resulting from an irregular condensation of isoprene units. There is the third

interesting alternative that it could arise from farnesol if a series of allylic hydroxylations were to occur before ring closure. This is illustrated in LXXXII.



LXXXII

Trichothecin is an antifungal antibiotic, having no action against bacteria (497), and it has shown considerable promise in field trials against fungal plant diseases (498). Pharmacological experiments have demonstrated that it has severe irritant properties (499). Several analogues of trichothecin have been prepared (499) and it has been demonstrated that replacement of the isocrotonyl moiety by the acetyl, butyryl or crotonyl radicals does not greatly affect the antifungal properties. Tetrahydrotrichothecin is, however, devoid of antifungal activity.

Fumagillin is a potent amoebicide (500) both *in vitro* and *in vivo* and has been shown to be of use in the treatment of *Nosema* infection of bees (501) although it is claimed that a complete cure is not effected (502). Various amine salts of fumagillin are also active against *Nosema apis* (503).

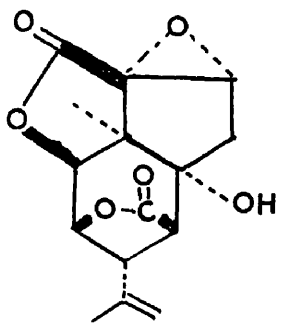
Fumagillin has been claimed to show promising carcinostatic activity against various types of carcinoma (504) as has fumagillin alcohol (505). Fumagillin was, however, without effect on Crocker sarcoma 180 in mice (506).

Other studies have shown that it is toxic to spermatozoa (507), that it prevents phage multiplication in infected *Micrococcus pyrogenes* var *aureus* (508), and that it is active against *Plasmodium gallinaceum* in chicks (509). A review of the biological properties of fumagillin which emphasises its clinical application and tolerance has been published (510).

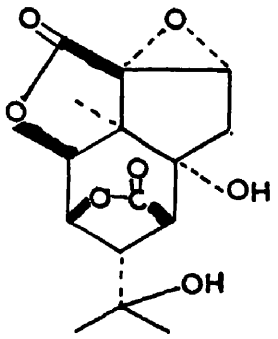
Picrotoxin

Santonin has on occasion been compared in its biological properties with picrotoxin, which is a molecular compound of the two substances picrotoxinin

(LXXXIII) (511) and picrotin (LXXXIV). Although both

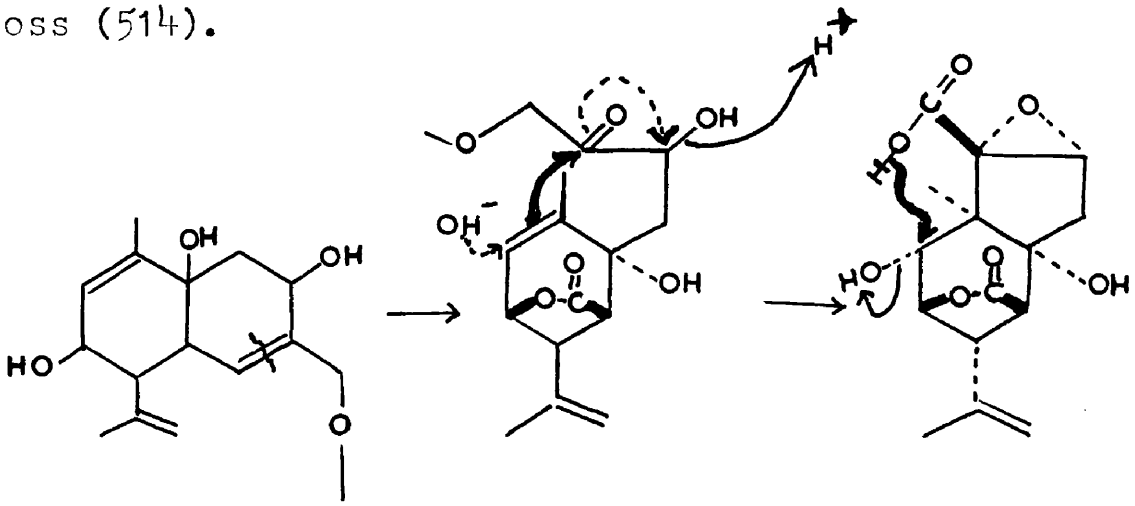


LXXXIII



LXXXIV

picrotoxinin and picrotin have been subjected to separate biological investigations (512,513), as have several derivatives from them, nearly all the studies have employed picrotoxin. Both picrotoxinin and picrotin are C_{15} compounds and can probably be regarded as sesquiterpenoids. An interesting possibility is that they could arise in anture from a suitably oxygenated cadinene-type precursor, as shown in LXXXV. An alternative biogenetic scheme from farnesol has been proposed by Cross (514).



LXXXV

Picrotoxin has been extremely well investigated biologically, there being well over 200 publications concerned with its pharmacological and clinical properties. The earlier work has been reviewed by Trendelenburg (515). A considerable proportion of the work has been concerned with the physiological antagonism between picrotoxin and various central nervous system depressant drugs. Picrotoxin is a convulsant drug (516) and acts both on the midbrain (517) and on the medulla oblongta where it stimulates the respiratory, vasomotor and other autonomic centres. The drug does, however, show some depressant properties (518). The similarity between the respiratory, convulsive and circulatory changes occurring with picrotoxin and those occurring in epileptic attacks has been remarked upon (519).

Picrotoxin has been shown to antagonise the narcotic effects of chloral hydrate (520,521), paraldehyde (522), urethane (523), sodium bromide (524) and the barbiturates (525). The degree of respiratory stimulation produced has been found to depend upon the depth of the depression originally present (526). It would appear that picrotoxin is unable to antagonise the narcotic effects of magnesium sulphate (527) or morphine, although it does

counteract the respiratory and circulatory depressant actions of morphine (528). Actually morphine has been shown to exert a diphasic action, being able to enhance the action of both depressant and stimulant drugs, and it has been demonstrated to render subconvulsant doses of picrotoxin convulsant (529). The fact that picrotoxin shows no antagonism towards the action of myanesin has been advanced as supporting evidence that myanesin acts selectively on the spinal cord (530).

The ability of picrotoxin to antagonise barbiturate narcosis has been utilized clinically and the drug has been used as an antidote to barbiturate intoxication (531), although its use in this connection is not without danger (532) and it has now been replaced by amphetamine and β-ethyl-β-methylglutarimide. Conversely barbiturates have been employed to control the convulsions produced by picrotoxin (533)

Picrotoxin has seen application in the evaluation of anti-convulsant drugs (534,535), and has seen limited trial in the convulsion treatment of schizophrenia. Considerable effort has been devoted in attempts to locate the exact site of action of picrotoxin in the central nervous system (536), and many of the experiments have been essentially of a biochemical nature.For instance,

investigations have been made on the distribution of sympathin in the brain during picrotoxin-induced convulsions (537), as well as on the ammonia (538), phosphate (539), lactic acid (540), glycogen (541) and acetylcholine (542) contents of the brain. Picrotoxin has also been shown to inhibit the oxidation of glutamate, succinate, fumarate and pyruvate by the cat brain in vitro (543) and to produce a marked decrease of the acid phosphatase activity in the large cells of the motor cortex of the dog (544).

As a result of experiments in which picrotoxin was shown to reversibly block peripheral inhibition in crayfish muscle (545), it has been postulated that picrotoxin competes for receptors with an "inhibitory transmitter" and that by analogy, the central action in mammals may occur by a similar process. In this connection it is interesting that picrotoxin has been shown to be a competitive antagonist of γ-aminobutyric acid (546).

An interesting property of picrotoxin which is thought to be associated with its convulsant action is its ability to induce ovulation in rabbits via an indirect stimulation of the hypophysis and release of luteinizing hormone (547,548). That the convulsions themselves are not sufficient to ensure ovulation has been shown by

Kasdon (549), who found that although picrotoxin undoubtedly can induce ovulation, this action is rather unpredictable. It would appear that picrotoxin does not provoke the release of luteinizing hormone in rats (550).

Picrotoxin has an antipyretic action (551) which is thought to result from stimulation of a cooling centre in the diencephalon which is closely related to the centres controlling the parasympathetic system (552). It has been claimed, however, that picrotoxin at the convulsant level can produce an increase in the temperature of the skin (553).

Another result of the central action of picrotoxin is the production of hyperglycaemia (554), the effects being mediated via the splanchnic nerve and adrenal glands (555). The increase in the concentration of sugar and lactic acid in the various body fluids which is induced by picrotoxin has been studied in detail by Hata (556). There is also evidence that picrotoxin can act directly on the adrenal gland itself (557). Similarly picrotoxin is thought to stimulate the rabbit uterus mainly by a central action, but there would appear to be a peripheral component (558) as well. Other peripheral actions of picrotoxin are reported by Ogawa (559) and Radouco-Thomas et al. (560).

Amongst the many other detailed studies with picrotoxin may be mentioned those concerned with its effects on vasoconstriction (561), on the rhythmic activity of leech muscle (562), on the permeability of frog skin (563) and on the glomerular filtration and tubular reabsorption (564). Picrotoxin also produces an increase in the potassium level of the blood (565), as indeed do other analeptic agents.

Like camphor, picrotoxin has afforded good examples of how the potency of a drug varies with the temperature (566), with species and sex (567), with the route of administration (568), with age (569) and with dietary history (570).

Other compounds which are closely related chemically to picrotoxinin and picrotin are coriamyrtin, tutin (571) and mellitoxin. The biological properties of these compounds are similar to those of picrotoxinin (512). Thus coriamyrtin appears to have a similar action on the central nervous system to that of picrotoxin (521,572) and to be able to antagonise the action of barbiturates (573) although it is unable to induce ovulation in rabbits (548). Many other studies of the pharmacological properties of coriamyrtin have been reported (574,575)

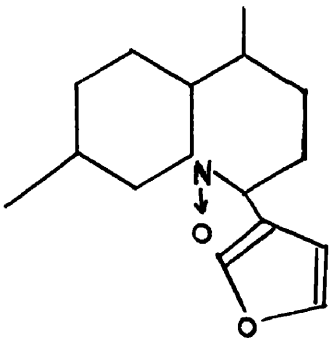
and several of its derivatives have been investigated (576).

Tutin has also seen considerable pharmacological investigation (575,577) and both tutin and mellitoxin show antagonism to barbiturates (578).

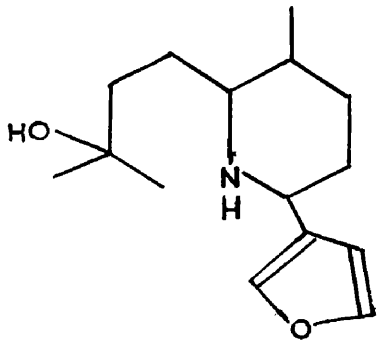
Other Sesquiterpenoids

Relatively little biological work appears to have been carried out on the other sesquiterpenoids which are at present known.

Two sesquiterpenoid alkaloids nupharidine (LXXXVI) (579) and nupharamine (LXXXVII) (580) have been isolated from the roots of Nuphar japonicum and pharmacological



LXXXVI



LXXXVII

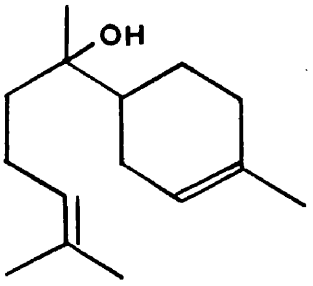
studies (581) on deoxynupharidine hydrochloride have shown that this derivative constricts peripheral blood vessels, increases the blood pressure in small doses and lowers the blood pressure in large doses. It also

depresses the respiratory centre and exhibits anti-acetylcholine and anti-histamine activity.

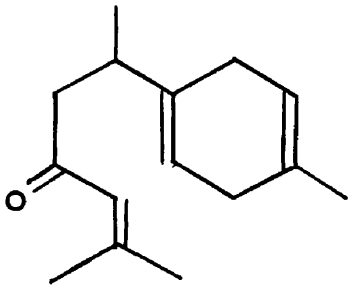
The sesquiterpenoids farnesenic acid, farnesal, farnesol and nerolidol have been shown to inhibit the conversion of acetate (582) and mevalonic acid (583) into cholesterol by rat liver homogenates. Although the inhibition was probably due to alternative incorporation of the preformed sesquiterpenoid in the squalene synthesis since farnesyl pyrophosphate is known to be an intermediate (584), these experiments raise the question as to whether other sesquiterpenoids can be found which will be capable of blocking the squalene synthesis in vitro . As was pointed out by Robinson (585) such compounds might be of value in the treatment of conditions like atherosclerosis, although animal experiments would tend to show that exogenous and not endogenous cholesterol is involved in this affliction (586). A demonstration that farnesenic acid itself can show an inhibitory anti-metabolite effect was given when it was shown to antagonise growth promotion by mevalonic acid in Lactobacillus acidophilus (587).

α-d-Bisabolol (LXXXVIII) is active in vitro against Mycobacterium tuberculosis (588) and possesses

antiphlogistic (589) and spasmolytic (590) properties. Turmerone (LXXXIX) has seen use as a cholagogue and is

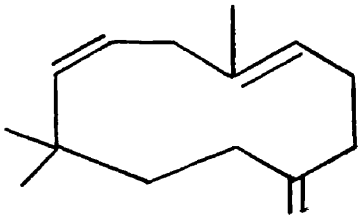


LXXXVIII

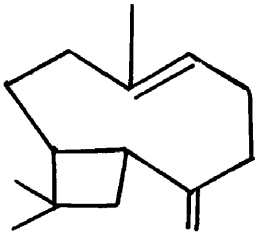


LXXXIX

of interest as it has been stabilised by means of its thiourea inclusion compound (591). Humulene (XC) has been shown to depress the nervous system of the frog in low dosage and to stimulate it in higher concentrations (592) whilst caryophyllene (XCI) exhibits anti-anaemic



XC

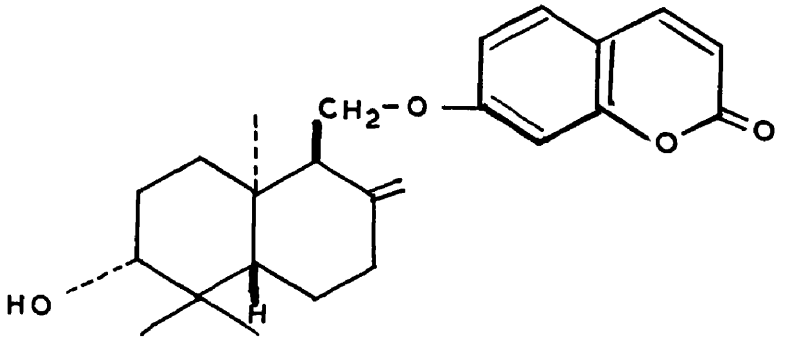


XCI

activity in the toluenediamine-anaemic rabbit (593).

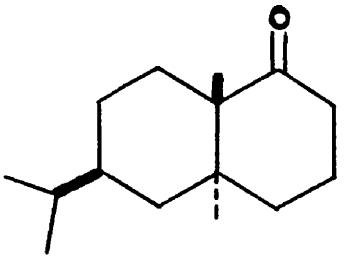
Asafoetida, a resin obtained from Foerula spp. was at one time used in the treatment of hysteria and as a stimulant, expectorant and carminative (594). Recent

work has shown this resin to contain several interesting sesquiterpenoid ethers of umbelliferone, a representative member being farnesiferol A (XCII) (595). A similar monoterpenoid derivative, auraptene, which is the geranyl ether of umbelliferone (596), possesses antibacterial activity (597).



XCII

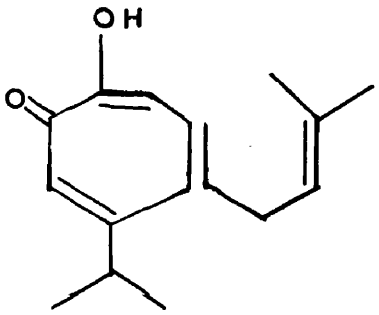
Valeranone (syn jatamansone) (XCIII) (598) is a



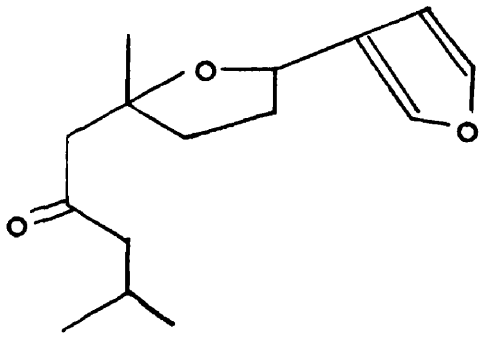
XCIII

sesquiterpenoid not obeying the head-to-tail isoprene coupling rule and may well result from the rearrangement of a precursor in which the isoprene units are arranged

in the regular manner. It has been claimed to exhibit antiarrhythmic and anticonvulsant properties (599). Other sesquiterpenoids worthy of mention are the various acids showing antibacterial properties (600), nootkatin (XCIV), ngaione (XCV) which may be the active principle

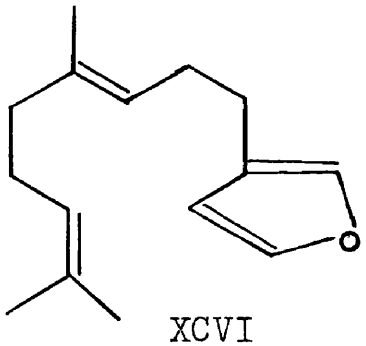


XCIV



XCV

of the toxic essential oil Myoporum laetum Forst (601), and dendrolasin (XCVI) which is the odoriferous principle

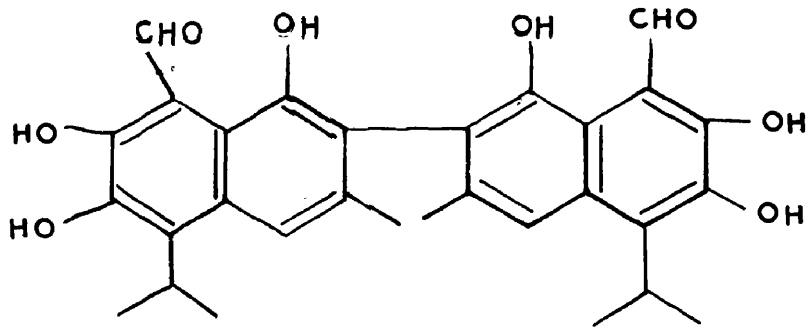


XCVI

secreted by the mandibular glands of the ant Dendrolasius fuliginosus Latr (602).

Dimeric Sesquiterpenoids

This group includes the dimeric guaianolides absinthin and anabsinthin (603) which are the bitter principles of wormwood (Artemisia absinthum) and certain dimeric naphthalene compounds (604) occurring in various species of cotton (Gossypium spp.) which are to be regarded as dimeric dehydrocadalene derivatives. The best known of these dehydrocadalene dimers is gossypol (XCVII) (605)



XCVII

which is the principle colouring matter of cottonseed.

Gossypol has seen extensive biological investigation on account of its toxic properties (606) and its implication in the poisoning of cattle fed on cottonseed meal, although recently work has been published in which it is claimed that the free gossypol content of cottonseed meal does not completely account for its toxicity (607).

Fortunately, during the processing of the cottonseed meal, which is such a valuable protein foodstuff, the gossypol is largely inactivated (608) by interaction with the free amino groups of the proteins (609). In this connection it has been shown that the compound formed between gossypol and glycine is non-toxic (610).

The pharmacology and toxicology of gossypol have seen considerable study. In low doses ingestion of gossypol produces a loss of appetite and fall in body weight in various species (611). As it has been shown that gossypol interferes with the in vitro digestion of cottonseed globulin by pepsin and trypsin (612) these symptoms of starvation in vivo are probably due in considerable degree to an inability to absorb proteins from diet. Somewhat higher doses induce diarrhoea, intestinal haemorrhage and ulceration (613). These haemorrhages have been shown not to be primarily due to hypoprothrombinaemia (614), although gossypol does induce this condition in rabbits and pigs (614), and haemolytic anaemia in chicks (615).

In addition to exerting a haemolytic effect on erythrocytes, gossypol has been shown to prevent the liberation of oxygen from oxyhaemoglobin (616), which

would account for the observation that rats sometimes die in a state resembling suffocation (617). Anoxia of circulatory origin has also been observed in pigs (618).

Intravenous or intraperitoneal injection of the sodium salt of gossypol produces a fall of blood pressure, cardiac irregularities and pulmonary oedema, death resulting from circulatory failure (619). Other pharmacological studies have shown that gossypol stimulates the isolated longitudinal muscle of the small intestine (620) and induces paralysis of the limbs with myelin degeneration of the nerves (619,621). Attempts to discover whether gossypol would inhibit the oxidative inactivation of adrenaline were frustrated by its low solubility (622).

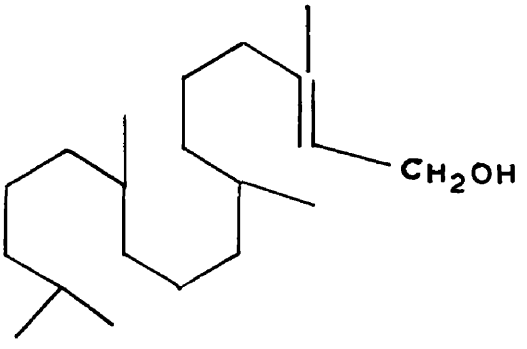
Gossypol was tested as an insecticide but found to be ineffective against the species employed (623). More detailed reviews of the biological properties of gossypol have been published elsewhere (624).

DITERPENOIDS

As with the mono- and sesqui-terpenoids, the diterpenoids show considerable variation in their chemical structure. Excellent reviews of the chemistry of diterpenes have recently appeared (398,625), including one which emphasises their industrial applications (626).

Vitamins A, E and K.

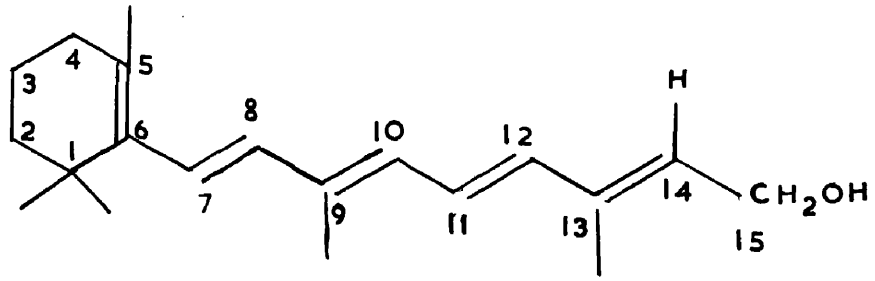
Without doubt the most important diterpenoids and diterpenoid derivatives from a biological point of view are the vitamins A, E, K_1 and K_2 [20], but as these compounds have been the subject of numerous reviews (49,627) very little need be said concerning them here. These compounds can all be formally regarded as being chemically related to the acyclic diterpenoid alcohol phytol (XCVIII) which has itself been shown to be a nutritional lipotropic factor (628). The phytyl radical



XCVIII

can probably be regarded as being of the nature of a supporting moiety in the chlorophyll molecule.

