STUDIES ON TRITERPENOIDS

by

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INTRODUCTION

The terpenoids are a class of organic compounds widely distributed in the plant kingdom, and to a lesser extent in animals. The best definition[1] of a terpenoid is that it is a compound whose carbon skeleton is either (a) theoretically constructed from isoprenoid units, or (b) at some stage in its biogenesis had a carbon skeleton so constructed.

The terpenoids may be subdivided into major classes depending on the number of carbon atoms they contain.

The lower terpenoids: monoterpenoids (ten carbon atoms per molecule) and diterpenoids (twenty carbon atoms) have been reviewed comprehensively by Simonsen[2], and by de Mayo[3,4]. Specialized topics within the field have been reviewed[1,5] in greater detail.

The triterpenoids[4], containing thirty, or exceptionally thirty one carbon atoms may be subdivided according to the number of rings in the molecule.

Only two triterpenoids having less than four rings are known: these are squalene (1) and ambrein (2). They are unusual in that they are of animal origin, squalene was first isolated[6] from shark liver oil, and ambrein from ambergris. The importance of squalene in the biogenesis of steroids and triterpenoids will be discussed later.

The tetracyclic and more particularly the pentacyclic triterpenoids are the subject of this thesis, and will be con-

sidered in more detail. The existence of a hexa-carbocyclic triterpenoid, phyllanthol, which has a cyclo-propane ring, but lacks a double bond, has been proved[7].

Of the still higher terpenoids, the carotenoids (forty carbon atoms) are frequently symmetrical branched chain polyenes having few carbon rings. One C_{45}-polyterpene, solanesol[8], has been isolated from flue-cured tobacco.

REVIEW OF LITERATURE

The Biogenesis of Triterpenoids

The biogenesis of triterpenoids has been receiving considerable attention, and provides a convenient starting point in this survey. It not only presents a logical framework for the diverse structures to be met among the known triterpenoids, but also accommodates the skeleton of the γ-onocerin series to which it is considered that zeorin belongs. The mechanism of biogenesis of triterpenoids is being studied in two different ways, which are being developed notably by Ruzicka, and by Bloch.

Ruzicka[9-11] has studied the carbon skeletons of the various groups of naturally occurring triterpenoids, and has proposed a scheme in which, by ionic cyclisation of squalene to a tetracyclic carbonium ion, followed by stereospecific carbonium ion rearrangements, the skeletons of the various triterpenoid groups can be obtained.

The second method involves the study of the biochemical steps in the conversion of simpler molecules to terpenoids and steroids *in vivo*, or with enzyme systems *in vitro*. In this the use of isotopic tracers has been invaluable.

Biochemical studies

It is now established that acetic acid is converted in animals to mevalonic acid (3), isopentenyl pyrophosphate (4), farnesyl pyrophosphate (5), and thence to squalene (1), lanosterol (6) 4:4-dimethylcholesta-8:24-dien-3β-ol, (7) and cholesterol

(8). Although it appears likely that a similar series of transformations from acetic acid to squalene takes place in plant systems, the considerably greater practical difficulites have prevented rigorous proof of this.

The conversion of acetic acid to cholesterol was first clearly established in 1945 by Bloch and Rittenberg[12]. Acetic acid labelled with C^{13} at both the carboxyl and methyl carbons, and with deuterium yielded, in mice, cholesterol containing both isotopic carbon and deuterium in amounts which indicate that acetic acid is a major source of carbon atoms for cholesterol. Incorporation of acetic acid into cholesterol was not depressed by addition of ethanol, acetoacetic acid, or leucine to the diet, although these molecules could themselves be incorporated into cholesterol, probably by degradation first to acetate.

Cholesterol biosynthesized from 1-C^{14}-acetate and from 2-C^{14}-acetate showed radioactivity corresponding in both cases to a slightly greater utilization of metnyl carbon atoms (M) than carboxyl carbons (C). The ratio 1.75:1 is that of 15 carbon atoms of cholesterol of methyl origin, and 12 of carboxyl origin. As a result of many degradations of labelled cholesterol, the labelling pattern (8) has been established. A concise review[13] of these degradations has recently been published.