There are several vitamins A, all of which are closely related chemically, the most active being vitamin A_1 (IC)



IC

in which the double bonds of the side chain all have the trans configuration. Neovitamin A_1 differs from vitamin A_1 only in the configuration at C-14 and vitamin A_2 differs from vitamin A_1 by possessing an additional double bond in the 3-4 position.

As vitamin A_1 is formed by degradation of the α-, β-, and γ-carotenes in the liver of herbivores these tetraterpenoids are often referred to as provitamins A. A review of our knowledge concerning the way in which the provitamins A are transformed into vitamin A has recently appeared (629) and the provitamin A effect of carotenoids in relationship to cis-trans isomerism has

also recently been reviewed (630).

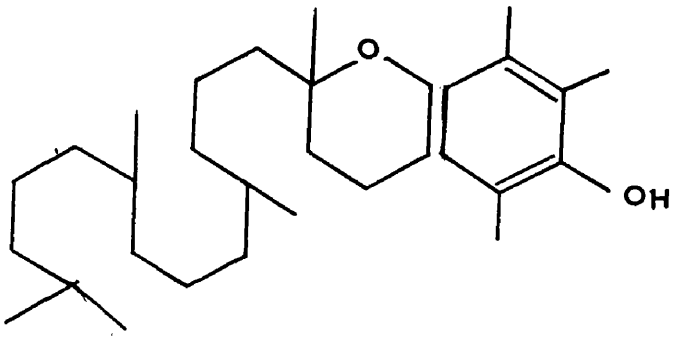
The vitamins A are the vitamins responsible for the maintenance of healthy epithelial tissue and normal growth in mammals (631). They also aid in maintaining resistance to infection. Vitamins A_1 and A_2 have another important function in that they are precursors of the 11-*cis* retinenes (neoretinenes b) which on combination with specific proteins of the rods and cones called opsins form the visual pigments of vertebrate vision. The first symptom of vitamin A deficiency in man and other animals is the rise of visual threshold known as night-blindness, and continued vitamin A deficiency leads to degeneration of the rods and cones. Excellent accounts of the parts played by the vitamins A and the 11-*cis* retinenes in vision are available (632). A detailed account of hypervitaminosis A has also appeared (633).

Numerous modifications of the vitamin A molecule have been synthesised in attempts to establish relationships between chemical structure and vitamin A activity. Most of the work has been concerned with alterations in the structure of the side chain although some attention has been paid to the ionone ring system, including preparation of the cyclohexenyl (634) and benzene (635)

ring analogues. A summary of the structural modifications of the vitamin A molecule which have been tested for biological activity is given by Baxter (636).

Recently it has been shown that vitamin A acid will maintain normal growth of rats, but will not protect them against night-blindness (637). This has led to the interesting postulate that vitamin A itself is only a storage precursor, giving rise to 11-cis retinene on the one hand for the maintenance of normal vision and to vitamin A acid on the other for the maintenance of normal growth and tissue function. The animal is unable to convert vitamin A acid into 11-cis retinene.

The vitamins E or the α, β, γ, δ, ε, ζ, and η tocopherols have been shown to be essential to the fertility of rodents, but it has never been clearly demonstrated that they are essential to man, although there are reports of women subject to spontaneous abortion having experienced successful pregnancies after vitamin E therapy. The vitamins E can all be regarded as derivatives of phytol and they differ only in the number and position of the methyl substituents on the benzene ring. The structure of α-tocopherol is shown in Fig.C.



C

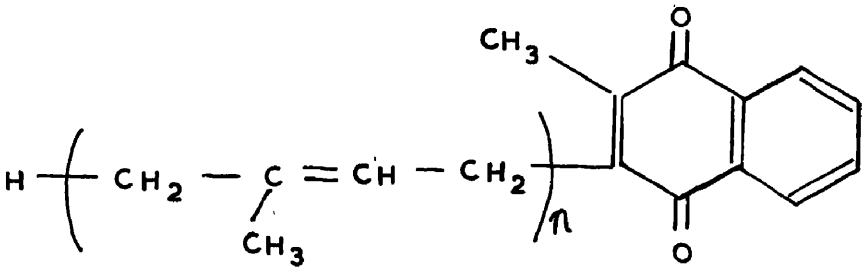
The tocopherols appear to participate as hydrogen carriers in the oxidative cycles of muscle metabolism (638) and they have been shown to function as in vivo antioxidants for fats (639). The typical symptom of E-hypovitaminosis in animals is muscular dystrophy and creatinuria.

As in the case of vitamins A numerous synthetic analogues of the tocopherols have been prepared and tested. These studies have clearly shown that the structure of the phytyl side chain cannot be altered without considerable loss of activity and so provide an excellent illustration of the supporting moiety concept. Key references to vitamin E analogues are to be found in the review by Mason (640).

Both α-tocopheryl acetate and phytol are of interest from the antimetabolite concept as they impair the utilisation of β-carotene as a provitamin A although

they do not affect the utilisation of vitamin A itself (641).

The vitamins K are the antihaemorrhagic vitamins, regulating the synthesis of prothrombin in the body. Actually only two vitamins K, viz: vitamin K_1 which occurs in plants and vitamin K_2 [20] (CI, n=4) which



CI

occurs in mammalian tissue are diterpenoid derivatives. The other vitamins K_2 which occur in fish meal and mycobacteria are higher isoprenalogues fitting formula C.

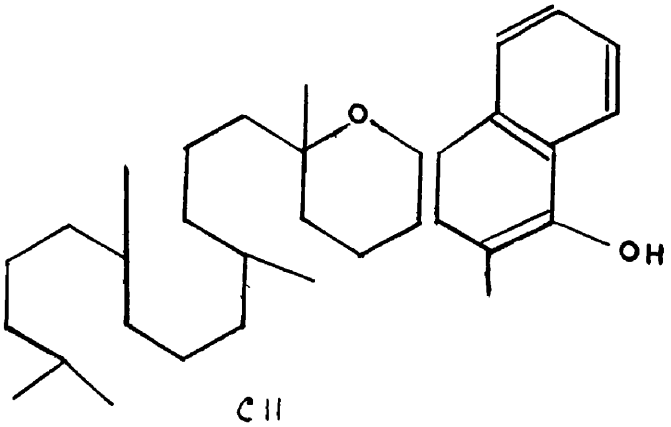
The vitamins K are employed prophylactically to prevent post-operative bleeding in patients with obstructive jaundice. As new-born infants have low prothrombin values vitamin K is often administered to expectant mothers before birth in order to prevent haemorrhage in the child. The vitamins K also find application in overcoming the effect of overdoses of dicoumarol which is employed as an anticoagulant in

patients prone to thrombosis.

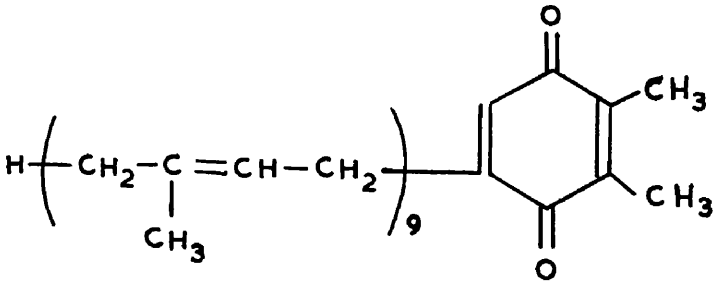
Considerable work has been devoted to the preparation of various vitamin K analogues, especially by Isler's school (31,32,642).

An interesting naphthotocopherol (CII) was synthesised by Tishler et al. (643) and found to exhibit vitamin E activity in moderate doses whereas in large doses it has vitamin K-like activity. This compound which incorporates structural units common to both series is thus very interesting from the point of view of structure-action relationships.

Other compounds closely related chemically to the vitamins K and E are the tocopherylquinones, Kofler's quinone (CIII) and the ubiquinones or coenzymes Q.



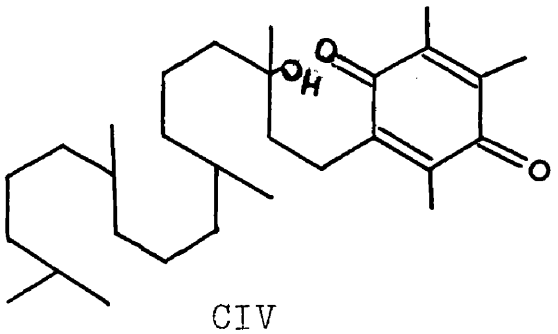
CII



CIII

∝-Tocopherylquinone (CIV) is of interest as it has recently been claimed to possess hypotensive properties

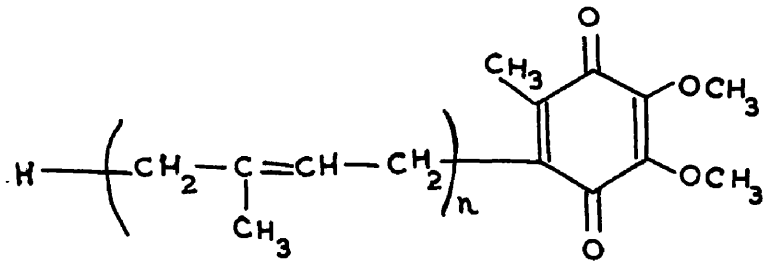
which are thought to result from a direct adrenocortical



CIV

inhibition (644).

The ubiquinones (645) can be represented by the general formula CV where n= 6, 7, 8, 9, 10.



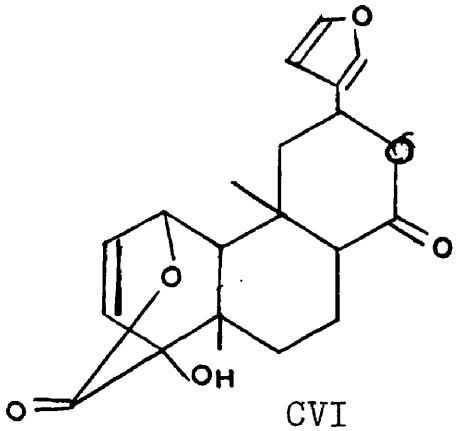
CV

The effects of the vitamins A and E on cholesterol metabolism and their usefulness in the treatment of atherosclerosis are summarized by Lin and Chen (646).

Diterpenoid Bitters

Several diterpenoids have been identified as the bitter principles of various plants. Examples are

andrographolide, marrubin and columbin (CVI). Marrubin



CVI

is the bitter principle of the horehound (Marrubium vulgare) galenical preparations of which were at one time used as an expectorant and laxative (in large doses) (647). Columbin occurs together with the chemically related bitter principles palmarin and chasmanthin in Colombo root (Jateorrhiza palmata, Miers) which has seen use as a bitter tonic. Columbin is not strictly a true diterpenoid, but is thought to arise in nature by migration of methyl groups in a diterpenoid precursor (648). Another bitter tonic prepared from quassia, the stem wood of Picroena excelsa, contains the rearranged diterpenoids quassin (644) and neoquassin.

The popularity of bitter tonics stems largely from the belief that bitters stimulate appetite and aid digestion by increasing gastric secretion. Experiments

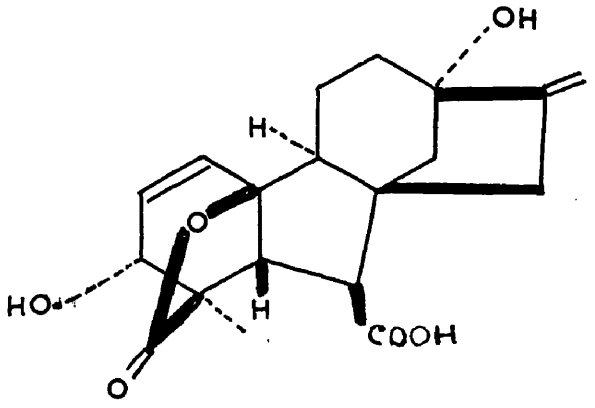
have shown, however, that in normal human beings and animals bitters actually inhibit hunger contractions and abolish hunger sensations (650), and are without effect on gastric secretion (651). On the other hand, in cachetic dogs they do increase gastric secretion (652) and this observation may provide some support for their use in anorexic states. It is also conceivable that their continued use by healthy human beings sets up a conditioned reflex of appetite.

Quassin has seen trial as an insecticide, but it proved to be ineffective (439,653). It was also used at one time against threadworm infections, and has seen limited trial as an alternative to emetine in the treatment of amoebic dysentery (654). It slows the beat of the isolated frog heart and produces coronary constriction in the isolated mammalian heart (655). On administration to rabbits quassin produces muscular tremor followed by paralysis (656).

Gibberellins

An extremely interesting compound which has been postulated (657) to arise in nature by a route involving diterpenoid intermediates is gibberellic acid which has recently been shown to possess the structure CVII (658).

Gibberellic acid is one of at least nine naturally



CVII

occurring gibberellins, all of which are closely related chemically, and all of which possess growth-promoting action on plants. Six of the gibberellins viz: gibberellic acid and gibberellins A_1, A_2, A_4, A_7 and A_9 are metabolites of the fungus Gibberella fujikuroi, the organism which causes bakanae disease in rice, and the other three gibberellins A_5, A_6 and A_8 have been isolated from the seeds of the runner bean (659). Gibberellin A_1 has been positively identified as a constituent of certain plants (660) and numerous crude extracts possessing gibberellin-like activity have been prepared from other plants (661) so it would appear that the gibberellins are natural plant growth hormones.

Qualitatively all the gibberellins appear to exhibit the same type of biological activity, but gibberellic

acid has seen the widest investigation as it can be produced in quantity from the filtrates of deep cultures of G. fujikuroi (662).

There is considerable species variation in the action of gibberellic acid on plants, but this will not be discussed here as an excellent, comprehensive and up-to-date review has just appeared (663). The practical aspects of the use of gibberellins in agriculture and horticulture have also been recently reviewed (664) and numerous other reviews are available (665).

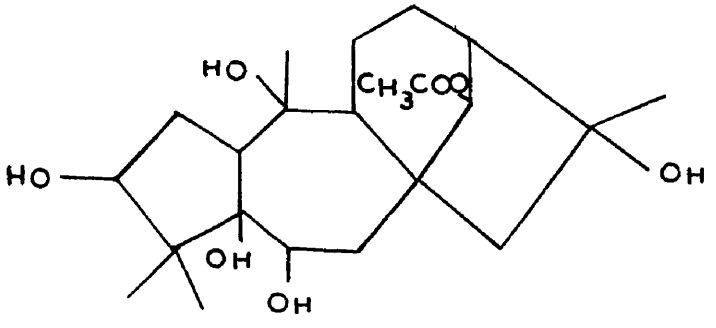
Recently there has been considerable interest in the effects of gibberellic acid on the biosynthesis of natural products, particularly those of medicinal importance. For example, it has been shown that gibberellic acid depresses the alkaloidal content of Datura stramonium (666,667), and of Atropa belladonna (666), although the alkaloid production is not affected in the second year of growth in the case of the second plant (668). With Hyoscyamus niger, however, it is claimed that gibberellic acid produces an increase in alkaloidal content (669). Other experiments have shown that gibberellic acid produces an increase in glycoside content of Digitalis purpurea (670) and a decrease

in the rutin content of Fagopyrum esculentum Moench (671).

Despite its spectacular physiological effect on plants, pharmacological and toxicological studies (672) have shown that gibberellic acid is without any pronounced activity in mammals and is virtually harmless. It is also without action on bacteria or moulds (673) and gibberellin A has been shown to have no effect on Ehrlich mouse carcinoma (674).

The Grayanotoxins

An interesting series of toxic principles which appear to be rearranged diterpenoids occurs in plants of the family Ericaceae. These compounds are the grayanotoxins I, II and III and the pieristoxins A, B and C. The most widely distributed member is grayanotoxin I (CVIII),



CVIII

which is better known by its alternative name andromedotoxin. It has also been designated asebotoxin, rhodotoxin and acetylandromedol. Andromedotoxin has been

identified as a constituent of various species of Andromeda, Kalmia, Leucothoe, Lyonia, Pernettya, Pieris and Rhododendron (675), and is the agent responsible for the toxicity of these plants to livestock (676). It is also the toxic principle of the Chinese drug Nao-Yang-hua (677). Andromedotoxin has been positively identified as the compound responsible for the poisonous nature of certain honeys (678), and as the agent present in Rhododendron nectar responsible for the outbreaks of bee poisoning (679).

In mammals the symptoms of acetylandromedol poisoning are emesis and purging with severe respiratory disturbance and convulsions, followed by death through respiratory arrest

The grayanotoxins are of considerable taxonomic interest for although diterpenoids and plant sterols frequently occur together in nature, diterpenoids and true triterpenoids rarely do so. Hence the occurrence of the triterpenoid taraxerol (680), the pieristoxins A, B, C (681) and grayanotoxin III (682) in the leaves of Pieris japonica is of some significance.

Andromedotoxin was early subjected to considerable pharmacological investigation (683). The results of

these experiments showed that it was without effect on unicellular organisms such as Paramoecium and Opalina but that it had digitalis-like action on the mammalian and frog hearts and that it depressed the excitability of the motor nerves of the frog and rabbit, in addition to depressing respiration.

More recently investigations have shown that in many ways andromedotoxin has similar activity to certain of the Veratrum alkaloids (684), possessing hypotensive properties, and producing both direct and indirect positive inotropic effects on the heart. In high doses it produces ventricular arrhythmia, the nature of which has been investigated in detail (685).

Diterpene Alkaloids

The diterpene alkaloids on present day knowledge would appear to fall into four main classes. The first class, comprising the Erythrophleum alkaloids are esters of diterpene acids with alkanolamines. The second class is represented by ryanodine which is an ester of pyrrole-α-carboxylic acid (686) and ryanodol which has been postulated to be a diterpenoid of unusual type (687). The third class is represented by thelepogine which is apparently biogenetically related to manoyl oxide (688).

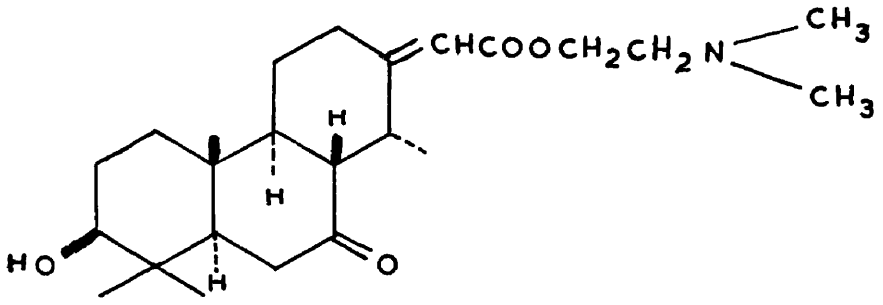
The fourth class, which is the largest, is represented by the Aconitum, Delphinium, and Garrya alkaloids, and in this group the nitrogen atom is bound directly to the diterpenoid carbon skeleton as in thelepogine. These alkaloids (like the monoterpenoid, sesquiterpenoid and steroidal alkaloids) are of considerable interest from the biogenetic point of view as they do not possess structural fragments recognisably derived from essential amino acids as do the vast majority of alkaloids (689). Instead the nitrogen atoms are attached directly to an isoprenoid skeleton and the term "alcaloida imperfecta" has been coined to describe them (690). It is to be noted that the absolute configuration of the diterpene alkaloids corresponds to the kaurene and steviol type and not the phyllocladene type (691).

Within the aconite-delphinium group of alkaloids, two main subclasses are recognised. The first consists of alkaloids with a C_{20} skeleton which are relatively non-toxic and the second of the alkaloids characterised by a C_{19} skeleton which are highly toxic. The C_{19} group in turn consists of a heptacyclic perhydrophenanthrene group represented by deltaline and delpheline (692) and a group with a rearranged hexacyclic perhydrophenanthrene

skeleton represented by lycoctonine and aconitine.

Comprehensive reviews on the chemistry of the Aconitum Garrya alkaloids are available (693), although care must be taken as they employ the mirror image of the true absolute configuration of the alkaloids.

The Erythrophleum alkaoids, typical members of which are cassaine (CIX), norcassaidine, homophleine, erythrophleine and coumingine, have been well investigated



CIX

biologically and all would seem to show qualitatively similar physiological activity (694) although there is quantitative variation. These alkaloids exert a pronounced action on the heart which is similar to that shown by digitalis (26,695). Crude preparations of the Erythrophleum alkaloids have been used clinically in the treatment of congestive heart failure (696), and in his review of the cardiac properties of the Erythrophleum alkaloids McCawlay (697) points out that if these compounds could be obtained cheaply, they hold promise

as digitalis substitutes.

In addition to their cardiac activity the Erythrophleum alkaloids possess local anaesthetic activity (698), increase mammalian blood pressure (both by vaso-constriction (699) and by their action on the heart muscle (700) , and stimulate the isolated rabbit intestine (701,702), and uterus (701,703). They also act on the respiratory and emetic centres. The statement by Tsutsui and Tsutsui (704) in which they attribute the local anaesthetic and cardiac activities of cassaine to cassaic acid is an unfortunate error.

Studies with Erythrophleum alkaloids have afforded excellent examples of species (705) and seasonal variations (706) in drug response.

Ryanodine is obtained from Ryania speciosa Vahl. and crude extracts from this plant are used commercially as an insecticide (707) being especially effective against the European corn borer (Pyrausta nubilalis) (708) and the oriental fruit moth (Grapholitha molesta) (709). The pure alkaloid was isolated in 1948 (710), and has seen considerable pharmacological investigation. Earlier work using crude preparations is summarized by Kuna and Heal (711) and by Procita (712) who, in the course of

detailed pharmacological investigations, showed pure ryanodine to be highly toxic to mammals.

The most remarkable property of ryanodine is its ability to induce an irreversible contracture in the skeletal muscle of mammals and frogs (713) and in mammalian intestinal smooth muscle (714). This action occurs without the resting membrane potential nor the ATP-ase activity being affected and is independent of the innervation. Oxygen uptake is, however, increased (715). Detailed studies of the influence of temperature upon the kinetics of ryanodine-induced contracture have been reported, using the rat diaphragm (716).