As the pattern of distribution of the two carbon atoms of acetate was being revealed, evidence was accumulating which indicated that the conversion of acetate to cholesterol is not

necessarily direct. An early experiment with deuterium labelling[14] showed that the isoprene-like isovaleric acid, but not isobutyric acid, can serve as a precursor for cholesterol in rats. Experiments with isotopic carbon showed that the methyl groups of isovaleric acid are incorporated into cholesterol five times more efficiently than is the methyl group of acetic acid[15]. Cholesterol from methyl-labelled isovalerate has the label more or less evenly distributed in the molecule and not solely in the side chain[16].

The distribution of acetate methyl and carboxyl carbons in the side chain of cholesterol (8) was found to conform to the pattern postulated[17] for a polyisoprene chain and led to speculation[18] as to a possible biogenetic relationship between the terpenoids, for which an isoprenoid precursor had long been suggested, and the sterols. In particular, the speculation[19] that squalene is a precursor of cholesterol was revived. In agreement with this it had been found that addition of squalene to the diet lead to an increase in liver cholesterol in rats[20].

Strong support for the squalene hypothesis was obtained by Langdon and Bloch[21], who demonstrated the presence of labelled squalene in rat liver, shortly after feeding 1-C^{14} or 2-C^{14} acetate to the rat. The biosynthetic C-^{14} squalene was efficiently converted to cholesterol in mice[22], although squalene regenerated from the hydrochloride cannot be metabolized. Regenerated squalene has been shown to contain some cis-double bonds and methylene double bonds $C{=}CH_2$, while the natural material is all trans[23].

Popják[24] confirmed the biosynthesis of squalene from acetate and together with Cornforth[25] established the distribution (1) of methyl and carboxyl-derived carbons in squalene biosynthesized from acetate.

Although it is possible that the biosynthesis of the pentacyclic triterpenoids and euphol type tetracyclic triterpenoids differs from that of the lanosterol group and the steroids at some point in the sequence earlier than squalene, it appears likely that they do not. In the cyclisation step from squalene to a tetracyclic intermediate the two series must diverge and the results of the investigations of the sequence from squalene to cholesterol are of uncertain value in considering the biogenesis of the larger class of triterpenoids. The squalene to cholesterol series will therefore be described only briefly and the earlier stages in more detail.

Squalene to cholesterol

Two schemes of cyclisation of squalene to cholesterol have been proposed. In 1934 Robinson[26] suggested a cyclisation of squalene folded as shown in (9). This scheme was in agreement with the acetate distribution pattern of cholesterol to the extent that it had been revealed in 1953. Work on the structure of lanosterol[27] now showed that it was related to the steroids on the one hand and to the triterpenoids on the other. In particular the 4-gem-dimethyl group of lanosterol (6) could not satisfactorily be explained by Robinson's cycli-

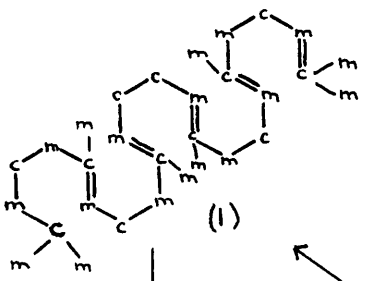
sation scheme. Woodward and Bloch[28] therefore proposed an alternative scheme for cyclisation of squalene with the chain folded as shown in (1) to give lanosterol, which by demethylation, migration of the nuclear double bond, and saturation of the side chain would give cholesterol.

The two schemes called for different arrangements of acetate carbon atoms at C(7), C(12) and C(13). By degradation of cholesterol derived from methyl labelled acetate, Woodward and Bloch[28] showed that either C(10) or C(13) originated from acetate methyl. Their theory predicts that C(13) should be derived from methyl carbon, whereas Robinson's cyclisation scheme suggests that both C(10) and C(13) are of carboxyl carbon origin. The cholesterol degradation completed by Cornforth's group, leading to the labelling pattern (8) are in agreement only with the Woodward-Bloch cyclisation scheme.

The postulated biogenetic relationship of lanosterol to squalene and cholesterol was confirmed by biosynthesis of lanosterol from labelled acetate[29] and its conversion to cholesterol in rat liver tissue[30].

The mechanism of cyclisation of squalene to lanosterol was examined in detail by Bloch[31]. Hog liver homogenate in a deterium oxide medium converts squalene to lanosterol without incorporation of deuterium in the product; in H_2O^{18} no O^{18} was found at the lanosterol 3-position. If the cyclisation is carried out in ordinary water with an O_2^{18} atmosphere, the labelled oxygen is then incorporated[31]. The cyclisation and rearrangement of squalene to lanosterol therefore does not involve addition of protons from the water at any stage, although hydroxylation of a carbonium ion and subsequent cleavage of the C-O bond is not ruled out.

The rearrangement of the two central methyl groups of squalene to (finally) the positions at C(13) and C(14) of lanosterol can take place either by two 1:2 shifts of the methyl groups (10) or a 1:3 shift of the methyl group at the position 8 (steroid numbering) to position 13, while the methyl at C(14) would remain in place (11). In order to decide between the two mechanisms Bloch[32] synthesized squalene doubly labelled with C^{13} in the two migrating methyl groups and the carbon atoms to which they were attached in all three combinations in which the labelled atoms are not both in the same half of the squalene



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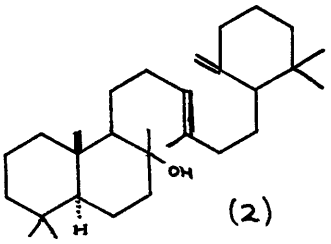

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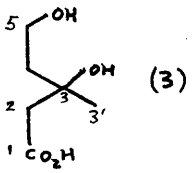

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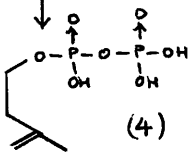

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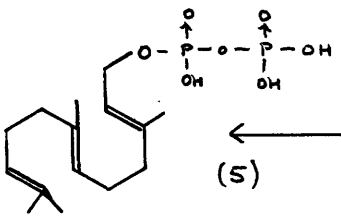

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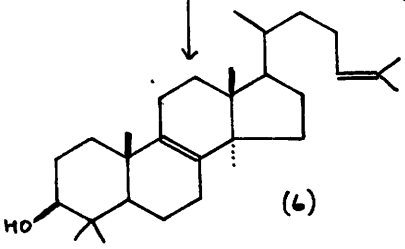

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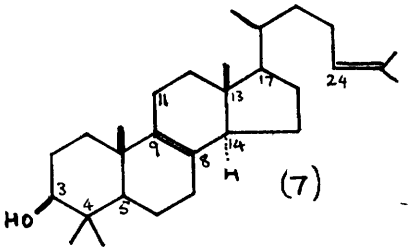

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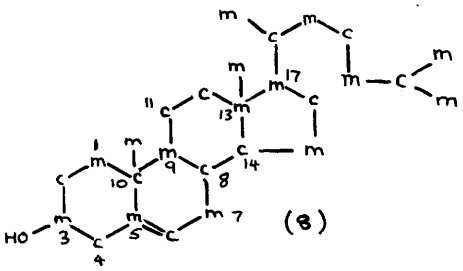

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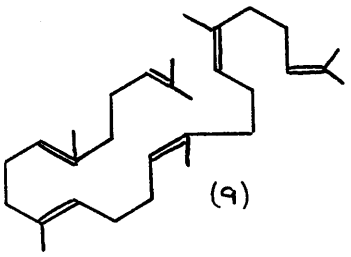

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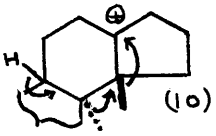

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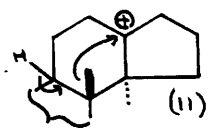

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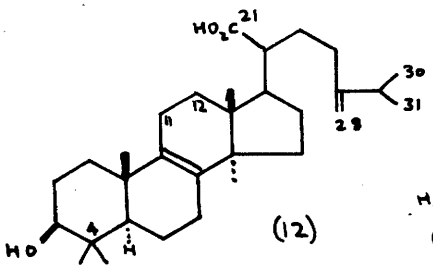

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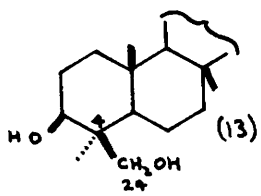

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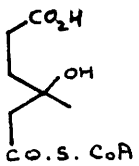

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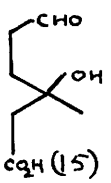