With insect muscle ryanodine induces a flaccid paralysis (717) accompanied by an increase in oxygen uptake (717,718). Ryanodine also acts on mammalian cardiac muscle (719) with resultant hypotension, but in this case there is a depression of contractility without contracture. The alkaloid has further been shown to block the spontaneous rhythmic contraction of isolated skeletal muscle cells in tissue culture (720).

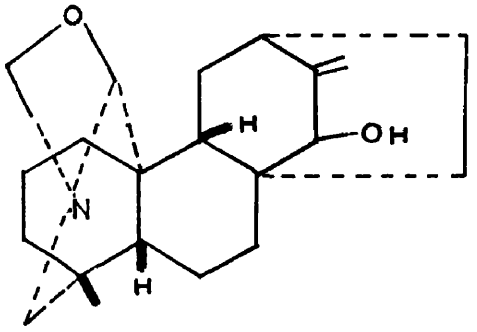
Another interesting action of ryanodine is its ability to block mitosis by retarding the anaphase movement of chromosomes (721). It has also been shown to inhibit

development and depress oxygen uptake in developing embryos of Amblystoma punctatum (722).

Most of the earlier biological work on the aconite and delphinium alkaloids employed crude extracts from the plant, and presumably the activity was mainly due to alkaloids of the C_{19} group. Such galenical preparations were formerly used medicinally as salves in the treatment of neuralgia, arthralgia and inflammation (723), and as anodynes, diaphoretics and diuretics. The Chinese drugs Wu-T'ou and T'sao-wu-t'on are preparations of aconite alkaloids. Preparations of aconite alkaloids were also employed as arrow poisons by the ancient Chinese and Gauls.

The Aconite and Delphinium alkaloids appear to possess insecticidal properties against a variety of insects (724), although not against all insects (725).

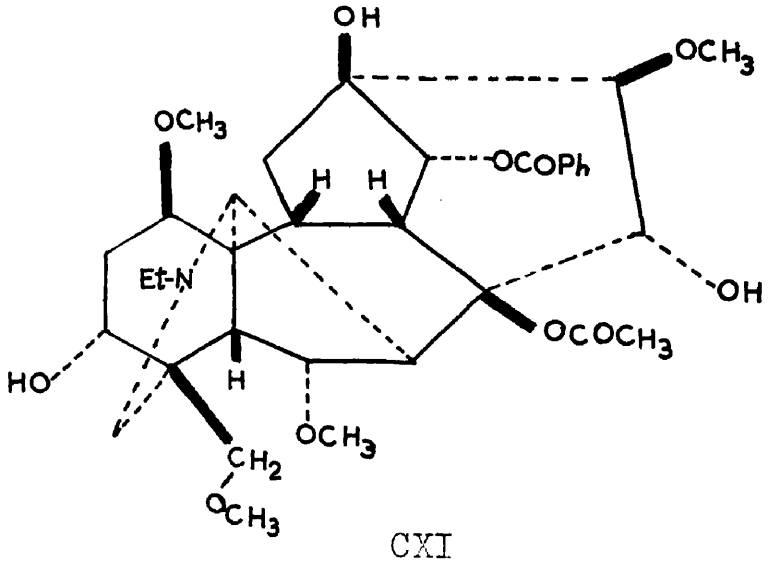
The C_{20} alkaloids of which atisine (CX), garryine,



CX

veatchine, garryfoline and cauchichicine are typical examples, have been shown to be without significant action on smooth muscle, to briefly lower the blood pressure of the anaesthetized cat and to produce respiratory failure when administered in large doses (726). Garryine has also been shown to be devoid of antimalarial activity (727). The most interesting property of atisine is its ability to suppress the bradycardia accompanying adrenaline hypertension whilst increasing the slowing of respiration, thus dissociating these two reflex actions of adrenaline hypertension (728).

The most intensively investigated alkaloid of the C_{19} group from the biological point of view is aconitine, which has been shown to possess structure CXI (729), and



CXI

which is one of the most toxic alkaloids known (730).

Aconitine has anaesthetic properties which produce a peculiar sensation of tingling and numbness. It was this action of aconitine that led to the use of aconite preparations in the treatment of rheumatism and neuralgia. The alkaloid also shows pronounced action on the emetic centre (731).

Systematically aconitine slows the heart by central vagus stimulation and it was at one time employed medicinally in asthenic fevers. Toxic doses arrest respiration and the heart. Numerous detailed studies of the action of aconitine on the heart have been reported (732,733).

The disturbance in cardiac function induced by aconitine are antagonised by antihistamine drugs (734), and the ability of aconitine to produce auricular arrhythmias is utilized in evaluating antifibrillatory drugs (735).

Recent studies with aconitine have been concerned with its action on intracisternal injection (736), and with its effects on aerobic glycolysis (737), and the activity of muscle membranes (738). The morphological changes occurring in acute experimental aconitine poisoning have been reported (739). A concise account of the pharmacology

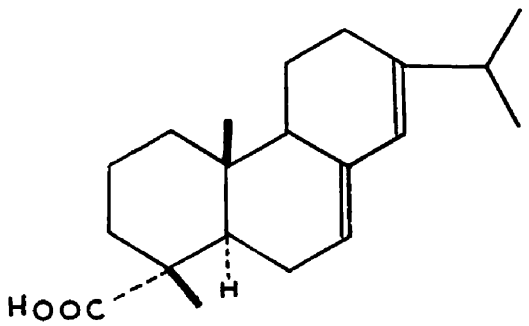
of aconitine is given by Sollmann (740), and a fuller account of the early work is given by Boehm (741). Related alkaloids such as indaconitine (742) and japaconitine (743) have also been well investigated.

Aconine, which is des-acetyl, de-benzoyl aconitine, differs considerably in its activity from aconitine (732,744).

Quaternary salts formed from various alkaloids of the lycoctonine group show the curariform activity characteristic of quaternary nitrogen compounds in general (745).

Other Diterpenoids

Several diterpenoids such as podocarpic acid (746), ferruginol (746), and neoisodextropimaric and 7-isodextropimaric acids (747,748), possess antifungal properties and various metal salts of abietic acid (CXII)



CXII

are bactericides (749) and fungicides (750). Ozonised

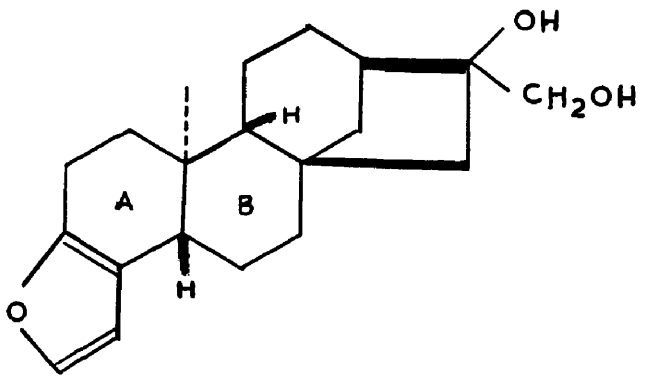
polyethenoxy abietate was tested as a bactericide against Staphylococcus aureus, but found to be relatively inactive (751). Sodium abietate, although active in vitro as an anti-viral compound, has been shown to be inactive in vivo (752).

An interesting application of a diterpenoid as a supporting moiety has been the utilization of several amines derived from abietic acid to prepare new penicillin derivatives (753) which have an extremely low solubility in water, thus leading to prolonged effective blood concentrations. These derivatives can be employed as stable aqueous suspensions for oral use and several are available as proprietary drugs. Penicillin salts with monoterpenoid bases have also been prepared (754).

Various amines derived from abietic acid showed molluscidal activity when tested against Australorbis glabratus (755), and 6-aminodehydroabietinol and its methylated derivatives were prepared as potential morphine-like analgesics (756).

Cafestol (formerly known as cafesterol) (CXIII), which is of considerable chemical interest because the A/B ring junction like that of farnesiferol A is antipodal to that of the steroids and diterpenes of the abietic acid class

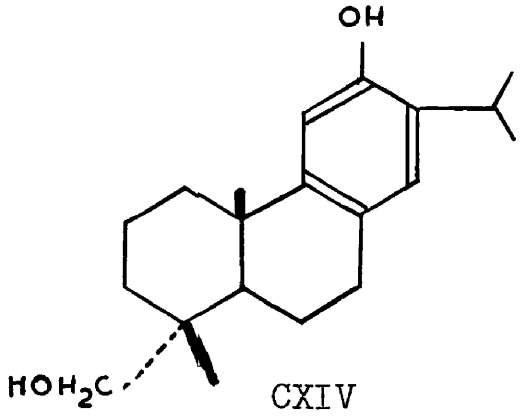
(757), aroused biological interest when it was claimed to



CXIII

possess oestrogenic activity (758). Later work, however, has proved these claims to be erroneous (759).

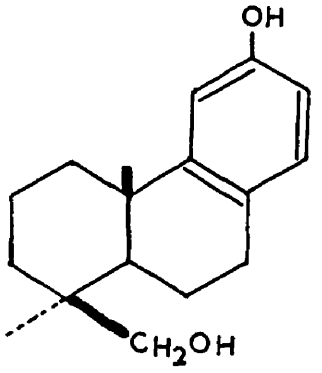
Nevertheless oestrogenic activity does occur within the diterpenoid series. One compound possessing marked oestrogenic properties is 6-hydroxydehydroabietinol (CXIV)



CXIV

which was prepared by Fieser and Campbell (756) in the course of a series of investigations as to the possibility of utilizing abietic acid as a raw material for the synthesis of physiologically active compounds. 6-Hydroxydehydroabietinol does not bear any marked structural similarity to the natural oestrogens but

construction of models shows that the two hydroxyl groups can readily assume an intergroup distance ranging from ca 7.5 A° to ca 10.0 A°. The compound thus supports the contention of Schueler (760) that oestrogenic activity is associated with strong hydrogen bond forming groups such as hydroxyl groups when separated by an optimum distance of ca 8.5 A°, although more recent work (65) would tend to place this distance somewhat higher viz: 10.5 A°. Subsequently Brandt and Ross (761) prepared podocarpinol (CXV) which also was claimed to possess an oestrogenic activity as was 7-isopropylpodocarpinol (762). Other



CXV

diterpenoid oestrogens include certain esters of 6-aryloxyacyldehydroabietic acid which are claimed to be of value in the treatment of hypercholesterolaemia (763).

The effect of cafestol on the metabolism of the rat has been studied (764), and it was at one time recommended for the treatment of psoriasis and eczema (765).

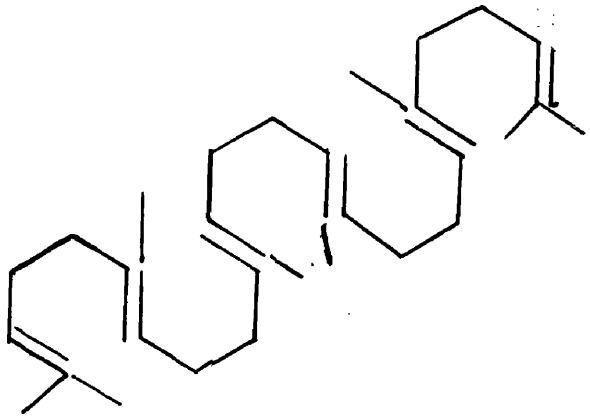
Several diterpenoid compounds were tested as choleretic agents (492) and podocarpic acid, podocarpic acid 0-propionate and 6-carboxymethoxy-1, 12-dimethyl-1,2,3,4,9,10,11,12-octahydrophenanthrene-1-carboxylic acid, were found to have potencies very close to that of dehydrocholic acid. Abietic acid-maleic anhydride adduct was also tested but it showed a lower activity. An interesting observation was that these compounds all produced a fall in blood pressure immediately after injection.

TRITERPENOIDS

As a class the triterpenoids are somewhat less interesting biologically than the lower terpenoids when consideration of the steroids is omitted. The exact function of the triterpenoids in the economy of the plant kingdom, like that of the diterpenoids, is not well understood. The possibility exists, perhaps, that phosphorylated precursors play a role in the transport of the water insoluble protective plant waxes to the exterior and so fulfil a function somewhat analogous to that of the bile salts in animals. Evolutionary differences in the ability to reabsorb and recycle the phosphorylated compounds could be invoked to explain the

different ratios of higher terpenoids to paraffins and straight chain esters found in different plants.

Squalene (CXVI) has been extremely well studied in

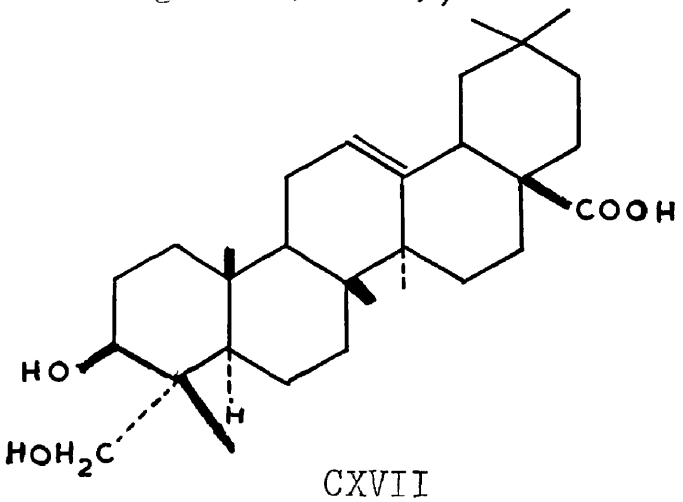


CXVI

connection with its role as a steroid precursor but several other interesting actions have been reported. For instance it has been assigned a possible role as a protective agent in sebum on account of its mild fungistatic (766) and antibacterial (767) properties. It is also thought that peroxidised squalene may play a defensive role against carcinogens (768). Another interesting hypothesis is that squalene may be involved in the aetiology of baldness (769) for like vitamin A, lycopene and carotene it has been shown to have a depilatory action on animals (770). Squalene diperoxide has been shown not to function as an antimetabolite in the biosynthesis of cholesterol (771). A review of the

biological properties of squalene has appeared (772).

Various triterpenoid saponins such as hederin (773), a glycoside of hederagenin (CXVII), have seen pharmacological



CXVII

study. In general these saponins are bitter and exert an irritant action on the mucous membranes. They are also haemolytic agents (774).

The saponins hydroxyasiaticoside and asiaticoside have been reported to possess weak antituberculous properties (775) and asiaticoside has seen clinical trial in the treatment of leprosy (776). The aglycone, asiatic acid, is however inactive (777). Asiaticoside is further claimed to stimulate the reticuloendothelial system and so promote wound healing (778), and produce a thickening of the skin with increased vascularisation of the connective tissue in healthy animals (779). Glycyrrhizinic acid has, however, been claimed to decrease function of the reticuloendothelial system (780).

The adverse effect of high concentrations of triterpenoid saponins in the diet on the growth of chicks has been demonstrated (781). This action is believed to occur as a result of the interference with cholesterol absorption from the intestine. A similar effect is observed with trimethyl sterols of wool fat (782).

It has been concluded that triterpenoid saponins play an important contributory role in the production of bloat in ruminants grazing on legumes (783), although a complex combination of factors is involved (784). Work with the sapons from alfalfa (Medicago sativa) which is known to contain triterpenoid saponins (785) would indicate that a three-fold mechanism is involved in which fermentation by the rumen bacteria (786), interference with the mechanism of eructation (787) and the formation of froth play a part. Other workers, however, have not been able to induce experimental bloat with triterpenoid saponins (788). Detailed toxicity studies of the alfalfa saponins have shown that they produce congestion of lung tissue and inflammation, haemorrhage and hyperaemia of the small intestine and abomasum (788). Severe kidney and liver damage also result. Other investigations have been concerned with the effects of the alfalfa saponins on the

cardiovascular and central nervous system and on the motility of the intestines and ruminant stomach (789).

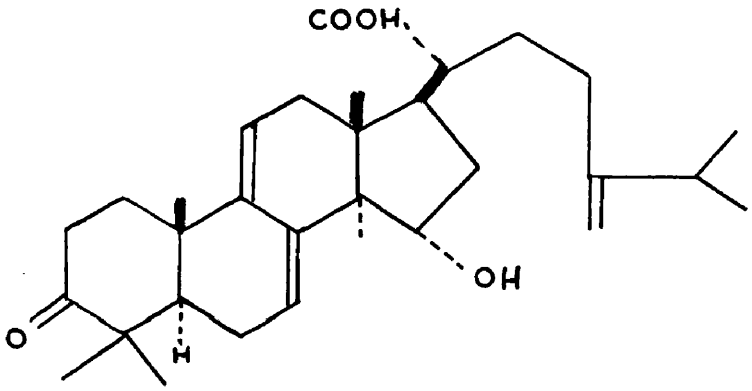
The report that glycosides of ursolic acid and oleanolic acid were responsible for the cardiotonic and diuretic action of the leaves of Nerium odorum (790) is obviously in error. These properties are attributable to steroidal cardiac glycosides which occur both in the leaves (791) and the bark (792) of the plant.

Similarly the claims that the triterpene acids, oleanolic, ursolic and crataegolic acids, have a beneficial action on coronary flow (793) appear to be without foundation as other workers have found them entirely devoid of pharmacological activity (794). Glycyrrhetinic acid has also been claimed to exert an effect on the heart (795), but once again later work using the potassium salt of glycyrrhizinic acid failed to show any positive cardioactivity (796).

The role of glycyrrhetinic acid as a potentiator of the adrenocorticoid steroids has already been discussed. Other investigations have shown that it possesses bacteriostatic properties (797). A detailed study of its general pharmacology has been made by Tocco-Tocco (798), and a further interesting property of the compound is its ability

to effect detoxification of tetanus toxin (799).

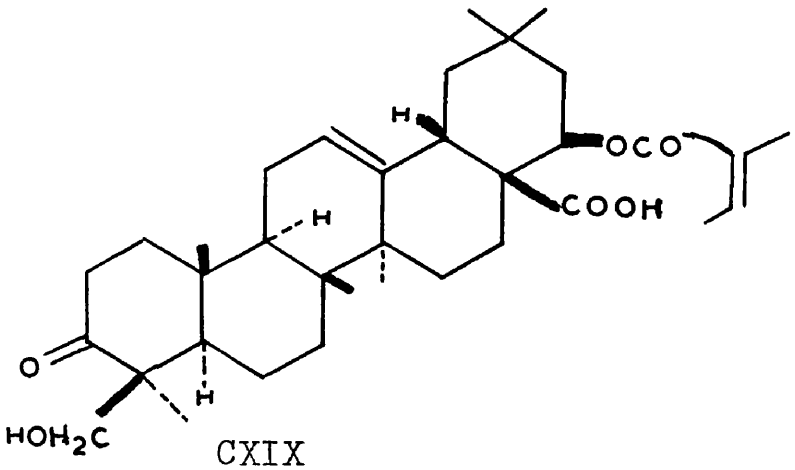
The triterpenoids obtained from Polyporus betulinus have been claimed to inhibit the growth of neoplastic cells (800). Polyporenic acid C (CXVIII) has been shown to possess antibacterial activity (801) and the alkali



CXVIII

metal salts of various triterpenoid acids are reported to be highly toxic to fish (802). Oleanolic acid fed to Drosophila melanogaster has been reported to lead to a marked increase in propagation (803).

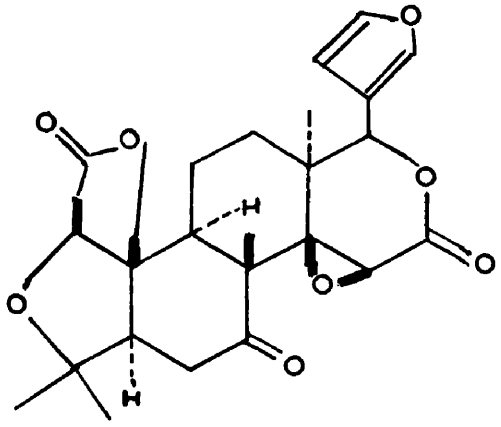
Two triterpenoids, lantadene A (syn rehmannic acid) and icterogenin (CXIX) are of somewhat unusual interest as they



CXIX

have been shown to produce liver damage in sheep leading to an inability to excrete phylloerythrin (a degradation product of chlorophyll formed by microbial action in the fore-stomach) with the subsequent production of photosensitivity (804,805). Icterogenin also shows a paralytic action on isolated rabbit duodenum (804).

Various triterpenoids such as the cucurbitacins and compounds like limonin (CXX), which are believed to arise



CXX

in nature from triterpenoid precursors (806), constitute the bitter principles of some plants.

The cucurbitacins are highly oxygenated bitter triterpenes belonging to the trimethyl steroid group (807) and are of widespread distribution in the family Cucurbitacaae where they occur as glycosides.

Various plants of the Cucurbitaceae have been used medicinally (808) and cucurbitacin E or α-elaterin is a

constituent of elaterium which is one of the most drastic purgatives known, and which at one time was used in the treatment of uraemia. The cucurbitacins are cytotoxins and some have been shown to have a necrotizing effect on experimental tumours (809). Toxicity determinations on pure cucurbitacins A, B and C have been reported (810), and it has been shown that these compounds produce acute pulmonary oedema at or near the lethal dose.

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Section II.

II. Hemisuccinates of Mono-, sesqui- and di- terpenoids as potential general anaesthetic agents.

It is now over 100 years since anaesthesia was first introduced into medicine by Crawford W. Long in Georgia, William T. G. Morton in Massachussetts and James Y. Simpson in Edinburgh, but despite the expenditure of much time and effort, there is still no completely satisfactory explanation as to the precise mechanism of action of anaesthetic agents. Of the various theories which have been proposed, each suffers from certain shortcomings, and in an attempt to further our knowledge it was considered desirable to investigate certain hemi-succinate derivatives of predominantly lipid-soluble alcohols as potential general anaesthetics. The choice of mono-, sesqui- and di- terpenoid alcohols for this project was influenced by the fact that terpenoids do not appear to have been fully utilised in fundamental studies of drug action, as has been made clear in the introduction of this thesis, and also by the fact that such compounds possess molecular weights of a particularly suitable order of magnitude as will be apparent from the following discussion.

One of the first attempts to explain the mode of action of general anaesthetics was that of Overton and Meyer (1) who emphasized the need for pronounced lipid solubility in a general anaesthetic agent. Accordingly their ideas have become known as the Overton-Meyer Lipid Solubility Theory. This theory obviously is unable to take account of the activity of certain ions such as Mg^{++} and Br^{-} without some modification, but by and large, the need for lipid solubility in an organic general anaesthetic is still accepted today. Inorganic ions can be accommodated if one considers general anaesthetics to affect the properties of cell membranes where certain ions are closely associated with a structure showing both ionic and lipid properties. Mullins (2) has given a generalised picture of the cell membrane, which, even if not a literal representation of the actual state of affairs, does permit us to see how both ions and lipid soluble molecules can alter the normal state of the membrane.