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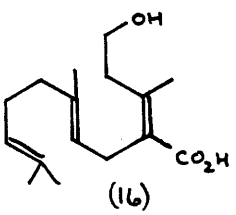

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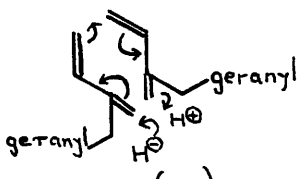

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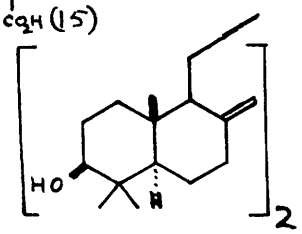

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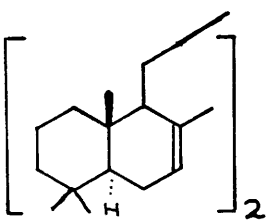

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molecule. Enzymatic conversion of the labelled squalene to lanosterol and extraction of the angular methyl groups as acetic acid by Kuhn-Roth oxidation gave some acetic acid shown to contain two C^{13} atoms in the same molecule. This could not occur if the C(8) angular methyl group had moved to the completely unlabelled position (13) and could occur only if the partly labelled C(8) angular methyl group had moved to the partly labelled C(14) angular position, by the double 1:2 shift mechanism (10).

Further support for mechanism (10) was provided by Cornforth[33] who showed that in the conversion of squalene (1) to cholesterol (8) the C(18) angular methyl group remaining at C(13) in cholesterol has come from the same isopentane unit as the C(13) carbon atom. This he did by mixing mevalonic acid labelled at both the 3′ and 4 positions (3) with completely unlabelled mevalonic acid and converting the mixture to cholesterol. C(13) of cholesterol is then the only partly labelled carbon atom to which is attached a methyl group. If mechanism (10) is correct, then whenever the C(13) atom is labelled, it would be predicted that the angular methyl group attached to it would also be labelled. Were the 1:3 shift mechanism (11) correct then the occurrence or non-occurrence of a labelled methyl group attached to a labelled position, C(13), would depend on the origin of the adjacent mevalonic acid unit, whether it came from unlabelled or doubly labelled mevalonic acid. Kuhn-Roth oxidation of the cholesterol yielded acetic acid derived from inter alia C(13) and its attached methyl group, C(18). Part of this

was subjected to mass spectrometry to determine the proportions of doubly labelled acetic acid (mass 62) to singly labelled (mass 61) and unlabelled acetic acid (mass 60). Combustion of a portion of the acetic acid gave the total proportion of unlabelled carbon, while the remainder on oxidation with bromine gave the proportion of carboxyl labelled acetic acid. The variation in the proportion of mass 62 acetic acid with varying proportions of doubly labelled mevalonic acid in the starting material fully supported mechanism (10). In practice the labelled mevalonic acid contained singly as well as doubly labelled molecules. This, while altering the calculations, in no way decreases the validity of their conclusion.

The Cornforth experiment is complementary to that of Bloch in that it deals with the migration of different methyl groups, and it has the advantage that it also rules out the possibility of intermolecular methyl shifts.

The conversion of lanosterol to cholesterol involves removal of the C(14) angular methyl group, migration of the nuclear double bond to the 5-position, and the 4-gem-dimethyl group and saturation of the side chain. There is considerable evidence that the sequence of conversions occurs in the order described.

Injection of C^{14} acetate to rats and killing them within a few minutes yielded the expected labelled lanosterol and cholesterol together with a third substance "X_1"[34], which could be separated from lanosterol only with difficulty. The minute yield made handling of X_1 alone impossible, but by mixing it with unlabelled lanosterol it was possible to investigate it. The diluted X_1 could be cleaved to yield radioactive acetone

from the side chain. The retropinacol reaction and cleavage also yielded radioactive acetone, so both the isopropylidine group of the side chain and the 4-gem-dimethyl group were still present. The possibility that the 14-methyl group alone had been lost was investigated by conversion of X_1, together with its inactive lanosterol carrier, to cholesterol. In the conversion of methyl labelled lanosterol to cholesterol, the three methyl groups lost appear as labelled carbon dioxide. Conversion of the X_1 and the carrier to cholesterol yielded only two thirds of the usual amount of labelled carbon dioxide relative to the labelled cholesterol produced. X_1 must therefore contain only two oxidisable methyl groups which are known to be at the 4-position[34].

There remained the question of the position of the nuclear double bond in X_1. On mechanistic grounds it was considered possible that the double bond might have shifted to the 8(14) position during removal of the C(14) methyl group as the corresponding carboxylic acid. A mixture of labelled dihydro X_1 and synthetic 4:4-dimethyl cholest - 8(14) en-3-ol clearly separated on recrystallisation. Separation of dihydro X_1 and 4:4-dimethyl cholest-7-en-3-ol was less marked, while with the double bond at 8(9) still less separation was observed. By conversion of the dihydro X_1 and 4:4-dimethyl cholest-7-en-3-ol carrier to the corresponding 7:8-glycol and cleavage to the keto aldehyde, chromatography clearly showed that the radioactive material separates from the carrier, proving their non-identity. On similar cleavage of the dihydro X_1 plus 4:4-dimethyl-cholest-8(9)-en-3β-ol, however, the intensity of radioactivity precisely followed the weight of the carrier in the

chromatographic fractions. X_1 is therefore 4:4-dimethyl-cholest-8:24-dien-3β-ol(7)[35].

Two substances have been proposed as intermediates between 4:4-dimethyl-cholest-8:24-dien-3β-ol and cholesterol. The first, zymosterol is synthesized from acetate by yeast and contains the rare double bond pattern of lanosterol, but lacks the three methyl groups at 4,4, and 14. The second, demosterol (cholesta-5:24-dien-3β-ol), is synthesized from acetate by chick embryos[36]. Both zymosterol and desmosterol are readily converted to cholesterol by rat liver.

Of the triterpenoids having more than thirty carbon atoms, only one has so far been investigated. Eburicoic acid (12) has been biosynthesized by allowing Polyporus sulphureus to grow in a medium containing 1-C^{14}-acetate. By stepwise degradation, Dauben has shown that, as in the case of cholesterol, C(4), C(11) and C(12) are labelled and that C(21), C(28), C(30) and C(31) are not labelled[37]. Using methyl labelled (2-C^{14}) acetate in a similar synthesis and degradation, it was found that C(21), C(30) and C(31) become labelled[38], but the "extra" carbon atom at C(28) remains unlabelled. Formate was found to be an efficient source of C(28)[39,40]. It appears probable that the same squalene cyclisation path is involved here as in the biosynthesis of lanosterol. It is particularly interesting that C(11) and C(12) are both derived from carboxyl carbon, since this is a necessary requirement if squalene is to be utilised as an intact molecule coiled as in (1) in the manner proposed by Woodward and Bloch[28].

Acetic acid to squalene

The search for intermediates in the conversion of acetic acid to squalene in vivo met with little success until 1956 when Folkers and his group[41] at Merck isolated mevalonic acid (MVA) (3) from "distillers solubles" while searching for "vitamin B_{13}"[42]. The acid was recognised as an acetate-replacing growth factor for certain lactobacilli.

As the structure of mevalonic acid (3) was becoming evident from degradations, its relationship to the isoprenoid hydroxymethylglutaric acid led another Merck group[43] to investigate its biosynthetic activity in cholesterol synthesis. Racemic mevalonic acid was converted in 43% yield to cholesterol, almost quantitative conversion of the (+) isomer[44].

Mevalonic acid labelled at the carboxyl group (1-C^{14} mevalonic acid) on incubation with cell-free rat liver homogenates yielded unlabelled cholesterol, the radioactivity being accounted for in the liberated carbon dioxide; 2-C^{14}-mevalonic acid, on the other hand, gave the incorporation of five radioactive carbon atoms into the cholesterol. In the complete absence of oxygen the conversion of 2-C^{14}-mevalonic acid was blocked at the squalene stage with incorporation of six labelled carbon atoms per molecule. The positions of the labels in the squalene[45,46], and cholesterol[47] indicate that the farnesyl chains (see (5)) are formed by linkage of C(5) of one mevalonate unit to C(2) of the next. The two central carbon atoms of squalene, which alone are converted to the methylene groups of succinic acid on ozonolysis by Bloch's technique[47], originate in

C(2) of mevalonate, showing that squalene here contains a C(2) to C(2) linkage of mevalonate units.