Overton and Meyer further suggested that there was a relationship between anaesthetic potency and the distribution coefficient of the compound between olive oil or cottonseed oil and water and considered that these oils could be taken as representative of the brain lipids. Indeed they

found a good correlation between anaesthetic activity towards tadpoles and the olive oil/water distribution coefficient for a limited series of compounds which they termed " indifferent chemical agents ". However, certain substances such as camphor which possess favourable distribution coefficients are not anaesthetic agents, but central nervous system stimulants inducing convulsions. Although at first sight these compounds would appear to contradict the Overton-Meyer theory it must be borne in mind that central nervous system excitation is a property of many general anaesthetic agents themselves at low dosage levels and that one of the problems confronting the anaesthetist is to ensure that the patient quickly passes through Stage II of anaesthesia (the excitement stage) into Stage III. Again slight structural modifications to the side chains of certain barbiturates may lead to convulsant and not depressant properties (3). The explanation for the excitement stage in general anaesthesia lies in the fact that depression of the central nervous system occurs progressively from the higher centres down (4) and that inhibitory mechanisms are paralysed before the mechanisms which they inhibit. Thus central excitation may in fact result from a selective depressant action on the cerebral hemispheres facilitating

functional release of the normally inhibited subcortico-diencephalic layer with resultant increase in motor activity (5).

The Overton-Meyer theory is probably best regarded,not as a theory offering any basic explanation of the mechanism of anaesthesia, but rather as an expression of the experimentally observed fact that general anaesthetic activity is often present in lipid soluble compounds. There would seem to be a case for considering lipid solubility as related to an "affinity" with a secondary property, the "intrinsic activity", determining the degree of central nervous system depression evoked by the drug. Ariens has introduced these concepts to afford a clear picture of drug action in other situations including action at the neuromuscular junction (6) and at the neuro-effector junctions of the parasympathetic system (7).

An extension of the Overton-Meyer theory was advanced in 1939 by Ferguson (8) who treated the problem from a thermodynamic standpoint. Ferguson pointed out that if narcotic action was primarily dependent upon a suitable equilibrium between the concentration of the drug in an external phase (e.g. aqueous body fluids) and the affected phase (e.g. the brain lipids) then equi-potency should

result when different narcotic agents were present in the affected phase in such concentrations that their thermodynamic activities were the same. Moreover there is no need to know the exact nature of the affected phase nor to create artificial models of it, since provided there is equilibrium between the external and affected phases the partial molal free energies in each phase for any given narcotic agent will be identical.

$$\text{Ie} \quad \bar{F}_{Ext} = \bar{F}_{BIO}$$

and as $$\bar{F}_{Ext} \underset{def}{=} F_O + RT \ln a_{Ext}$$

and $$\bar{F}_{BIO} \underset{def}{=} F_O + RT \ln a_{BIO}$$

then $$a_{Ext} = a_{BIO}$$

where $\bar{F}_{Ext}$ = partial molal free energy in the external phase

$\bar{F}_{BIO}$ = partial molal free energy in the biophase

F_O = partial molal free energy in the standard state which is taken as the pure substance

R = gas constant

T = temperature in degrees Kelvin

a_{Ext} = thermodynamic activity in external phase

a_{BIO} = thermodynamic activity in biophase

The value of a_{Ext} can be measured from a knowledge of the partial and saturated vapour pressures where the anaesthetic is applied as a vapour $\left(a_{Ext} = \frac{Pt}{Ps}\right)$ and from a knowledge of the actual molal concentration and the saturated concentration at the same temperature where the anaesthetic is applied in solution $\left(a_{Ext} = \frac{Ct}{Co}\right)$, if activity coefficients are ignored.

The basic assumption in Ferguson's treatment, namely that true equilibrium pertains in general anaesthesia would seem valid in view of the fact that maximum physiological effect is rapidly attained and remains at the same level of intensity so long as the organism is in contact with a constant effective concentration of the drug. On removal at any time to drug-free surroundings, recovery is rapid. Moreover Ferguson's experimental results are in good agreement with his basic premise as not only did equipotency of action occur at virtually identical thermodynamic activities for homologous series such as n-alkanes, primary alcohols, and certain esters, ethers, ketones and amides before cut off was reached due

to insolubility, but a number of chemically unrelated general anaesthetics were equipotent when their thermodynamic activities fell within the narrow range of 0.01 - 0.07. These compounds included nitrous oxide, acetylene, dimethyl ether, diethyl ether, methyl chloride, ethyl chloride, ethylene oxide, methylal, dimethylacetal, diethyl formal, dichloroethylene, carbon disulphide and chloroform.

The diverse chemical nature of these general anaesthetic agents has led to several workers (9) classifying pharmacologically active compounds into 2 groups, the structurally non-specific and the structurally specific. With structurally specific drugs minor changes in the stereochemistry or electron density are accompanied by profound changes in activity - as is often observed with individual optical antipodes. For example the l form of morphine is the active analgesic, the d form being entirely devoid of analgesic properties (10). With structurally non-specific drugs such changes in electron density and stereochemistry are without appreciable effect as evidenced by the general anaesthetics previously mentioned. Although structural specificity is properly regarded as relating to molecular structure, it so happens

that structurally non-specific drugs tend to be less selective in their actions than structurally specific drugs, and they also tend to exert an effect on a wide variety of biological phenomena. Complete specificity of action is never encountered in drug action, however, as is emphasized by the theory of biological relativity (11).

A number of other theories relating anaesthetic activity to physical properties have been advanced. One such theory seeks to relate hypnotic activity to the Van der Waal's volume constant as when the compounds studied were listed in order of increasing molecular volume, it was found that hypnotic activity also increased in the same order (12,13). Helium and neon apparently have too small a molecular volume, as unlike the other rare gases, they are inactive as anaesthetic agents. Other theories of narcosis have postulated that anaesthesia results from lowering of surface tension (14) or decrease in cell permeability (15) but these theories are not considered seriously today. On the one hand it has been shown (16) that the surface tension between a cell-membrane and the water surrounding it is already zero so that no further lowering is possible

and on the other hand decrease in cell permeability is not universally observed with narcotics (17).

Several attempts have been made to more closely define the sequence of events once the anaesthetic agent has gained access to the biophase and foremost amongst these is the theory that production of anaesthesia is dependent upon blockade of oxidative carbohydrate metabolism. Indeed Quastel and his collaborators (5) have demonstrated that there is a parallelism between the hypnotic potency of certain barbiturates and their ability to depress the oxygen consumption of minced guinea pig brain, but the question remains as to whether such inhibition of respiration in brain tissue is the cause or the result of anaesthesia. Not all oxidative processes are affected - the main inhibitions occurring with glucose lactate and pyruvate - and not all drugs which inhibit oxygen uptake are anaesthetics (17). More detailed studies have been concerned with the effect of narcotics upon cytochrome oxidase (18), flavoprotein (19) the synthesis and release of acetylcholine in the brain (20,21) and upon the synthesis of ATP (22,23) but the overall results obtained have not given rise to any clarification of the position.

The demonstration that a number of predominantly lipid-soluble steroids possessed general anaesthetic properties (24) posed the question as to whether certain molecules (other than the barbiturate and oxazolidine 2;4 dione type) possessing molecular weights lying between those of the simple gaseous anaesthetics and the steroids would exhibit structurally non-specific narcotic activity if they could be introduced into the central nervous system in sufficient concentration. In view of the successful clinical introduction of hydroxydione (21-hydroxy pregnane- 3, 20-dione) in which the hemisuccinate moiety confers a high initial aqueous solubility on the compound before the molecule is rapidly hydrolysed by the non specific esterases of the serum permitting the parent lipid soluble steroid to cross the blood-brain barrier and build up an anaesthetically-active concentration (25) it was decided to prepare a number of hemi-succinates of representative mono-, sesqui- and di-terpene alcohols. Such terpene alcohols would be expected to be structurally non-specific agents and moreover it is well established that a number of terpenoids exhibit central nervous system stimulation in low doses and depression at higher doses. Other terpenoids such as camphor and thujone exhibit predominantly stimulant properties. This could be due

either to a low intrinsic activity or to their being present in too low a concentration for depression to be evoked. It is to be noted that in the steroid field, whilst the majority of steroids act as central nervous system depressants - a few such as cortisone produce stimulation.

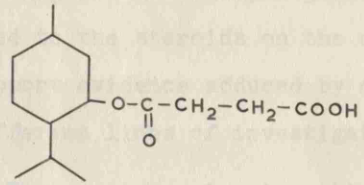
Encouragement was given to the current project involving the terpenoid hemisuccinates by the fact that the hemisuccinate of π hydroxycamphor has been reported to be a hypnotic (26).

The terpenoid hemi-succinates were all prepared by refluxing the requisite alcohol with succinic anhydride in a basic solvent (pyridine or quinoline). The formulae of the compounds studied are shown in Fig.1 together with those of several long chain alcohol hemisuccinates which were included in the investigation for the purposes of comparision.

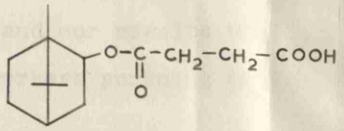
The compounds prepared were kindly tested by Dr. K. Ahmad and found to be inactive as general anaesthetics on intraperitoneal injection into rats at dose levels of 5-10 mg/100g of body weight (27).

Hence it would appear that there is not a continuous spectrum of structurally non-specific anaesthetic activity

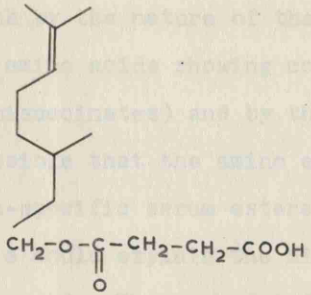
Fig.1.



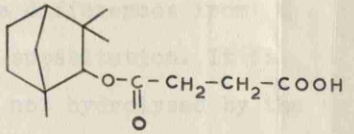
1. Menthyl hemisuccinate.



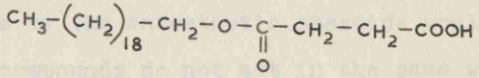
2. Bornyl hemisuccinate.



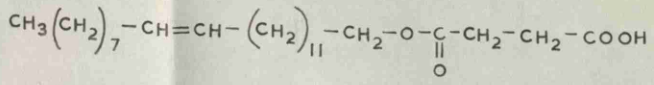
3. Citronellyl hemisuccinate.



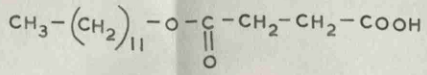
4. Fenchyl hemisuccinate.



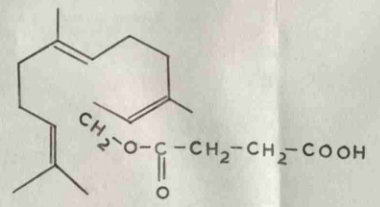
5. Arachidyl hemisuccinate.



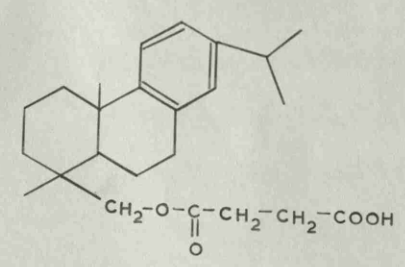
6. Erucyl hemisuccinate.



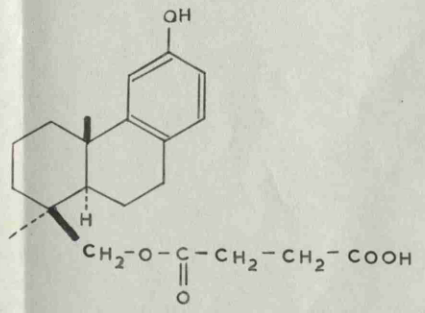
7. Dodecyl hemisuccinate.



8. Farnesyl hemisuccinate.



9. Dehydroabietyl hemisuccinate.



10. Podocarpyl hemisuccinate.

running from the simple gaseous anaesthetics on the one hand to the steroids on the other, and our results would support evidence adduced by other workers pursuing entirely different lines of investigation.

Thus an extensive investigation of the pharmacological properties of the hemisuccinate of glycyrrhetinic acid prompted no mention of anaesthetic properties (28) and the work of Figdor and his acollaborators (25) has shown that anaesthetic potency in the steroid field is influenced both by the nature of the water-solubilising group (esters of amino acids showing considerable differences from hemisuccinates) and by the nuclear substitution. It is possible that the amino esters are not hydrolysed by the non-specific serum esterases as are hemisuccinates and this could explain the high acute toxicity of these compounds. The marked influence of nuclear substitution on activity (25) however would perhaps indicate a structurally specific mechanism. On the other hand, the influence of stereochemical changes appears to be slight.

Moreover studies on the inhibition of oxidation in brain tissue by the anaesthetic steroids would indicate that these compounds do not act in the same way as do other anaesthetics (29).

Experimental

(-) Menthyl hemisuccinate (30)

(-) Menthol (15.0 g.) in dry freshly distilled pyridine (150 ml.) was refluxed with succinic anhydride (10.0 g.) with exclusion of moisture. After 6 hrs. the pyridine was distilled off, the menthyl hemisuccinate taken up in ether and the ethereal solution washed once with water and once with dilute hydrochloric acid. The product was then extracted with cold aqueous sodium bicarbonate and the solution carefully acidified with dilute hydrochloric acid and extracted with ether. The ethereal solution was dried and after removal of the solvent the product was sublimed and recrystallised from ether (10.2 g., 40.8 %) with m.p. 64° [Lit., (31) 62°; (32) 64°].

(+) Bornyl and citronellyl hemisuccinates

(+) Borneol (15.4 g.) and (+) Citronellol (12.6 g.) were converted into their corresponding hemisuccinates by refluxing for 6 hrs. with 10.0 g. and 8.0 g. succinic anhydride respectively, in redistilled quinoline (150 ml.), followed by standing overnight. Isolation in the usual manner gave :-

(+) Bornyl hemisuccinate (33) m.p. 60-67° (11.6 g., 45.6%) [Lit., (33) 61-2°] and

Citronellyl hemisuccinate m.p. 60-70° (5.99 g., 29.1%)

Fenchyl hemisuccinate

As preparation of fenchyl alcohol by direct catalytic hydrogenation of fenchone in acetic acid with a platinum catalyst in the presence of perchloric acid proved unsatisfactory, the following procedure was adopted.

Fenchone (15.0 g.) was refluxed with lithium aluminium hydride (11.0 g.) in 400 ml. of anhydrous ether for 6 hrs. Excess lithium aluminium hydride was destroyed by careful dropwise addition of water and the fenchyl alcohol reclaimed from the ether. This was converted to the hemisuccinate by refluxing with succinic anhydride (10.5 g.) in quinoline (150 ml.) and the product (10.3 g., 40 %) recrystallised from benzene with m.p. 115-120°.

Arachidyl-, erucyl-, and dodecyl- hemisuccinates

These three straight chain esters were prepared by refluxing the alcohol (1 mole) with succinic anhydride (1 mole) in freshly distilled quinoline and isolating the products as previously described.
Arachidyl hemisuccinate (52.5 %) had m.p. 88-96° ; erucyl hemisuccinate (38 %) had m.p. 45-54° and dodecyl hemisuccinate (39 %) had m.p. 66-74° .

Farnesyl hemisuccinate

Refluxing farnesol (4.4 g.) with succinic anhydride (1.9 g.) in freshly distilled quinoline (150 ml.) yielded a dark oil (3.1 g., 49 %) which could not be induced to crystallise. The sodium salt was semi solid.

Two diterpenoid alcohols prepared by reduction of the carbonyl functions of podocarpic acid and dehydro abietic acid were converted into their hemisuccinates. The acids as their methyl esters were reduced by refluxing with lithium aluminium hydride (2-3 g.) for 6 hrs. in anhydrous ether. Excess lithium aluminium hydride was destroyed by dropwise addition of water and the products were extracted with ether.

The alcohols, podocarpyl (2.8 g.) and dehydroabietyl (3.4 g.) were then converted to the hemisuccinates with succinic anhydride (1.1 g. and 1.19 g. respectively) in 150 ml. quinoline and crystallised.

Podocarpyl hemisuccinate (1.9 g., 48.7 %) with m.p. 205-15°.

Dehydroabietyl hemisuccinate (3.0 g., 65 %).

The infra red spectra of the hemisuccinates in nujol showed broad absorption bands between the region 3400-3180 cm^{-1} and sharp peaks at 1734 cm^{-1}, and 1250 cm^{-1} characteristic of both the ester and carboxylic acid functions.

All the hemisuccinates synthesized in the above manner were converted to their sodium salts by treating them with equimolecular proportions of sodium bicarbonate in aqueous solution and shaking vigorously for 30 mins. The aqueous solutions were treated with ether to remove impurities, separated from the ethereal layer and evaporated to complete dryness. The sodium salts were obtained as white powders, freely soluble in water.

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Section III.

III. Utilization of the Bornane Skeleton as a Rigid Molecular Framework for the Investigation of Acetylcholine-like action.

Acetylcholine as a neurohormone

Perhaps the greatest single fundamental advance in the science of Pharmacology was the establishment of the fact that conduction and translation of nerve impulses involves the intercession of "chemical transmitters" or neurohormones, as this discovery enabled the action of a large number of drugs to be rationally interpreted in terms of their interference with the normal physiological functioning of these neurohormones. The existence of chemical mediators in nervous conduction was first suggested by Elliot (1) who postulated the existence of sympathin to explain transmission at the sympathetic neuroeffector junction. The same concept was subsequently applied to parasympathetic innervation by a number of workers. At first muscarine was thought to be the parasympathetic transmitter, although Hunt and Taveau (2) and Dixon (3) had early appreciated the similarities between the effects of acetylcholine and those of parasympathetic stimulation. Following the thorough examination of the pharmacological properties of

acetylcholine by Dale (4) and Loewi (5) acetylcholine correctly became considered the parasympathetic transmitter, and in 1936, Dale, Feldberg and Vogt (6) were able to demonstrate the existence of acetylcholine in the perfusion fluid from the mammalian neuromuscular junction following stimulation of the motor nerve.

In addition to sympathin (which is actually a mixture of noradrenaline and adrenaline) , and acetylcholine whose role as neurohormones in the peripheral nervous system is now well established, three other compounds, histamine, 5-hydroxytryptamine and γ-aminobutyric acid have been postulated to act as chemical transmitters (7) but so far unequivocal proof that these compounds are indeed neurohormones has not been forthcoming.

Whereas sympathin appears to be involved in only three situations in the body viz,, in the central nervous system, at the neuroeffector junctions of a number of nerves belonging physiologically to the sympathetic division of the autonomic nervous system and as a secretion by the cells of the adrenal medulla released into the blood stream, acetylcholine is known to be involved in seven separate situations. These are :-

1. In the brain where its exact role is still not fully ascertained.

2. At the synapses of the ganglia of the sympathetic nervous system.

3. At the synapses of the ganglia of the parasympathetic nervous system.

4. In the cells of the adrenal medulla which are in actual fact modified ganglionic cells.

5. At the neuroeffector junctions of all post-ganglionic nerve fibres belonging to the parasympathetic nervous system.

6. At the neuroeffector junctions of certain post-ganglionic nerve fibres belonging anatomically to the sympathetic nervous system, notably those innervating the sweat glands and certain vascular beds of the skin.

7. At the neuroeffector junctions of the voluntary nervous system, i.e. at the neuromuscular junctions.

In order to illustrate the precise role of acetylcholine in nervous transmission, one can consider the emission of a nerve impulse from the central nervous system along a fibre belonging to the parasympathetic system. With the arrival of the nerve action potential at the terminals of the preganglionic axon, acetylcholine is liberated from an intra-axonal storage site, and acts initially at the

same presynaptic terminals to bring about the liberation of more acetylcholine. The secondarily released, increased amount of acetylcholine then acts at the post synaptic site to initiate transmission in the post ganglionic fibre, i.e. the acetylcholine crosses the narrow synaptic cleft and combines with receptor groups on the ganglion cell membrane, causing a localized non-propagated depolarization, known as the post-synaptic potential. This in turn initiates electrogenically a nerve action potential, which is propagated along the post ganglionic fibre. The polarized state of the post synaptic membrane is restored with the rapid destruction of the synaptic transmitter by the enzyme, acetylcholinesterase (8). When the post ganglionic impulse arrives at the neuroeffector junction acetylcholine is again liberated, crosses the gap between nerve terminal and the effector cell, affects the resting potential of the latter and brings about the characteristic response of the effector cell.

A similar situation pertains at the neuromuscular junction of voluntary nerve which has been studied in great detail. Thus, histological studies of the neuromuscular synapse have revealed an intensive branching of the motor nerve prior to its termination (9), and a

lack of direct cytoplasmic continuity between nerve endings and the muscle fibre (10). The presence of vesicles in the terminal portions of the motor neurons has also been established (11), and it is presumed that these store the bound acetylcholine.

The process of liberation of acetylcholine is however neither fully understood nor easily investigated (12) but it is known to be influenced by the concentration of certain cations in the environment (13). Moreover a spontaneous and intermittent release of discrete quanta of acetylcholine occurs at rest (14) continually eliciting minute changes in the potential of the post junctional membrane and it is only the synchronous release of a large number of quanta of acetylcholine which is sufficient to excite the muscle.

This excitatory depolarizing effect produces alterations in the permeability of the membrane to inorganic ions (15) and produces the so called "spike" action potential propagating in both directions along the membrane of the muscle fibre inducing contraction.

An excellent summary of present day knowledge of events at the neuromuscular junction has been given by Muir (16).

Drugs Interfering with the Function of Acetylcholine.

Drugs capable of interfering with the action of acetylcholine may act in one of several ways :-

1. By blocking the synthesis of acetylcholine e.g. the hemicholiniums (17).

2. By preventing the liberation of acetylcholine from its bound storage form,e.g. botulinum toxin (18).

3. By interference with the formation of the complex between acetylcholine and its receptor e.g. tubocurarine and decamethonium. These two drugs represent two different modes of action, one, tubocurarine being termed a "competitor" and the other a "depolariser". These different mechanisms of action have been rationalised in terms of intrinsic activity values (19), i.e. the depolarisers mimic the action of acetylcholine and the competitors antagonise it.

4. By blocking the response to acetylcholine through occupation of receptors other than those utilized by acetylcholine itself e.g. the "non-competitive" neuromuscular blocking agents, prodeconium (20).

5. By preventing the destruction of acetylcholine by acetylcholinesterase thus allowing the acetylcholine to accumulate and exert its effect in an exaggerated manner,

e.g. neostigmine, eserine and various organic phosphate esters.