In a unique study of pentacyclic triterpenoid biogenesis, Arigoni[49] showed that 2-C^{14}-mevalonic acid is converted by soya bean seedlings to the soyasapogenols. Partial degradation of soyasapogenol A(see p. 38) has shown that two of the methyl groups are labelled as would be predicted by the squalene hypothesis. Of particular interest is the finding that the labelled soyasapogenol D(partial formula 13) contains a negligible proportion of the labelling at the 4β-hydroxymethylene group C(24), which must therefore originate from the C(3′) methyl group of mevalonic acid (3). Positions 2- and 3′- of mevalonic acid therefore do not become equivalent at any stage of the conversion to soyasapogenol D. In agreement with this, the diterpenoid rosenonolactone, biosynthesized from 2-C^{14}-mevalonate contains C^{14} at the 4α-methyl group equivalent to C(23) of the pentacyclic triterpenoids, while the 4β-lactone group equivalent to C(24) does not[50,51].

The formation of mevalonic acid from acetic acid is not yet completely understood, but the most probable sequence[52] is the conversion of acetyl co-enzyme A(acetyl CoA) to acetoacetyl CoA, which then reacts with a further molecule of acetyl CoA to give hydroxymethyl glutaric acid CoA(14). This is reduced either directly, or through the 5-aldehyde stage, mevaldic acid (15) to the 5-alcohol, mevalonic acid (3).

The possibility that the incorporation of mevalonic acid in terpenoids took place <u>via</u> mevaldic acid (15) has been con-

sidered for some time. Mevaldic acid is incorporated into terpenoids at the same rate, (within experimental error), as mevalonic acid itself; mevalonic acid, however, depresses the incorporation of mevaldic acid to a greater extent than the reverse. The incorporation of labelled mevalonic acid into terpenoids is only slightly reduced by the presence of large quantities of the 5-aldehyde.

More convincing support for the direct incorporation of mevalonic acid has recently been described. Mevalonic acid, doubly labelled with deuterium at the 5-position (3) is converted into squalene with incorporation of 9 to 10 of the 12 deuterium atoms[48]. An oxidative step at C(5) would have prevented incorporation of at least half of the deuterium into the squalene.

By conversion of unlabelled mevalonic acid to squalene, in a deuterium oxide medium, and degradation of the product, the exchange of hydrogen with the aqueous solution has been shown to be connected with the coupling of the two farnesyl chains to form the central link of the squalene molecule, and with the formation of the gem-dimethyl terminal groupings. The head to tail couplings, C(2) to C(5) in the formation of the farnesyl residues take place without exchange, and hence without incorporation of deuterium in the laevulinic acid moiety on ozonolysis of the squalene formed[48].

The conversion of mevalonic acid to squalene could, in principle, proceed either by building up multiples of the C_6 units of mevalonic acid to carboxylated long chain intermediates,

with subsequent decarboxylation, or by first decarboxylation to a C5 unit which would then polymerise. That the latter is indeed the case has been demonstrated both by Bloch[53] and by Lynen[54].

The formation of squalene from mevalonic acid was found to require the presence of a yeast or liver enzyme, adenosine triphosphate (ATP), a divalent metal cation and either reduced di- or triphosphopyridine nucleotides (DPNH or TPNH). In the absence of the reducing agent, Tchen[55] isolated the 5-monophosphate of mevalonic acid and confirmed its identity by synthesis.

The monophosphate, on further incubation with a yeast enzyme fraction, ATP and the manganese cation, yielded a diphosphate, mevalonic acid-5-pyrophosphate which again required phosphorylating conditions (enzyme, ATP, and a divalent cation) for conversion to yet another product[54,56].

The third product was shown by Lynen[54] to be isopentenyl pyrophosphate (4) and confirmed both by degradation and by synthesis. Lynen further converted the isopentenyl pyrophosphate to farnesyl pyrophosphate (5) using only a yeast enzyme and the magnesium cation. In the presence of a reducing agent (TPNH or DPNH) as well as the cation and enzyme, all of the above precursors were efficiently converted to squalene.