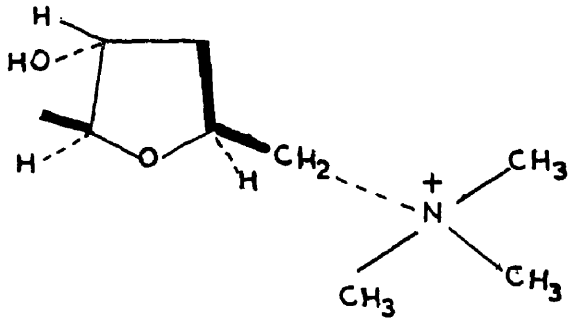
At first sight it would perhaps appear that drugs capable of interfering with the normal function of acetylcholine might do so at all the seven sites in the body where acetylcholine is implicated but in practice a certain degree of selectivity is observed. Complete selectivity for site of action does not occur, however, as is emphasized in the theory of biological relativity (21) and it is found that drugs which act by interference with acetylcholine at the neuromuscular junction often interfere with acetylcholine at the autonomic ganglia. The fact that drugs blocking the action of acetylcholine at the synapses of the sympathetic ganglia also usually block the action of acetylcholine at the synapses of the parasympathetic ganglia is one of the drawbacks to attempts to treat hypertension clinically by blockade of the sympathetic ganglia.

Nevertheless by and large it is possible to distinguish separate classes of drugs which act by interfering with the action of acetylcholine at different sites.

Thus the pharmacologist classifies drugs known to interfere with the action of acetylcholine as follows :-

1. Muscarinic

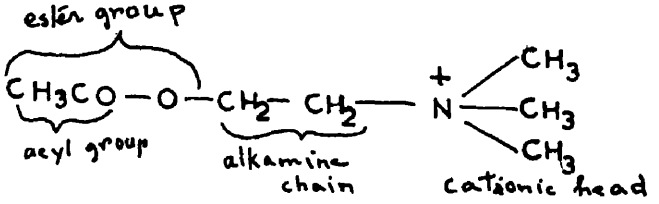
This group embraces those drugs such as the alkaloid L(+)-muscarine (I) (22) which mimic or intensify the action



I

of acetylcholine on smooth muscle and the exocrine glands i.e. at the parasympathetic neuroeffector junctions and certain sympathetic neuroeffector junctions.

Many attempts have been made to interpret the requirements for muscarinic activity in terms of structural modification of the acetylcholine molecule and these are summarized by Barlow (23), who considers the acetylcholine molecule as divisible into 4 parts, the cationic head, the alkamine chain, the acyl group and the ester group (II). The cationic head is essential for



II.

muscarinic activity but it need not be an ammonium ion as activity is also present in sulphonium, phosphonium and arsonium compounds, although the potency is greatly diminished (24).

It has long been known that replacement of the N-methyl groups of the acetylcholine molecule by ethyl or higher radicals causes decrease in its stimulant properties (25) and Ing (26) has stressed the importance of the size of the cationic head. He has pointed out that if the molecule is pictured as being adsorbed at a surface (Fig. 1) then only half the head is actually involved in the process, the other half will be free. Replacement of one methyl group attached to the nitrogen atom will lower the adsorbability, and hence the potency, because the probability of the head being in the right position for adsorption (with both methyl groups presented to the surface) will not be unity. Replacement of another methyl group, will be disasterous, as there will no longer be even half a "head" which fits the receptor surface.

The alkamine chain of 2 carbon atoms in acetylcholine is of optimal length for muscarinic activity and lengthening or shortening it leads to reduced potency. The introduction of methyl groups has pronounced effects.

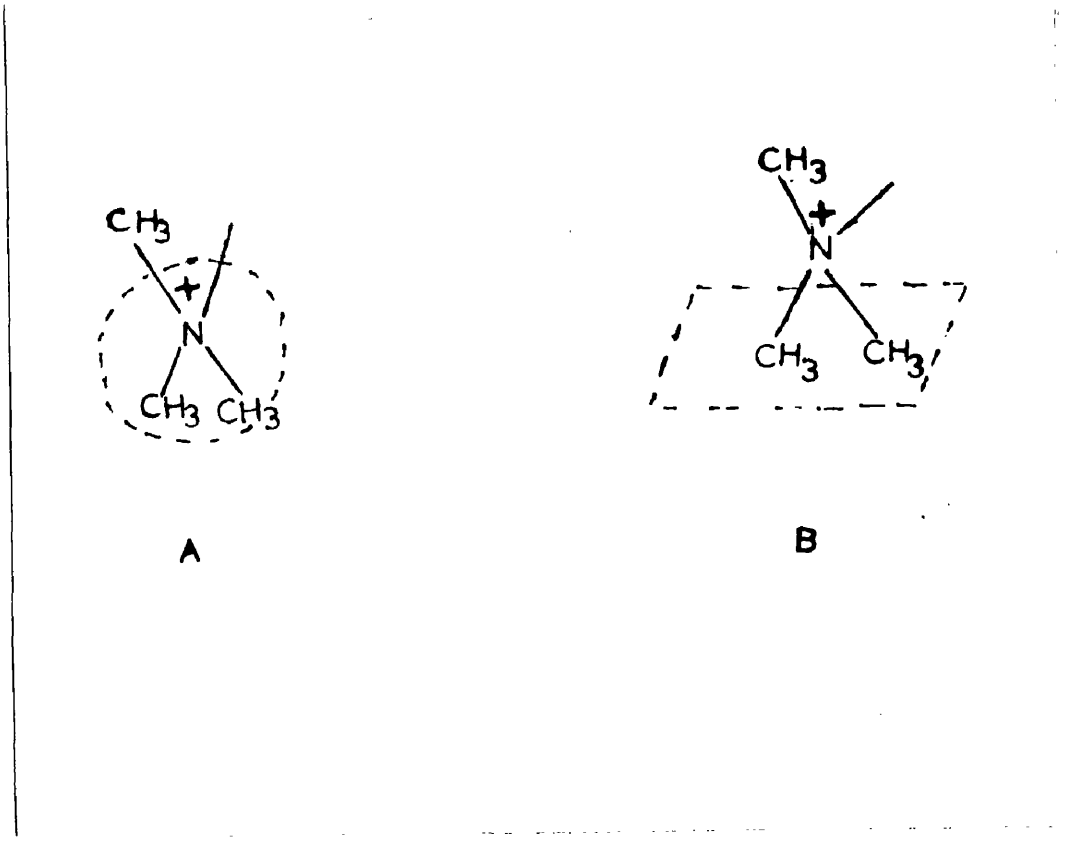
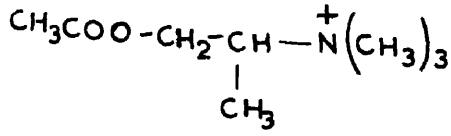


Fig.1.

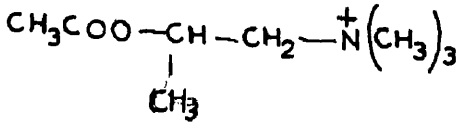
A. At hemihedral cavity. B. At a planar surface.

Thus acetyl ∝- methylcholine (III) has (23) only 1/20 th



III

of the muscarinic activity of acetylcholine and acetyl β - methylcholine (IV) has half the muscarinic activity of



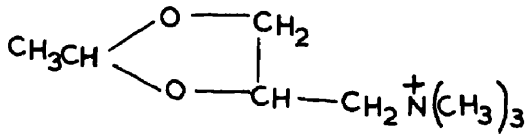
IV

acetylcholine.

The acetyl group appears to be the optimal acyl group for muscarinic activity as replacement by other acyl radicals leads to a pronounced loss of potency (27). It is to be noted, however, that choline, tropine and 1-methyl-3-piperidylmethanol, in which the acyl group is completely absent show some muscarinic activity. Very low muscarinic activity is present in phenylacetylcholine and phenylpropionylcholine (28).

The ester link of acetylcholine can be replaced by certain other groups with retention of muscarinic properties. Thus the n-butyltrimethylammonium ion and the

n-amyltrimethylammonium ion show acetylcholine-like action on certain heart preparations (29) and the ethoxyethyl-trimethylammonium ion has potent muscarinic properties. The compound F2268 (V) (30) has 10-50 times the muscarinic

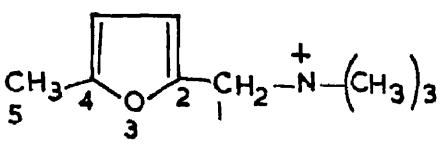


V

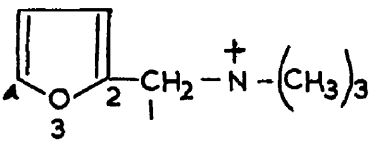
activity of acetylcholine itself.

It would also appear that the presence of an ether oxygen or thioether sulphur atom favours muscarinic activity (31).

In an attempt to briefly summarize the molecular requirements for muscarinic activity several workers (32) have proposed the 5-atom rule which states that for muscarinic activity there should be 5 atoms in a chain bearing a cationic head. This rule has been applied to explain why compound (VI) shows strong muscarinic properties



VI



VII

whereas compound (VII) does not.

Other workers have attempted to express the molecular requirements for muscarinic activity in terms of a detailed representation of the muscarinic receptor. Recently Waser (33) has reviewed these attempts to picture the receptor in terms of the structure of muscarine and its derivatives (Fig.2) (22). The potency of muscarine is considerably higher than acetylcholine which may be due to the absence of the ester linkage rendering the compound immune to hydrolysis by cholinesterase. The receptor is considered to enter a 3-point attachment with the muscarine molecule, which involves the OH group, the ether oxygen and the cationic head. It has been shown (34) that stereo isomers of muscarine are several hundred times less active than the natural alkaloid and it appears that steric hindrance of the approach of the ether oxygen or the OH group, to the receptor leads to a pronounced reduction in activity. The larger the sterically interfering group, the less potent is the compound.

The (+) isomers of acetyl-α- and acetyl-β-methyl-choline are about 10 and 250 times more potent as muscarinics respectively than their corresponding enantiomorphs (22). If the quaternary nitrogen group and

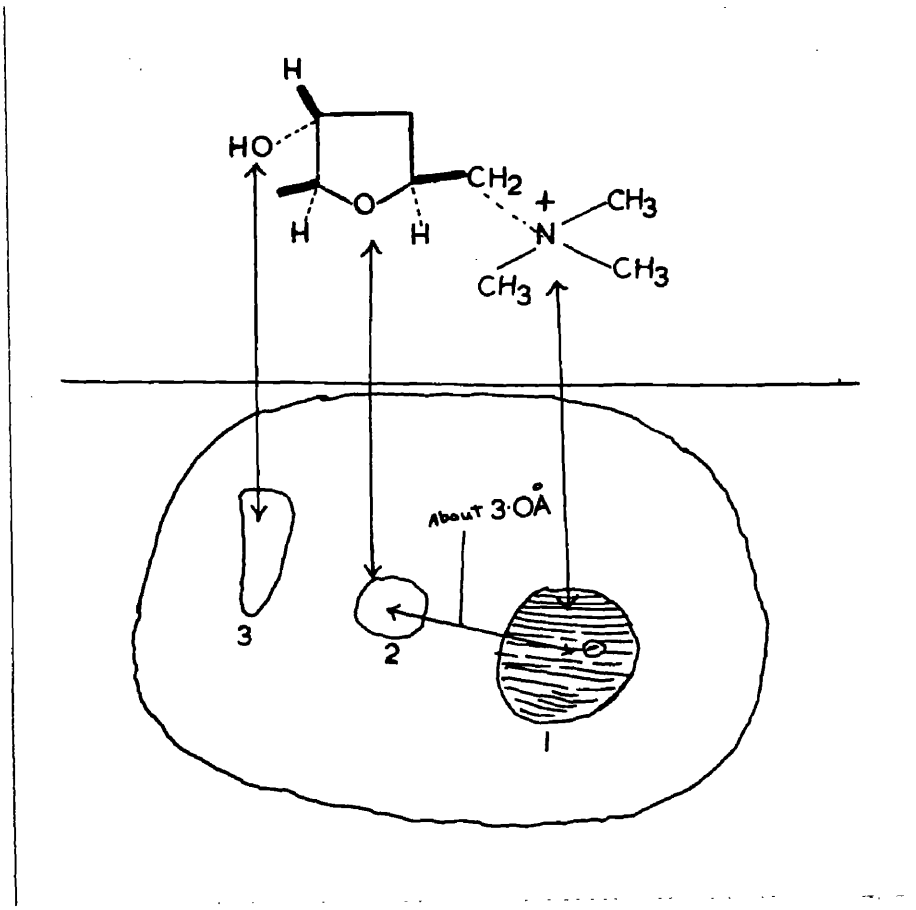


Fig.2.

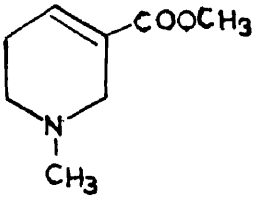
1. Anionic cavity negatively charged to accommodate quaternary nitrogen.
2. Positively charged point accommodating ether linkage of muscarine.
3. Positively charged area to accommodate OH of muscarine.

the ether oxygen of muscarine, lying almost in a plane, form the two main centres for drug receptor association (the hydroxy group acting as a secondary association site), then association with the receptor must involve the lower surface of the molecule. Inversion of the methyl group would then produce a steric barrier against such association. Replacement of this methyl group by a hydrogen atom considerably reduces activity probably because of a reduction in the electron density on the ether oxygen atom rather than by reduction in the availability of van der Waal's forces for bonding (35).

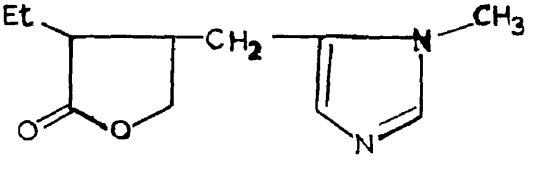
Certain muscarone derivatives (34) are more potent than muscarine and its derivatives and exhibit strong stimulating and blocking effects at both ganglionic synapses and the neuromuscular junctions. In atropinized animals, higher doses block the sympathetic ganglia and the end plates in a curare-like fashion. Here stereospecificity appears to be less important as the different isomers possess more or less comparable activity in contrast to muscarine and its derivatives. Thus conversion of the hydroxyl group in muscarine to the carbonyl group in muscarone exerts pronounced changes in the properties of the molecule. From the steric point of view, the oxygen of the carbonyl group

lies in the ring plane so is less influenced by substituents on either side of the ring. Moreover, the carbonyl group has a marked polar character which enables it, like the electrophilic carbon atom in an ester group to form a bond with a basic group in the receptor. Such bond formation which is not possible in muscarine (35) has been proposed by Wilson (36) to account for the binding of acetylcholine to acetylcholinesterase.

The proposals just summarised emphasize the steric and electronic requirements for muscarinic activity but there are many anomalies and the diverse chemical structures of the three best established muscarinic agents muscarine (I) itself, arecoline (VIII) and pilocarpine (IX), serves to



VIII



IX

underline the shortcomings of approaches to structure-action relationships such as those outlined above.

2. Atropinic

This group embraces those drugs which antagonise the action of acetylcholine at the parasympathetic neuroeffector junctions and at certain sympathetic neuroeffector junctions i.e. it consists of the drugs which antagonise the muscarinic effects of acetylcholine. Characteristic of the group is the possession of bulky molecules such as are represented in the series atropine, homatropine, eucatropine and the dialkylaminoalkanol esters of tropic, mandelic, atrolactic, diphenylacetic, benzilic and cycloalkylmandelic acids. These dialkylaminoalkanol esters can be regarded as derivatives of the acetylcholine molecule in which the acetyl group has been replaced by acyl groups possessing at least one ring system. A large number of heterocyclic ring derivatives have also been studied and a summary of the thiophen compounds of this class has been given by Martin-Smith and Reid (37).

The atropinic group includes a large number of quaternary ammonium derivatives (38), including higher alkyl trimethyl ammonium salts (39).

A detailed picture of the requirements for atropinic activity in terms of the muscarinic receptor has been given by Lands and his colleagues (40).

3. Nicotinic

The alkaloid nicotine has the ability to first stimulate and then depress ganglia and skeletal muscle so the selection of this agent as pharmacological prototype has been rather unfortunate insofar as a more precise division of drugs into ganglion stimulants, ganglion blockers, neuromuscular stimulants and neuromuscular blocking agents would represent a more fundamental classification. In practice such a division tends to be used today, and nicotine can be regarded as a drug showing a considerable lack of specificity.

Nicotinic action has been discussed in terms of the molecular structure of acetylcholine by Barlow (23) in an analogous fashion to his treatment for muscarinic activity. Thus replacement of the acetyl group of acetylcholine by the propionyl, benzoyl, phenylacetyl, phenylpropionyl or trimethyl acetyl radicals exaggerates the nicotinic properties of the neurohormone (27) at the expense of the muscarinic properties. Carbamylcholine shows both muscarinic and nicotinic properties.

Changes in the ester linkage of acetylcholine may also lead to increased nicotinic activity relative to the muscarinic properties as in acetyl β - methyl-thiocholine,

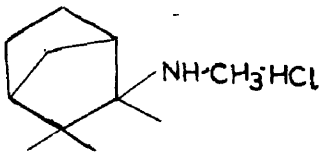
and in the butoxyethyl trimethylammonium ion. Dicholine esters of dicarboxylic acids also show nicotinic activity especially at the neuromuscular junction. Nicotinic properties are also present in a number of aromatic ethers of choline. These compounds have been studied in detail by Hey (41).

A further series of nicotinic compounds are afforded by certain aliphatic ketones bearing a quaternary trimethylammonium substituent where the nitrogen atom and the carbonyl group are in 1,4 relationship (42). It is of some interest that acetyl α - methylcholine although possessing only 1/20 th of the muscarinic activity of acetylcholine exhibits roughly the same nicotinic potency, whilst acetyl β - methylcholine is devoid of nicotinic properties, yet shows pronounced muscarinic activity.

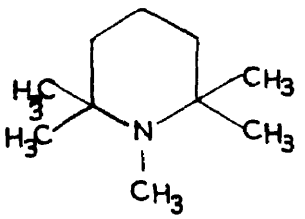
It has been suggested that the ideal requirements for nicotinic properties are a cationic nitrogen atom bearing three methyl substituents and an atom bearing a partial positive charge 3 atoms removed from the nitrogen atom.

4. Ganglion Blocking Agents.

These compounds antagonise the action of acetylcholine in the autonomic ganglia and are usually characterised by the possession of a single quaternary nitrogen atom, or tertiary sulphur atom as in trimetaphan. However, certain bis quaternary compounds induce ganglionic blockade, (e.g. hexamethonium) as do certain hindered secondary or tertiary amines such as mecamylamine (X) and pempidine (XI). It is



X



XI

also of interest that the acetylcholine analogue in which ethyl radicals replace two of the N-methyl groups, is a ganglion blocking agent.

5. Neuromuscular Blocking Agents

These are the compounds which induce paralysis of skeletal muscle through interference with the function of acetylcholine at the neuromuscular junction. They are customarily divided into two classes, the depolarisers and the competitors (43) of which the depolarisers can be regarded as strictly nicotinic as they first stimulate and then block neuromuscular conduction. Examples of depolarisers are decamethonium and succinylcholine, whilst d-tubocurarine is an example of a competitive neuromuscular blocking agent. Unfortunately the classification of neuromuscular blocking agents as depolarisers and competitors is unsatisfactory due to variation in the response of different species (44) and of different muscles within the same species (45) to a particular neuromuscular blocking agent. Moreover certain compounds induce a two-phase block (46). Neuromuscular blocking properties are associated with onium functions as was first recognized by CrumBrown and Fraser in 1869 (47) and tend to be very pronounced in molecules possessing 2 or more onium functions, leading to the concept of pharmacologically "bivalent molecules" (23) and by inference "polyvalent molecules". There are exceptions to the need for an onium

centre however e.g. the tertiary base β - erythroidine.

A detailed picture of the acetylcholine receptor at the muscle end plate has been given by Waser (48) and recently Ehrenpreis (49) has isolated a protein thought to be the acetylcholine receptor from the electric tissue of the electric eel.

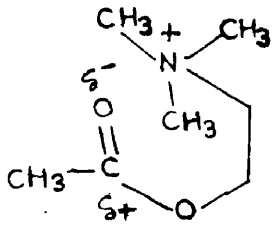
The fact that drugs interfering with the neurohormonal function of acetylcholine can be divided into the broad groups just outlined has by inference been mainly attributed to the varying abilities of the drugs concerned to cross permeability barriers in the body and so reach the various sites at which acetylcholine is involved. Thus the absence of a membrane barrier surrounding the neuromuscular junction (50) has been involved to explain the ready access of the ionic neuromuscular blocking agents to their site of action, whereas these compounds do not pass the blood-brain barrier for example to exert central effects.

There is however, another possible reason why drugs capable of mimicking or opposing the action of acetylcholine should fall into these catagories. It is conceivable that the receptors for acetylcholine at its different sites of action may be considerably different in their nature.

Acetylcholine is a conformationally non-rigid molecule and so theoretically capable of existing in an infinite number of conformations. It is possible therefore, that the different receptors are such as to permit interaction only with different conformational isomers of the neurohormone whilst it may not be energetically favourable for the different groups of drugs just discussed to achieve suitable geometry for interaction with all receptors.

It, therefore, seemed a worthwhile project to prepare a series of rigid acetylcholine-like molecules which would be incapable of conformational isomerism for pharmacological testing on different tissue preparations in order to ascertain whether there were marked differences in the activity of these isomers at the different acetylcholine receptors, and hence throw more light on the intimate nature of these various receptors. Even if little or no difference in activity on preparations such as perfused ganglia, the frog rectus abdominis, the chicken gastrocnemius, the rat diaphragm, the guinea pig ileum etc. were to be detectable the investigation would still be of value in-so-far as it would be the first indication that conformational isomerism played no part in the interaction of acetylcholine with its receptors.

It is of considerable interest that Fellman and Fujita (51) have very recently put forward evidence to show that in solution acetylcholine exists in the cyclic conformation (XII). Their conclusions were based on infrared measurements and on the high rate constant for the acylation



XII

of hydroxylamine by acetylcholine as compared to the rate constants observed with 3-dimethylaminopropyl acetate methiodide and 4-dimethylaminobutyl acetate methiodide indicating a high electron deficiency on the carbon atom of the carbonyl group in acetylcholine such as would result from the quasi 6-membered ring conformation (XII). Similar quasi ring conformations would not be expected to be energetically favourable for 3-dimethylaminopropyl acetate methiodide and 4-dimethylaminobutyl acetate methiodide by analogy with the fact that 7 and 8 membered cyclic transition states are well known to be unfavoured.

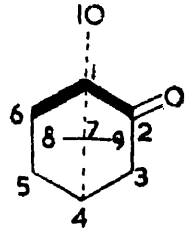
The infrared spectrum of acetylcholine measured in absolute ethanol showed strong ester carbonyl absorption at 1760 cm^{-1} with a less intense absorption at lower frequency whilst 3-dimethylaminopropyl acetate methiodide and 4-dimethylaminobutyl acetate methiodide showed split peaks of approximately equal intensity at 1752 cm^{-1} and 1732 cm^{-1} and 1748 cm^{-1} and 1728 cm^{-1} respectively. In all 3 cases the lower frequency peak can be interpreted as resulting from intermolecular hydrogen bonding between the ester molecules and ethanol molecules as hydrogen bond formation is well known to lower the frequency of ester carbonyl absorption (52). Thus the quasi 6-membered ring (XII) greatly decreases the ability of acetylcholine to hydrogen bond to ethanol giving further indication of its stability. At the same time the high value of the non hydrogen bonded absorption in acetylcholine at 1760 cm^{-1} probably indicates a greater degree of rigidity in the C = O bond in acetylcholine than is present in the higher homologues.

Fellman and Fujita quote as further evidence the fact that cis 2-dimethylaminocyclohexyl acetate methiodide absorbs at 1753 cm^{-1} whilst trans 2-dimethylaminocyclohexyl acetate methiodide absorbs at 1740 cm^{-1} in ethanol. Since quasi 6-membered ring formation from the cis (axial,

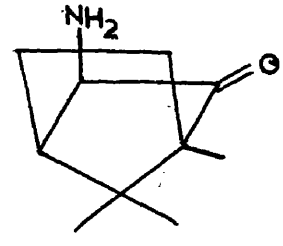
equatorial) and the trans in the equatorial equatorial chair conformations should be equally facile, the exact significance of these values is by no means clear.

It is to be borne in mind however that there is no justification for supposing that the thermodynamically most stable conformation of acetylcholine in solution is necessarily that actually adopted at the receptors.

The bornane skeleton appeared to offer the ideal rigid molecular framework or supporting moiety (53) on which to base certain of the desired inflexible acetylcholine-like molecules as the bicyclo heptane [2,2,1] system permits of no flexibility whatsoever. In particular the ready availability of D (+) camphor whose absolute configuration was shown to be (XIII) by Freudenberg et al (54) afforded a compound giving easy access to the desired derivatives, since it is readily converted to D-3-endoamino camphor (XIV).



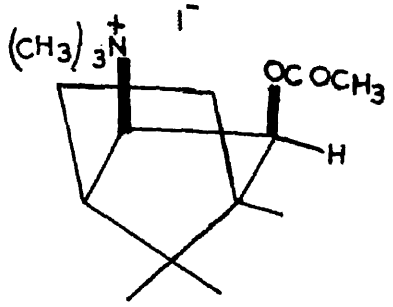
XIII



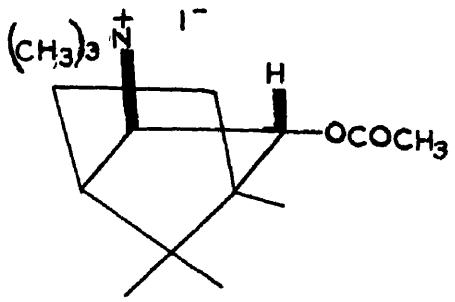
XIV

D-3-Endoamino camphor would then afford, after the appropriate synthetic steps, both 3-endodimethylamino-2-

endo-acetoxybornane methiodide (XV) and 3-endodimethylamino-2-exo-acetoxybornane methiodide (XVI), in which the

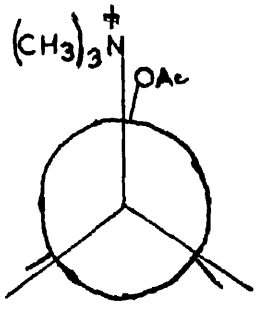


XV

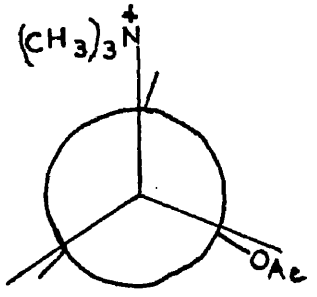


XVI

trimethylammonium and acetoxyl functions are in the fully eclipsed (XVII) and eclipsed (XVIII) conformations



XVII



XVIII

respectively, thus giving two extreme conformations to the incorporated acetylcholine unit.

Compounds (XV) and (XVI) are completely rigid molecules unlike the cis and trans cyclohexane and cyclopentane acetylcholine-like molecules prepared by Friess and his colleagues (56). The cyclohexane compounds can suffer ready conformational isomerism as in Fig.3, and the cyclopentane derivatives although not

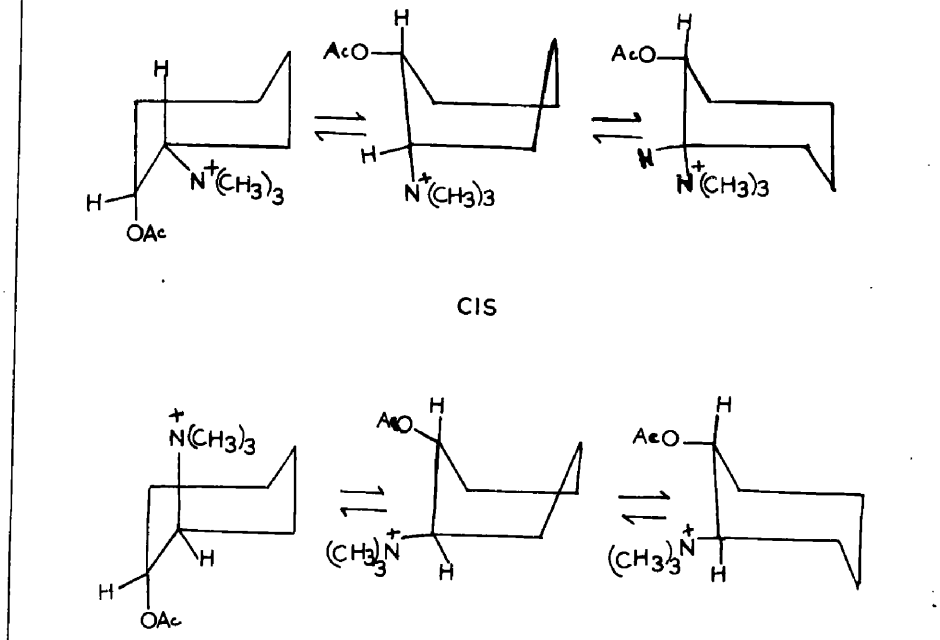
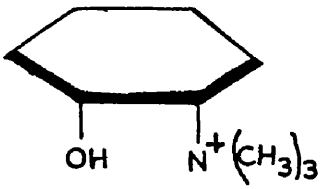


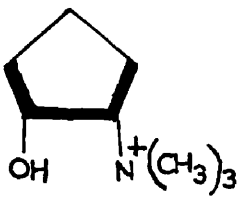
Fig.3.

subject to such extreme conformational isomerism as the 6-membered ring compounds, can nevertheless be expected to exist in multiplanar conformations as well as in the planar conformation (57). These cyclic derivatives of Friess and his colleagues have been tested for their ability to function as substrates of acetylcholinesterase (56), and for activity on the kitten phrenic nerve diaphragm preparation (58), but they do not appear to have been tested on ganglia, or on smooth muscle.

In both the cyclohexane and cyclopentane series the cis derivative was found to be more readily hydrolysed by acetylcholinesterase and to be more active on the kitten phrenic nerve diaphragm preparation. Similarly the cis alcohols (XIX) and (XX) were found to produce greater



XIX

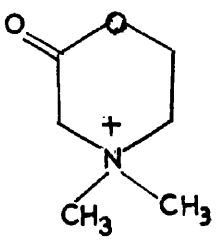


XX

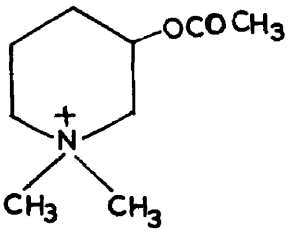
inhibition of acetylcholinesterase than their trans isomers.

On the other hand certain workers have emphasized the need for a degree of flexibility in acetylcholine like molecules. Thus Schueler (59) emphasized the flexibility

of two cyclic analogues of acetylcholine (XXI and XXII).



XXI



XXII

Gill (60) has postulated that completely rigid molecules should prove inactive due to variability in the receptors. This generalization which rests on the absence of ganglion-blocking activity in an extremely limited number of compounds such as the completely rigid N,N,N′,N′-tetramethyl-p-phenylenediamine dimethiodide (61), and certain virtually rigid furan derivatives still retaining a limited degree of rotational flexibility (62) certainly requires further substantiation before it can be unqualifiedly accepted especially as other receptors appear to accept rigid molecules as evidenced by the activity of the natural oestrogens and androgens.

Compounds (XV) and (XVI) therefore achieve a further significance in-so-far as they have direct bearing on Gill's hypothesis.

It is also of some interest that introduction of an acetylenic bond into certain acetylcholine-like molecules

gives a marked increase in potency (63) and this effect has been discussed in terms of the increased rigidity in such molecules.

Construction of models shows that for both compounds (XV) and (XVI) when the acetyl and quaternary nitrogen functions are laid on a planar surface the bulk of the molecule in no way interferes. Hence it can probably be assumed that utilization of the bornane skeleton as a supporting moiety will not introduce steric hindrance to the approach of the molecule to the acetylcholine receptors.

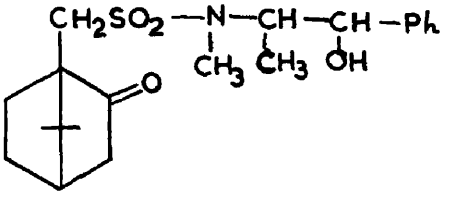
Measurements made on Dreiding and Bartonian models show that the $CH_3C(=O)-O\cdots\overset{+}{N}(CH_3)_3$ distance in compound (XV) is 2.56 A^o and in compound (XVI) is 3.6 A^o.

These distances can be compared with the N——O interatomic distances in the cyclic compounds of Friess and his colleagues where data for the cis - trans alcohols in both cyclohexane and cyclopentane series were interpreted to indicate an enzymatically preferred N——O separation with a maximum distance of about 2.5 A^o between anionic and esteratic sites on the surface. But with the substrates i.e. the cis - trans acetates the N⟶O separation distance was considered not necessarily to mirror the distance between sharply defined adsorption regions on the

enzyme, because the possibility exists that the esteratic site binds the —OCO—entity as a single locus of overall high electron density rather than concentrating its binding capacity on a single atom of the group. In the former event the average distance between catalytic adsorption regions on the enzyme most probably would be reflected in the distance between the quaternary nitrogen and that geometrical portion of the —OCO— function characterised as the centre of highest electron density.

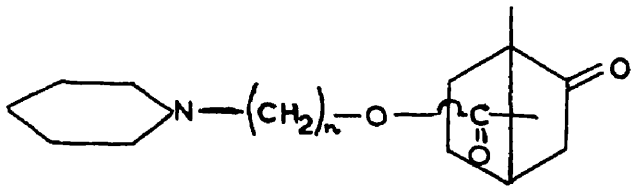
It is of interest that the bornane skeleton has been used as a rigid supporting moiety in a number of other biologically active molecules. For instance two isomeric 2-hydroxy-3-methylaminobornanes whose configurations were not established, exert a pronounced action on the respiratory centre of rabbits (64) and a 2,3-dihydroxybornane and its monocarbamate exhibit central nervous system depressant properties (65).

N-(2'-hydroxy-1'-methyl-2'-phenylethyl), N-methylcamphor sulphonamide (XXIII) was found to be a potent analeptic



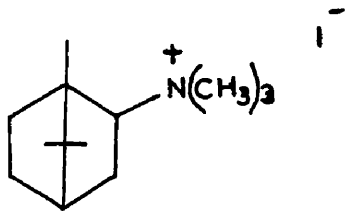
XXIII

(66), and esters of camphor-trans-π-carboxylic acid of type (XXIV) were found to be antitussives thus supporting



XXIV

the contention of Kase and Yuizono (67) that the piperidino group possesses the ability to function as an active antitussive moiety. Derivatives similar to those of type (XXIV) in which the piperidine ring was replaced by a morpholine ring or a piperazine ring were prepared as potential anti-hypertensive agents (68). Such anti-hypertensive activity is present in trimethyl-d-bornyl-ammonium iodide (XXV) (69) and one of the intermediates

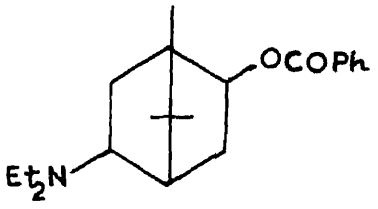


XXV

utilized in the present project, 3-endoaminocamphor in the form of its hydrochloride, exhibits feeble neuromuscular

blocking action and transiently lowers blood pressure (70).

Pronounced local anaesthetic properties have been shown to be present in 2-benzoyloxy-5-diethylaminobornane (XXVI) (71) and a number of 3-dialkylaminoacyl camphors like

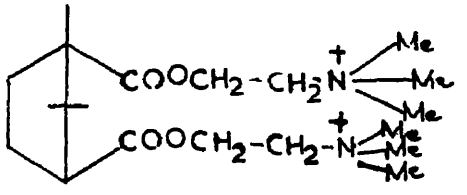


XXVI

diethylaminoethylbornyl ether exhibit smooth muscle and central depressant properties (72).

Various 3-amino-2-hydroxybornanes were prepared as potential antimalarial agents (73) and the benzoic ester of cis aminoborneol inhibits smooth muscle (74).

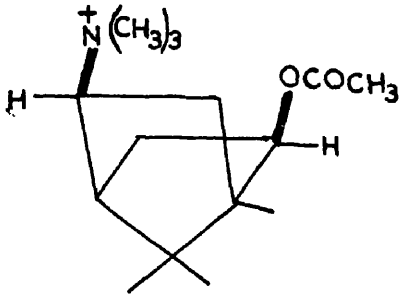
The 2,3-secobornane skeleton has also seen utilization as a supporting moiety in such compounds as (XXVII) which



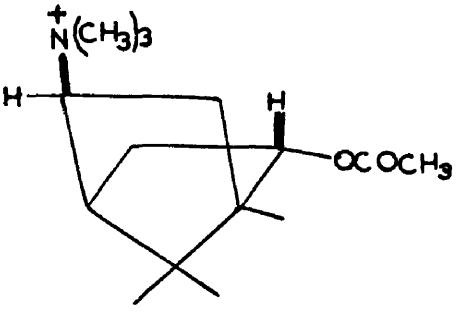
XXVII

is active as a neuromuscular blocking agent and in the mercurial diuretics mercaptomerin and mercurophylline.

In addition to compounds (XV) and (XVI) it was originally planned to include compounds of type (XXVIII) and (XXIX) i.e. isomeric 2-acetoxy-5-trimethylammonium



XXVIII



XXIX

bornanes in the present studies. Although these compounds are not strictly acetylcholine-like in structure as 4 carbon atoms separate the substituent groupings, they might be expected to throw more light on intergroup distances. For instance the $\overset{+}{N}$------$OCOCH_3$ distance in (XXVIII) is 3.44 A^{o} as measured on Dreiding models, which lies between the distances of 2.56 A^{o} in (XV) and 3.6 A^{o} in (XVI).

The fully opposed conformation for acetylcholine itself gives a separation of 3.8 A^{o}. In [illegible] (XXIX) the separation is 4.8 A^{o}.

However the low yields obtained of the key intermediates required for this project, either D (+) -2-endoacetoxy-5-oxobornane or dl-2-benzoyloxy-5-oxobornane, on chromic oxide oxidation of the corresponding bornyl esters by the

procedures in the literature proved an insurmountable obstacle to the completion of this project.

In the search for a more satisfactory method of preparation of a bornane derivative possessing oxygen functions in both the 2- and 5- positions a preliminary investigation was made of the action of molecular oxygen on D (+) camphor in the presence of ascorbic acid, the sodium salt of ethylenediamine tetraacetic acid, ferrous sulphate and a phosphate buffer.

The rationale for this approach lies in the observation of Brodie and his co-workers (75) that molecular oxygen in the presence of ascorbic acid, sodium ethylenediamine tetraacetate and ferrous sulphate in a phosphate buffer is able to mimic a number of biological oxidations involved in the biotransformation of aromatic compounds in the body to phenolic compounds. As 5-hydroxycamphor is known to be one of the end-products of the metabolism of camphor by the animal body (76), it was considered worthwhile investigating whether camphor would afford an acceptable yield of this oxidation product when exposed to the same reaction conditions. In the event oxidation of the camphor did occur as was evidenced by the uptake of oxygen but no pure 5-hydroxy camphor could be isolated from the reaction mixture.

Approach to the Synthesis of the Rigid Molecules.

The key starting material for the preparation of compounds (XV) and (XVI) was D 3-endoamino-camphor (XIV) which was prepared by the method of Duden and Pritzkow (77) from D isonitrosocamphor which is readily obtainable from D (+) camphor (XIII) by the action of isoamyl nitrite (78).

The configurational assignment of the compound as the 3-endoamino derivative follows from the work of Van Tamelen et al (55) and is in accord with the known greater stability of the endo configuration in the bornane series, where groups in the exo configuration suffer non bonded interaction with the methyl groups on the 1,4 methylene bridge.

The conversions of 3-endoaminocamphor into both 3-endoamino-2-endohydroxybornane (XXXI) and 3-endoamino-2-exohydroxybornane (XXXII) (Chart 1.) have been reported in the literature (79) and the N - p - nitrobenzoyl derivative of (XXXI) was shown to undergo smooth acyl migration under the influence of dry hydrogen chloride in dioxan. In the present work, however, attempts to achieve similar $N \rightarrow O$ acyl migration on D 3-endoacetamido-2-endohydroxybornane (XXXIV) prepared by sodium and wet

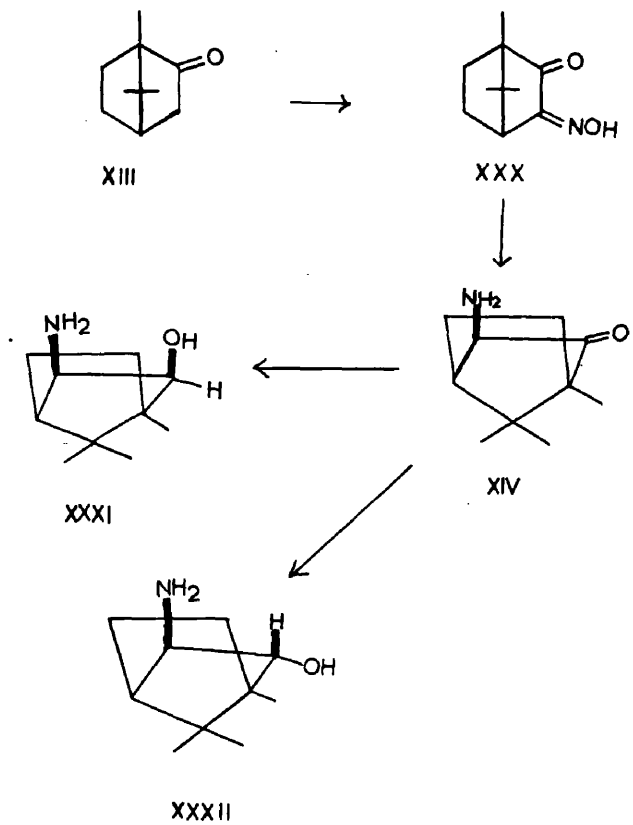


Chart. 1.

ether reduction of D 3-endoacetamidocamphor (XXXIII) (Chart 2.) were unsuccessful and so it was decided to achieve N - dimethylation before reduction of the carbonyl function. The choice of sodium and wet ether to effect transformation of (XXXIII) into (XXXIV) was based on analogy with the formation of 3-endoamino-2-endohydroxy-bornane by sodium and wet ether reduction of 3-endoaminocamphor (79) whereas sodium and alcohol reduction of 3-endoaminocamphor gives the 3-exohydroxy compound (79). That compound (XXXIV) was indeed 3-endoacetamido-2-endohydroxy bornane was shown by the infrared spectrum of the compound in carbon tetrachloride solution at dilutions (0.0005 M and 0.0015 M) sufficient to ensure the absence of inter-molecular hydrogen bonding. The broad peak with maximum at 3518 cm^{-1} showing intra-molecular hydrogen bonding proved the endo configuration of the hydroxyl group. Similar utilization of infrared spectra to assign configurations in the bornane-2,3-diol series has been reported by Angyal and Young (80).

The route followed to the desired compounds was therefore, that shown in chart 3.

D-3-Endoaminocamphor was dimethylated by a slight modification of the procedure described by Duden and

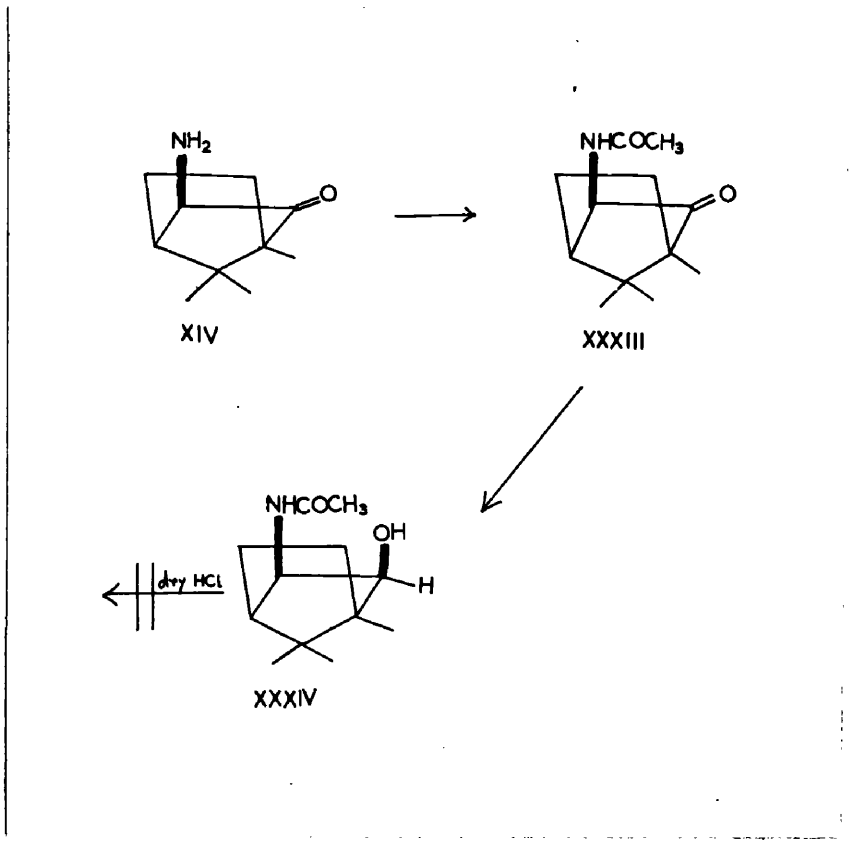


Chart. 2.

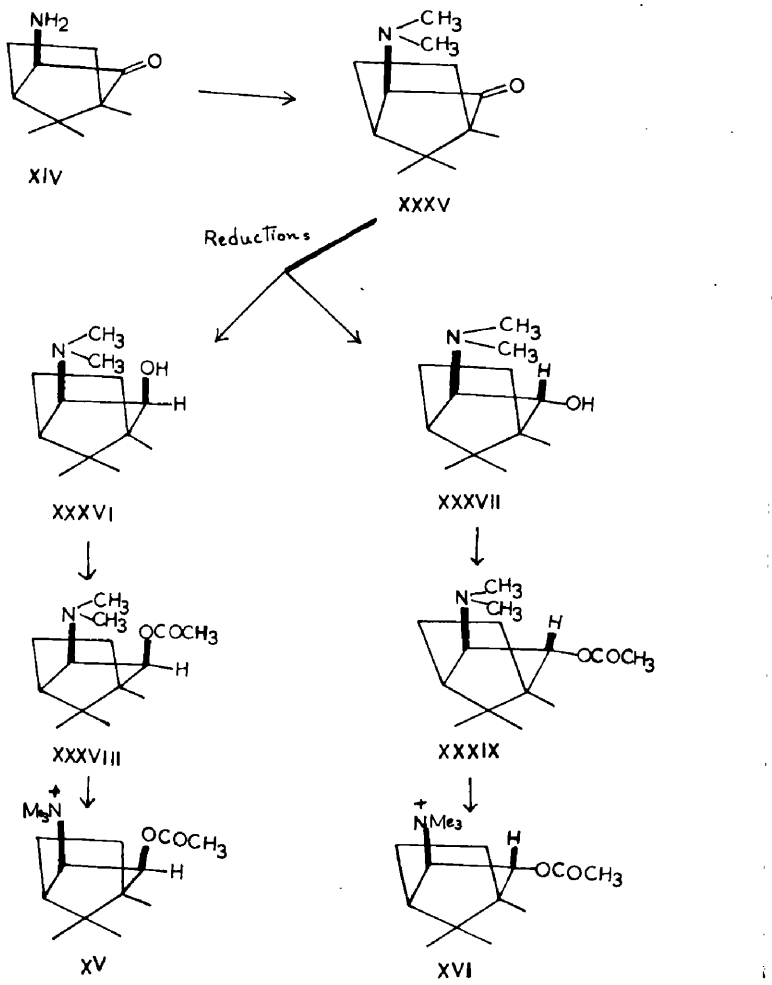

NH2
O
XIV
N
CH3
CH3
O
XXXV
Reductions
N
CH3
CH3
OH
H
XXXVI
N
CH3
CH3
H
OH
XXXVII
N
CH3
CH3
OCOCH3
H
XXXVIII
N
CH3
CH3
H
OCOCH3
XXXIX
Me3N
OCOCH3
H
XV
NMe3
H
OCOCH3
XVI

Chart. 3.

Pritzkow (81) to afford (XXXV) whose infrared spectrum showed strong peaks at 2800 and 2770 cm^{-1} characteristic of the dimethylamino group (Fig.4).

Reduction of (XXXV) by means of lithium aluminium hydride in dry ether afforded an oily mixture of the cis and trans isomers (XXXVI) and (XXXVII) clearly showing the presence of both free and intramolecularly hydrogen bonded hydroxyl groups when the infrared spectrum of the crude product was examined in carbon tetrachloride solution, at dilutions sufficient to ensure the absence of intermolecular hydrogen bonding. The presence of the cis isomer (XXXVI) was also indicated by the formation of a purple colouration (chelate formation) with copper sulphate solution. Chromatography of the mixture over alumina yielded the pure trans isomer (XXXVII) in 40 % yield, but the cis isomer could not be eluted from the column, presumably on account of chelate formation.

The cis isomer (XXXVI) was obtained in 55 % yield from the mother liquors after preferential crystallisation of the trans isomer from the mixture in light petroleum and in pure form by the following procedure. The crude reduction product was acetylated by the action of ketene and the mixture of acetyl derivatives chromatographed

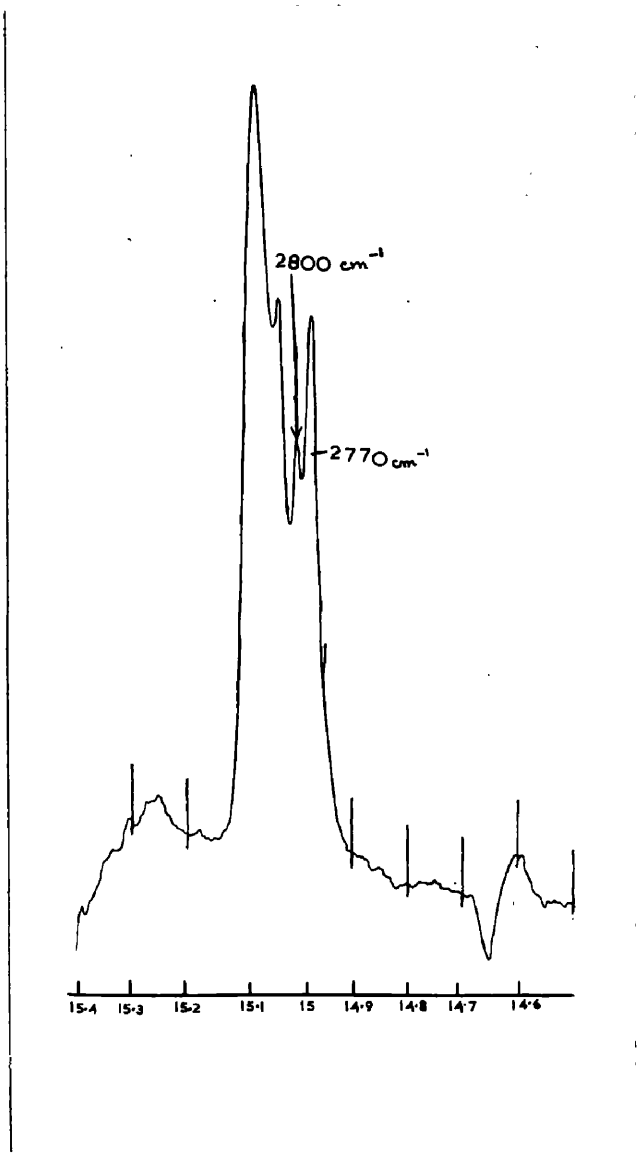
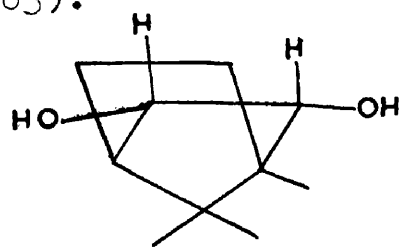


Fig.4.

Infrared spectrum of D-3-Endo-dimethylaminocamphor taken as a film showing absorption bands characteristic of the dimethylamino group.

over alumina (Woelm grade I neutral). The cis acetate eluted with light petroleum and the trans acetate suffered hydrolysis to the parent aminoalcohol which eluted with ether. Hydrolysis of the pure cis acetate (XXXVIII) then afforded the pure cis alcohol (XXXVI), which showed intra-molecular hydrogen bonding in dilute carbon tetrachloride solution (infrared, broad absorption with maximum at 3440 cm^{-1}) and gave a purple colouration with copper sulphate solution. The pure trans isomer (XXXVII) showed no hydrogen bonding when its infrared spectrum in dilute carbon tetrachloride solution was examined (OH stretching peak at 3620 cm^{-1}).

It is of interest to compare this ratio of exohydroxy (40 %) to endohydroxy (55 %) resulting from the lithium aluminium hydride reduction of 3-endodimethylaminocamphor with other results reported in the camphor field, where reduction of both camphor and epicamphor with lithium aluminium hydride is known to give predominantly exo alcohol (82) as is catalytic hydrogenation. Similarly reduction of camphorquinone with lithium aluminium hydride has been found to yield predominantly the cis di exo glycol (XL) (83).



XL

The higher ratio of the endo hydroxy compound observed in this work is presumably attributable to the bulk of the endo dimethylamino group which would be expected to hinder the rearward anionic attack of the keto group by the complex aluminiumhydride ion from the endo side. Such steric hindrance at the endo side is absent in camphor itself where the steric hindrance to rearward attack on the exo side due to the gem-dimethyl groups on C - 7 can be presumed to give rise to the preponderance of exo product, although this isomer is the thermodynamically least stable; i.e. kinetic control - not product control operates (84).

Reduction of 3-endodimethylaminocamphor (XXXV) by sodium and alcohol as previously described by Duden and Pritzkow (85) gave after alumina chromatography a 70 % yield of the trans isomer. The early workers reported a m.p. of ca 80° for their product and since the pure trans isomer was found in the present work to melt at 120°, it must be concluded that their material was contaminated with cis isomer.

Reduction of 3-endodimethylaminocamphor (XXXV) by means of sodium and wet ether gave a mixture of isomers and after alumina chromatography a 30 % yield of the trans

alcohol was obtained. The reaction mixture was not worked up for its content of cis isomer, but in view of the fact already established that the cis isomer does not elute from an alumina column, it can be assumed from the above results that reduction of 3-endodimethylaminocamphor (XXXV) with sodium and wet ether gives predominantly the endo hydroxy compound whilst reduction with sodium and alcohol gives predominantly the exo hydroxy compound. These results are in good accord with the results obtained by the 2 methods of reduction on 3-endoaminocamphor (79).

It is of some interest to compare these results with those already reported for camphor and epicamphor. Thus reduction of camphor with sodium and alcohol gives predominantly the endo alcohol (i.e. borneol) (86) as does reduction of epicamphor under the same conditions (87). It is to be noted that Angyal and Young (80) obtained the 2-endo, 3-endo, the 2-exo, 3-endo and the 2-endo, 3-exo bornanediols by catalytic hydrogenation procedures.

Whilst there can be no doubt as to the structure of 3-endodimethylamino-2-endohydroxybornane (XXXVI) since it displays the expected intramolecular hydrogen bonding, there exists a small chance that the compound described as 3-endodimethylamino-2-exohydroxybornane (XXXVII) is

in fact the rearranged camphane derivative (XLII) formed by a classical Wagner-Meerwein shift as shown in chart 4, where the 1,6 bond being trans and antiparallel to the exobond originating from the 2 position, migrates with extreme ease to form the tertiary carbonium ion (XLI). However as such Wagner-Meerwein shifts are to be expected under acid conditions it does not seem likely that sodium and alcohol, sodium and wet ether or lithium aluminium hydride which are all basic reducing agents would effect such a rearrangement.

Less certainty exists however with respect to the effect of the alumina columns used to purify this non intramolecularly hydrogen bonded dimethylaminohydroxy derivative and which produce hydrolysis of its acetate, and it is in this that any doubt concerning structure (XXXVII) arises, although the complete identity of the material eluting from alumina columns with that obtained by repeated direct crystallisations from light petroleum of the mixture of products from the lithium aluminium hydride reduction would tend to indicate that no rearrangement had in fact taken place on the columns.

As the usual procedures for acetylation proved unsatisfactory when applied to compounds (XXXVI) and

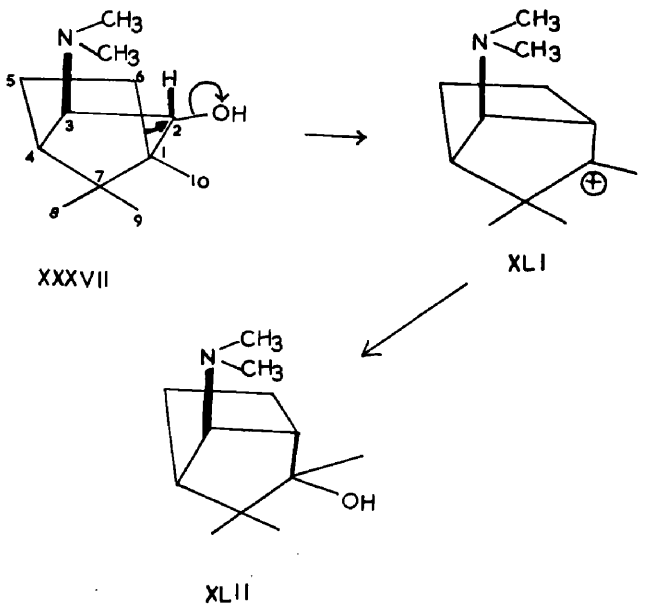


Chart. 4.

(XXXVII) both D-2-endo-acetoxy-3-endodimethylaminobornane (XXXVIII) and D-2-exo-acetoxy 3-endodimethylaminobornane (XXXIX) were prepared by the action of ketene in anhydrous ether on D-3-endodimethylamino-2-endohydroxybornane (XXXVI) and D-3-endodimethylamino-2-exohydroxybornane (XXXVII) respectively. Conversion of these compounds to the quaternary methiodides (XV) and (XVI) was achieved by means of methyl iodide.

In the case of the trans compound (XVI) it was found that hydrolysis of the acetyl function was exceptionally facile and the reaction was best performed in dry benzene. Both products were extremely hygroscopic.

As a sideline to the main problem the reduction of D camphor imine (88) (XLV) (Chart 5.) by means of lithium and ethanol in liquid ammonia was investigated since Ourisson and Rassat (89) had shown that reduction of camphor under these conditions gave 20 % of isoborneol (exo) whereas caesium in liquid ammonia gave 85 % of isoborneol and it seemed worthwhile to establish whether similar selectivity existed with respect to the imine. However the mixture of isomeric bornylamines formed could not be separated by chromatography. That reduction had taken place was shown by the complete absence of

C = N absorption in the infrared spectrum at ca 1700 cm^{-1}.

Experimental.

D (+)-α- Isonitrosocamphor (XXX) was prepared by slightly modifying the method of Claisen and Manasse (90).

D (+) camphor (40.0 g.) was dissolved in 200 ml. dry ether in a 2 litre flask. Freshly prepared sodium wire (6.1 g.) was added followed immediately by the slow and careful dropwise addition of a small quantity of isoamylnitrite.

Warming on the steam bath with concurrent scratching of the sodium by means of a glass rod ensured commencement of the reaction, as was evidenced by the solution turning yellow and the production of slight foaming.

Once reaction had set in, the reaction flask was placed in an ice bath. Further quantities of isoamylnitrite were added carefully with swirling at intervals (at such a rate that the reaction did not become too vigorous) until a total of 31.0 g. had been added.

The total time taken for the addition of the isoamylnitrite was 35-40 min.

The reaction mixture in which some sodium isonitroso-camphor had separated was allowed to stand for 1 hr. and then carefully treated with ice water whereby the sodium salt dissolved with the production of a red colour. The

solution was extracted twice with ether to remove traces of camphor and borneol and air was then blown through the aqueous solution to remove residual ether. On treatment of the aqueous solution with acetic acid (25 ml.) the crude product separated and was taken into ether. Removal of the organic solvent yielded an oily semi-crystalline mass of α-isonitrosocamphor (23.0 g., 57 %), which was used in the next step without any further purification. $[\alpha]_D$ = + 170.0° [C = 0.84 in chloroform][Lit., (91) 172.9° in chloroform].

D (-)- 3 - Endo-amino camphor. (XIV)

This compound can be prepared from α-isonitrosocamphor by reduction with either zinc and sodium hydroxide or zinc and acetic acid as reported by Duden and Pritzkow (77). Comparison of the two procedures showed that a higher yield resulted from the zinc and sodium hydroxide method (75 % as against 55 % from the zinc and acetic acid reduction).

α- Isonitrosocamphor (16.0 g.) was dissolved in 33 % sodium hydroxide solution (64 ml.) and water (64 ml.). To the solution powdered zinc (19.2 g.) was added in small quantities at intervals with warming on the steam bath so that the temperature of the reaction mixture remained in the range 50-60° C. As the reaction proceeded a thick

yellow oil separated. When the reaction was complete (1 hr.) the mixture was diluted with water (400 ml.) and extracted with ether. Removal of the organic solvent afforded a yellow waxy mass (12.0 g. 75 %) with indistinct m.p. The compound was therefore isolated as the carbonate salt which was obtained by passing carbon dioxide gas through a wet ethereal solution of the crude product. White crystals, feathery plates (11.0 g., 91.3 %) of m.p. 117-118°.

The amine hydrochloride from acetone as needles had m.p. 220-225° [Lit., (77) 223-225°].
(Found : C, 59.28; H, 8.95. Calcd. for $C_{10}H_{18}NOCl$: C, 58.95; H, 8.90 %).
The free base had $[\alpha]D=-18.9°$ (C=1.32 in chloroform) Harper in Rodd(92) states that this compound has $[\alpha]D$ +60° but it not clear from where he quotes this value.

The constitution of the amine as the 3-endoamino derivative of camphor follows from the work of Van Tamelen, Tousignant and Peckham (55).

D (+)-3-Endo-dimethylaminocamphor (XXXV) . This compound was prepared by a modification of the method of Duden and Pritzkow (81). 3-Endoamino-camphor carbonate (4.0 g.) was suspended in 30 ml. ether in a 100 ml. flask and to the flask was added a solution of sodium hydroxide (2.6 g.) in 30 ml. of water. Methyl iodide (5.8 g., 4 moles) was added

and after firmly stoppering the flask, the mixture was shaken overnight on a mechanical shaker. Separation of the ethereal layer and removal of solvent afforded the crude product of D (+)-3-endo-dimethylamino camphor as a yellow coloured oil. (3.1 g., 77.5 %). Chromatography over alumina (Woelm, grade I, neutral) in petroleum ether (b.p. 40-60°) removed the colour and treatment with hydrogen chloride in ether afforded the hydrochloride. Colourless needles from acetone had m.p. 220-224° [Lit., (81) 220-222°].

(Found : C, 61.35; H, 9.71. Calcd. for $C_{12}H_{22}NOCl$: C, 62.18; H, 9.57 %).

$[\alpha]D$ = + 32.8° (C = 1.0 in chloroform) [Lit., (96) + 50° in ethanol].

The infrared spectrum of the free base taken as a film showed strong absorption bands characteristic of the $-N\begin{matrix}CH_3\\CH_3\end{matrix}$ group (93) at 2800 cm^{-1} and 2770 cm^{-1} in addition to the peak at 1734 cm^{-1} characteristic of the five membered ring carbonyl group (Fig. 4).

D (-) - 3 - Endomethylaminocamphor.

To a suspension of D-3-endo-amino camphor carbonate (4.0 g.) in ether (25 ml.) in a 100 ml. quickfit flask was added a solution of sodium hydroxide (2.04 g.) in

water (20 ml.) and methyl iodide (2.8 g., 2 moles). The flask was firmly stoppered and shaken for 16 hr. on a mechanical shaker. Separation of the ethereal layer and removal of the solvent afforded the crude product (2.8 g., 70 %) which was converted to the hydrochloride by treatment with hydrogen chloride in ether. Several recrystallisations from acetone gave colourless needles with m.p. 225-228° [Lit., (94) melts and decomposes at 228°].

(Found : C, 60.22; H, 9.45. Calcd. for $C_{11}H_{20}NOCl$: C, 60.67; H, 9.25 %).

The infrared spectrum of the free base taken as a film showed 2 split peaks at 1580 and 1540 cm^{-1} and a broad band below 3300 cm^{-1} indicative of the secondary amino function as well as a band at 1734 cm^{-1} characteristic of the carbonyl group in a five membered ring.

The free base had $[\alpha]D = -26.8°$ (C = 1.1 in chloroform).

D (+) - 3 - Endodimethylamino - 2 - endo - hydroxybornane(xxxvi)

This compound was formed in admixture with D (+) - 3 - endodimethylamino - 2 - exo - hydroxy bornane by the action of lithium aluminium hydride on D (+) - 3 - endodimethylaminocamphor and was first obtained in the pure form after a multi-stage process. This involved acetylation of the crude reaction product and passage

of the resulting mixture of acetylated derivatives in ether over neutral alumina which effected preferential hydrolysis of D (-)-3-endo-dimethylamino-2-exo-acetoxy-bornane and gave a clean separation of the cis D (-)-3-endo-dimethyl-2-endo-acetoxybornane from the trans D (+)-3-endodimethylamino-2-exo-hydroxybornane. Finally hydrolysis under vigorous conditions of the D (-)-3-endodimethylamino-2-endo-acetoxybornane gave the cis hydroxy compound.

D (+)-3-Endo-dimethylaminocamphor (1.8 g.) was dissolved in 50 ml. of anhydrous ether and lithium aluminium hydride (1.3 g.) added in small portions to avoid too vigorous a reaction. After all the lithium aluminium hydride had been added the mixture was refluxed overnight with exclusion of moisture by means of a calcium chloride tube. On cooling the excess lithium aluminium hydride was decomposed by careful addition of several drops of water and the mixture of products obtained from the ethereal solution as a waxy solid with indefinite m.p. The mixture gave a purple colouration with copper sulphate indicative of chelation by the cis isomer. The infrared spectrum of the mixture in carbon tetrachloride solution at a dilution sufficient to ensure the absence of inter molecular hydrogen bonding (0.000508 M and 0.00253 M) showed O—H stretching

absorption at 3620 cm^{-1} and between 3470 - 3330 cm^{-1} indicating that both the intra molecularly hydrogen bonded cis isomer and the non-bonded trans isomer were present.

Chromatography of the mixture (1.8 g.) in ether over alumina (Woelm neutral grade I) resulted in the elution of only 720 mg. (40 %) of the material and this proved to be identical with trans D (+)-3-endo-dimethylamino-3-exo-hydroxybornane prepared as described below. Presumably the cis isomer had chelated to the alumina and could not be eluted.

In a separate experiment the crude reaction product from the lithium aluminium hydride reduction was acetylated by treatment with ketene (95).

The mixture of the two isomers (2.0 g.) was dissolved in anhydrous ether (50 ml.) and a continuous stream of ketene prepared as described later, passed through the solution for 2 - 3 hrs.

At the end of the reaction, on evaporation of the solvent a crude dark brown oil was obtained. On chromatography over alumina (Woelm, grade I neutral) the cis compound eluted in petroleum ether (b.p. 40-60°), (1.2 g., 50%), and the trans suffered hydrolysis to the corresponding alcohol which eluted with ether, (0.5 g., 25 %). The identity of this latter compound was confirmed

by its infrared spectrum and m.p.'s of the free base and its hydrochloride which showed no depression in admixture with authentic material.

The cis compound (1.2 g.) was hydrolysed by treating with sodium hydroxide solution (2·3 g., in 20 ml.) with stirring and refluxing for 2 hrs. The hydroxy compound was obtained as an oily semi crystalline mass.

The infrared spectrum in carbon tetrachloride solution at dilutions sufficient to ensure the absence of inter molecular hydrogen bonding (3.9 mg. in 50 ml. and 2.74 mg. in 5 ml.) showed the position of the O—H stretching as a broad band between 3460 - 3510 cm^{-1} with a maximum absorption at 3440 cm^{-1}.

$[\alpha]D = +27.7^{\circ}$ (C = 0.8 in chloroform)

It was characterised as the hydrochloride prepared by treatment with dry hydrogen chloride in ether. Recrystallisation from ethanol gave feathery needles with m.p. 253-55° (Found : C, 61.62; H, 10.12; $C_{12}H_{24}NOCl$ requires C, 61.64; H, 10.34 %).

It was later discovered that the cis hydroxy compound could be separated from the mixture of the two isomers obtained by lithium aluminium hydride reduction (2.0 g.) by treatment with hot petroleum ether (b.p. 40-60°) from

which the trans isomer crystallises out leaving the cis isomer in solution. Identification of the isomers follows from their infrared spectra and the m.p. of the trans isomer.

Cis - (1.1 g., 55 %) Trans - (0.8 g., 40 %).

D (+)-3-Endo-dimethylamino-2-exo-hydroxybornane (XXXVII)

(a) D (+)-3-Endodimethylaminocamphor (1.0 g.) dissolved in ethanol (50 ml.) was treated under reflux with sodium metal (3.0 g.) added at intervals in small aliquots. Refluxing was continued for 4 hr . when the reaction mixture was taken to dryness under reduced pressure. The reaction product was extracted from the sticky residue by addition of water (70 ml.) followed by extraction with ether. Removal of the solvent from the ethereal solution gave an oily mass slowly turning to a solid with indefinite m.p. (0.7 g., 70 %). On chromatography over alumina (Woelm, neutral, grade I) in ether eluted 92.8 % of the material applied to the column. Recrystallisation from petroleum ether (b.p. 40-60°) gave colourless needles with m.p. 120° [Lit., (85) Ca 80° presumably contained some cis isomer] .

(Found : C, 72.82; H, 11.2; Calcd. for $C_{12}H_{23}NO$: C, 73.04; H, 11.74 %).

$[\alpha]D$ = + 36° (C = 0.84 in chloroform)

It was converted to the hydrochloride and characterised as such. Colourless needles from acetone/ethanol had m.p. 277-280°.
(Found : C, 61.90; H, 10.56; $C_{12}H_{24}NOCl$ requires C, 61.64; H, 10.34 %).

(b) D (+)-3-Endo-dimethylaminocamphor (1.0 g.) was dissolved in ether saturated with water (50 ml.) contained in a three necked flask equipped with a dropping funnel and an efficient reflux condenser. Metallic sodium (3.0 g.) in small aliquots was carefully added at intervals whilst the mixture was kept under reflux. From time to time a few drops of water were added from the dropping funnel to ensure steady dissolution of sodium. Refluxing was continued for 4 hr. After cooling the ethereal solution was washed with water, dried and the solvent removed. Chromatography of the product in ether over alumina (Woelm, grade I, neutral) gave 300 mg. (30 %) of the trans - hydroxy compound.

This was recrystallised from petroleum ether (b.p. 40-60°) as needles with m.p. 120°.

There was no mixed m.p. depression with the material obtained by alumina chromatography of the mixture of products obtained by lithium aluminium hydride reduction

of D (+)-3-endo dimethylamino camphor described above and the infrared spectra were identical.

The trans configuration i.e. the 3-endo dimethylamino-2-exo-hydroxy configuration, was assigned to this compound as it gave no colouration on treatment with copper sulphate solution indicative of chelate formation, and as the infrared spectrum in carbon tetrachloride solution at dilutions sufficient to ensure the absence of intermolecular hydrogen bonding (5.3 mg. in 5 ml. and 2.55 mg. in 50 ml.) showed the position of the O—H stretching absorption to be at 3620 cm^{-1} proving that there was no intramolecular hydrogen bonding.

D (-)-2-Exo-acetoxy-3-endo-dimethylaminobornane (XXXIX)

Conventional methods of acetylation of 3-endo-dimethylamino-2-exo-hydroxybornane proved unsatisfactory, so this compound was prepared by the action of ketene on the parent alcohol.

3-Endo-dimethylamino-2-exo-hydroxybornane (1.0 g.) was dissolved in anhydrous ether (50 ml.) and a continuous stream of freshly generated ketene passed through the solution for 2 hr. At the end of this time removal of the solvent afforded the crude acetyl derivative as a dark oil. Dissolution in petroleum ether (b.p. 40-60°) followed by

filtration afforded the product as a colourless oil.

The infrared spectrum of the free base in thin film showed sharp absorption peaks at 1734 cm^{-1} and 1206 cm^{-1} characteristic of the acetate residue. There was no absorption in the 3600 - 3300 cm^{-1} region.

$[\alpha]D = -24^{\circ}$ (C = 0.9 in chloroform)

The acetoxy compound was characterised as its hydrochloride, needles from acetone had m.p. 265-270° (Found : C, 60.42; H, 9.11 $C_{14}H_{26}O_2NCl$ requires C, 60.96; H, 9.5 %).

D (-)-2-Endo-acetoxy-3-endo-dimethylaminobornane (XXXVIII)

This compound was obtained by direct acetylation of the mixture of 2-hydroxy-3-endo-dimethylaminobornanes resulting from lithium aluminium hydride reduction of 3-endo-dimethylamino camphor by means of ketene, followed by chromatography over basic alumina as previously described. The free base in thin film showed absorption in the infrared spectrum at 1734 cm^{-1} and 1260 cm^{-1} but no peak between 3600 and 3300 cm^{-1} indicating complete acetylation.

$[\alpha]D = -68^{\circ}$ (C = 1.26 in chloroform)

The acetoxy compound was characterised as its hydrochloride, needles from benzene had m.p. 245-247° (Found : C, 61.42; H, 8.8 $C_{14}H_{26}O_2NCl$ requires C, 60.96; H, 9.5 %).

D (+)-2-Exo-acetoxy-3-endo-dimethylaminobornane methiodide (XVI

D (-)-2-Exo-acetoxy-3-endo-dimethylaminobornane (350 mg.) was dissolved in dry benzene (30 ml.) and treated with excess methyl iodide (8.0 g.) and left overnight, when crystals of the methiodide had separated as colourless cubes, of m.p. 250-252° (160 mg., 31.3 %)
(Found : C, 47.06; H, 8.08. $C_{15}H_{28}O_2NI$ requires C, 47.24; H, 7.40 %).
$[\alpha]D = +62.5^{\circ}$ (C = 0.9 in ethanol).

D (-)-2-Endo-acetoxy-3-endo-dimethylaminobornane methiodide (XV)

D (-)-2-Endo-acetoxy-3-endo-dimethylaminobornane (500 mg.) dissolved in anhydrous ether (30 ml.) was treated with a large excess of methyl iodide (10.0 g.) and allowed to stand overnight when crystals of the

methiodide separated. These were collected by filtration. Colourless needles, extremely hygroscopic of m.p. 219-221°, without further purification (250 mg., 38 %)

(Found : C, 46.93; H, 6.95. $C_{15}H_{28}O_2NI$ requires C, 47.24; H, 7.40 %).

$[\alpha]_D = -25.4^\circ$ (C = 0.92 in chloroform)

D (+)-3-Endo-acetamidocamphor (XXXIII).

D (-)-3-Endo-aminocamphor (1.3 g.) in acetic acid (5 ml.) was refluxed with acetic anhydride (5 ml.) for 1 hr. At the end of this time the mixture was concentrated to less than one half the original volume and poured into cold water. The crystalline material which separated was filtered, dried and recrystallised from petroleum ether (b.p. 60-80°) as colourless feathery plates (1.0 g., 63 %) m.p. 122° [Lit., (97) 121-122°]. (Found : C , 69.15; H, 9.12 Calcd. for $C_{12}H_{19}O_2N$: C, 68.86; H, 9.15 %).

The infrared spectrum in nujol showed peaks below 3200 cm^{-1} and at 1734 cm^{-1}, 1640 cm^{-1} and 1570 cm^{-1}. The last two being characteristic of a monosubstituted amide.
$[\alpha]_D$ = + 50.5° (C = 0.88 in chloroform)

D (-)-2-Endo-hydroxy-3-endo-acetamido bornane (XXXIV).

This compound was formed in low yield by sodium metal reduction of 3-endo-acetamido camphor.

D (+)-3-Endo-acetamido camphor (0.5 g.) was placed in a 2-necked 250 ml. flask fitted with a reflux condenser and a dropping funnel and dissolved in wet ether (100 ml.), freshly prepared sodium wire (1.5 g.) was then added to it and refluxed on a steam bath for 2 hrs. with dropwise

addition of water till all the sodium went into solution. The ethereal extract was evaporated to dryness and the impure compound was chromatographed over alumina (Woelm, neutral grade I) in ether / methanol (50 : 1), to elute the desired product. Extensive variation of conditions (sodium in wet ether, sodium in ethanol, sodium shot in xylene / ethanol) failed to raise the yield of pure material above 20 %.

In all cases the crude product from the reaction required chromatographic purification. Colourless feathery plates from petroleum ether (b.p. 60-80°) or ethanol had m.p. 208-210°

(Found : C, 68.31; H, 10.75. $C_{12}H_{21}O_2N$ requires C, 68.20; H, 10.01 %).

$[\alpha]_D = -56°$ (C = 0.82 in chloroform).

Assignment of the endo configuration to the hydroxyl group follows from a study of the infrared spectrum in carbon tetrachloride at a dilution (0.0005 M and 0.0015 M) sufficient to ensure the absence of inter-molecular hydrogen bonding. The broad peak between 3540-3480 cm^{-1} with maximum at 3518 cm^{-1} showed the presence of intramolecular hydrogen bonding thus proving the compound to be the cis isomer (i.e. 2-hydroxyl group endo).

All attempts at N → O acyl migration by treatment with hydrogen chloride in dry dioxane (cf the successful N → O acyl migration of p-nitrobenzamide of β-aminoborneol to β-aminobornyl p-nitrobenzoate hydrochloride achieved by van Tamelen and his collaborators (55)) were unsuccessful.

D (+)-3-Endo-(2'2'2'-trichloro-1'-hydroxyethyl) amino camphor.

In an attempt to prepare D-3-endo-formylaminocamphor by the method of Blicke and Chi-Jung Lu (98), D (-)-3-endo-aminocamphor (2.0 g.) was dissolved in chloroform (100 ml.) in a 3-necked, 250 ml. flask equipped with stirrer, dropping funnel and condenser to which was attached a calcium chloride tube to exclude moisture. To the stirred mixture cooled in an ice bath, chloral (freshly prepared by distillation of equal weights of chloral hydrate and concentrated sulphuric acid) (1.6 g., 1 mole) was added dropwise. When all the chloral had been added stirring was continued for 4 hr. at room temperature and the mixture then refluxed for 30 min. Removal of the solvent under reduced pressure followed by crystallisation from petroleum ether (b.p. 60-80°) gave needles m.p. 108-113° .

(Found : C, 45.65; H, 5.88. $C_{12}H_{18}O_2NCl_3$ requires

C, 45.78; H, 5.76 %).

$[\alpha]_D = +30.8^\circ$ (C = 0.68 in ethanol).

The compound resisted all attempts to eleminate the elements of chloroform and generate the desired N - formyl derivative.

D-(+)-Bornyl acetate was prepared by heating D (+) borneol (50.0 g.) and acetic anhydride (40.0 g.) for 2 hrs. at 145° (72.9 g., 68 %), it had b.p. 99-100° [Lit., (99) 99-100°]

$[\alpha]_D$ = +43° (C=1.16 in chloroform) [Lit., (103) 44.38°]

D (+)-2-endo-acetoxy-5-oxobornane.

To a 3 necked 2 litre flask, fitted with a reflux condenser and a mechanical stirrer, was added bornyl acetate (50.0 g.) in acetic acid (75 ml.). The flask was half immersed in an electrically heated oil bath, the temperature of which was maintained at 140° (±5°). With vigorous stirring powdered chromium trioxide (125.0 g.) was added in spoonfuls down the condenser. With each addition, a violent frothing occurred and between each addition about 10 ml. acetic acid was poured down the condenser. This prevented blocking of the condenser and used in all about 250 ml. acetic acid. The total time of addition of chromium trioxide and acetic acid was 40 mins. approximately. After all the chromium trioxide had been added, heating and stirring was continued for another 30 mins. Thereafter the solution was cooled, dissolved in 2 litres of water, neutralized with sodium bicarbonate and extracted with ether. Evaporation of the ether yielded

a residue consisting of a mixture of camphor, unreacted starting material and the product. The mixture was distilled under vacuum and the fraction boiling at 97-117° at 0.7 mm was collected. This fraction crystallised on standing and rubbing with petroleum ether (b.p. 60-80°). Crystallisation from petroleum ether (b.p. 60-80°) gave colourless rods (5 g., 10 %) with m.p. 76-77° [Lit., (99) 76-77°].

$[\alpha]D = +113^{\circ}$ (C = 1.12 in chloroform)

dl Bornyl benzoate was prepared (100) by dissolving racemic borneol (15.0 g.) in dry pyridine and adding benzoyl chloride dropwise with careful exclusion of moisture. The reaction flask was kept in ice and stirred throughout the addition of the benzoyl chloride, the latter being added over a period of 30 mins. After the addition was complete the mixture was left for 2 hrs. After addition of water (250 ml.) the solution was acidified with dilute hydrochloric acid and extracted with ether.

The ethereal extract was washed once with water and once with dilute sodium hydroxide solution to remove traces of benzoic acid. The product was obtained as an oily liquid, (9.0 g., 38 %).

dl 2-endo-benzoyloxy-5-oxobornane was prepared in analogous manner to that described for the preparation of D-2-endo-acetoxy-5-oxobornane. Crystallisation of the oily product from petroleum ether (b.p. 60-80°) gave colourless needles with m.p. 115°. Despite minor modifications of the procedure the yield was always less than 5 %.

dl-2-endo-hydroxy-5-oxobornane.

Treatment of dl-2-endo-benzoyloxy-5-oxo-bornane (1.0 g.) with excess potassium hydroxide in ethanol under reflux for 2 hr. and removal of solvent gave after extraction of the residue with ether, dl-2-endo-hydroxy-5-oxobornane. Crystallisation from petroleum ether (b.p. 60-80°) gave colourless needles (500 mg., 75 %) m.p. 234-244° [Lit., (99) 238-246°].

The infrared spectrum in nujol showed peaks at 3200 cm^{-1} for the hydroxyl and 1734 cm^{-1} characteristic for five membered ring carbonyl.

D (+) Camphor oxime. (XLIII)

D (+) camphor (30.0 g.), sodium hydroxide pellets (45.0 g.) and ethanol (400 ml.) were refluxed with hydroxylamine (30.0 g.) in water (200 ml.) for 2 hrs. On cooling the reaction mixture was diluted by the addition of 1500 ml. of water and made acidic by the addition of acetic acid. The product separated as a crystalline mass which was collected by filtration. Crystallisation from petroleum ether (b.p. 60-80°) gave rods (26.0 g., 87 %) with m.p. 119° [Lit., (101) 119°].

$[\alpha]_D = +47^\circ$ (C = 0.84 in chloroform) [Lit., (101) 42.4° in ethanol].

D camphor nitrimine. (XLIV)

This compound was prepared by the method of Houben and Pfankuch (88). D (+) camphor oxime (25.0 g.) was dissolved in ether (250 ml.) in a 1 litre separating funnel and a solution of sodium nitrite (25.0 g.) and concentrated sulphuric acid (15.0 g.) in 160 ml. of water added and the mixture shaken. The by-product of camphor imine nitrite which separated, was removed by filtration (5.0 g., 20 %) and the ethereal layer concentrated under reduced pressure in the absence of heat in order to avoid decomposition of the product. Colourless needles subliming at 260° without

melting. The material was used in the next step without further purification.

D (-) Camphor imine (XLV)

D-Camphor nitrimine as isolated in the previous step was treated with sufficient ether to ensure complete liquifaction and 100 ml. of .880 ammonia added. After shaking the mixture vigorously it was allowed to stand overnight. Extraction with ether followed by removal of solvent gave the desired product which was converted to the hydrochloride as plates (14.0 g., 92 %) with m.p. 210°.

m.p. of free base 93-94° [Lit., (104) 95°].

The free base had $[\alpha]_D = -22°$ (C = 1.16 in chloroform). The infrared spectrum of the hydrochloride in nujol showed a strong C = N peak at 1700 cm^{-1}.

D-bornylamine and D-isobornylamine (XLVI)

D (-) camphor imine hydrochloride (3.5 g.) and ammonium chloride (1.0 g., 1 mole) were added to 100 ml. of liquid ammonia in a 250 ml. 3-necked flask fitted with stirrer and air condenser. Then small pieces of metallic lithium were added until the solution became permanently blue. The blue colour was carefully destroyed after some time with solid ammonium chloride which was dropped down the condenser and the solution was left until the ammonia had evaporated off completely. Water and ether were then

added and the mixture transferred to a separating funnel. The ethereal layer yielded a white crystalline solid which was soluble in ethanol and petroleum ether (b.p. 60-80°). The derived hydrochloride was obtained as a white powder which melted and sublimed at 244°. The infrared spectrum of the free base in nujol confirmed that complete reduction of the imine had taken place but the m.p. was not sharp (160-175°). The product was in fact a mixture of the isomers bornyl and isobornyl amine. Attempts to separate the isomers by chromatography over alumina (Brockman grade III) in petroleum ether (b.p. 60-80°) were unsuccessful.

Generation of Ketene. (Fig. 5)

Ketene was prepared according to the method of Williams and Hurd (95) by passing acetone vapour over an electrically heated chromel filament suspended from the top of a ground glass joint.

The filament, A, was prepared from a 175 cm length of B and S gauge 24 chromel A wire an alloy of nickel (80 %) and chromium (20 %) by stretching the spiral obtained by wrapping the wire around a 3 mm. diameter rod to a length of 70 cm. The filament so obtained was supported on platinum hooks, B, 15 mm. in length and sealed into a pyrex supporting rod. Two more platinum hooks, B, supported the filament at the bottom of the rod.

The ends of the filament were connected to tungsten leads of B and S gauge 24 wire by means of nickel sleeves, C, 10 mm. long and of 3.5 mm. internal diameter equipped with two set screws. These tungsten leads were sealed into the glass, and to them were soldered B and S gauge 24 copper wires (insulated by 6 mm. glass tubing held in place by the cork stopper D) which were connected to a source of 220 volts through a variac Rheostat.

The filament was contained in a chamber E, constructed from a 25 cm. length of glass tubing of 70 mm. internal

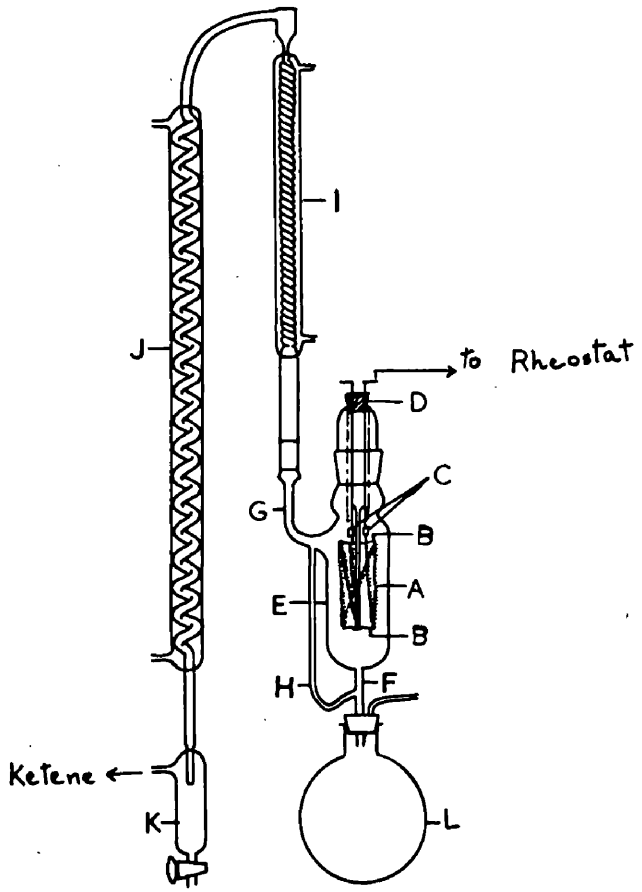


Fig.5.

Ketene generator.

A - Filament; B - Platinum hooks; C- Nickel sleeves; D - Cork stopper; E - Filament chamber; F - Connecting tube; G - Side arm; H - Reflux return tube; I - Double spiral condenser; J - Single spiral condenser; K - Liquid trap; L - Round bottomed flask for acetone.

diameter. The connecting tube at the bottom F, was 12 mm. tubing, the side arm G, near the top of the filament chamber was 15 mm. tubing and reflux return tube H, from side arm was 6 mm. tubing.

One condenser I, which was connected to the side arm of the filament chamber was a double spiral 50 cm. long, and the second condenser J, was a single spiral condenser 90 cm in length. The two were connected at the top. The liquid trap K, connected to the lower end of the single spiral condenser was provided with a stopcock for removal of liquid from the trap.

Operation - - Acetone was placed in the round bottomed flask L, which was attached to the lamp by means of a rubber stopper. The introduction into the flask of sufficient glass wool to extend a few cm. above the surface of the liquid served to prevent bumping. After the outlet tube had been connected to the reaction flask through a glass tube, the stopcock on the liquid trap was closed, and the liquid in the round bottomed flask was heated by a heating mantle until it refluxed gently from the condenser above. About 5 mins. refluxing was allowed to drive the air from the filament chamber. The current was then passed through the filament, which was heated to a dull red glow

(temperature 700-750°) to pyrolise the acetone.

After starting the operation the apparatus needed little attention. Occasionally condensed liquid had to be removed from the trap. If the condensers allow too much acetone to pass, a trap surrounded by ice water may be placed between the outlet for ketene and the reaction flask.

At the end of a run the following operations were carried out rapidly :- 1. The source of heat was removed, 2. the filament current was turned off and 3. the stopcock on the trap was opened.

In order to ensure that ketene was being generated, a steady and effluent stream of the gas was passed through excess aniline to ensure that crystals of acetanilide appeared immediately.

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