In a later study, Bloch[53] showed that the decarboxylation of mevalonic acid-5-pyrophosphate to isopentenyl pyrophosphate requires the participation of ATP and that the four products, isopentenyl pyrophosphate, adenosine diphosphate (ADP), carbon dioxide and orthophosphate are formed at the same rate and with-

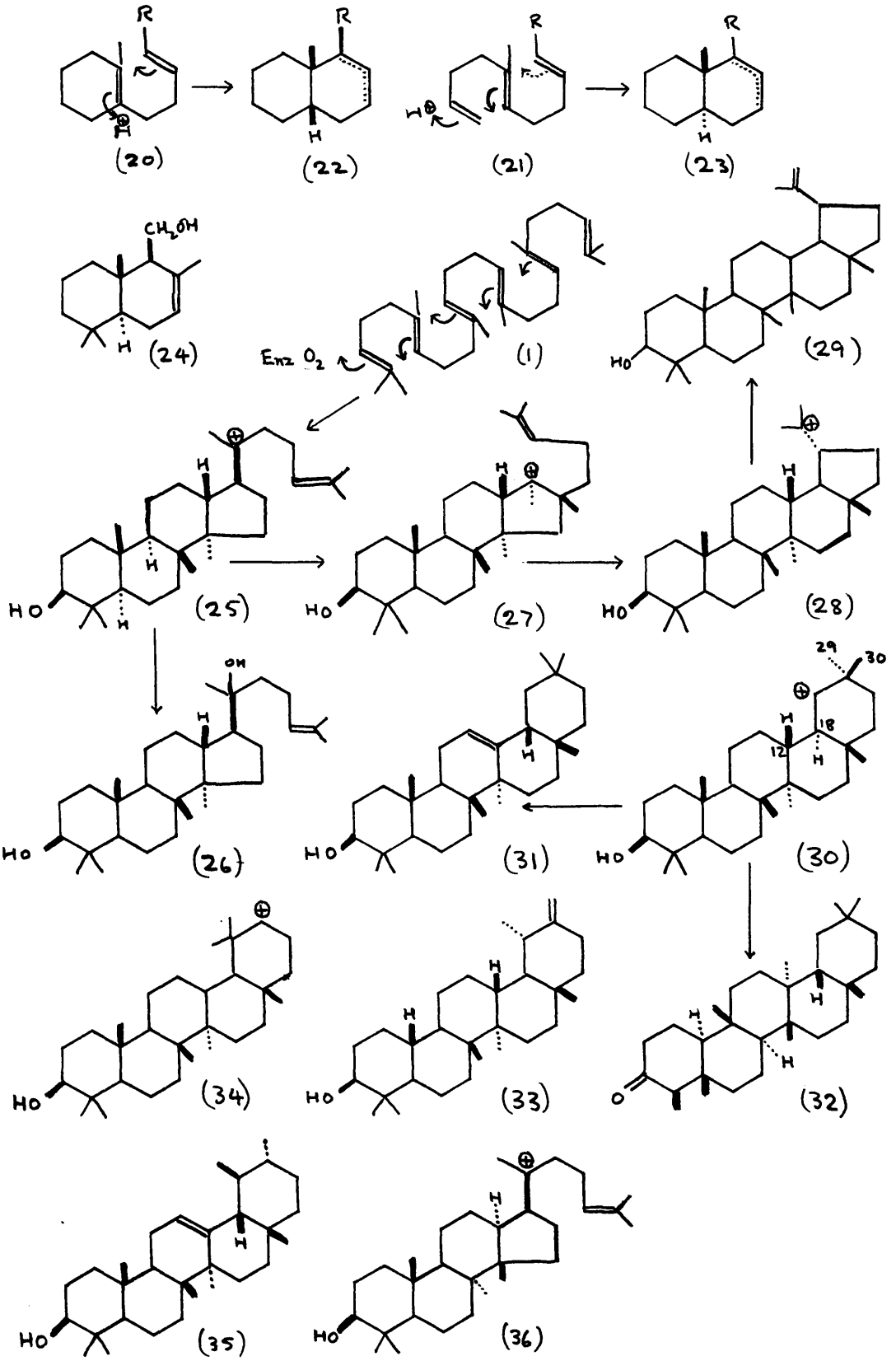
out an observable time lag. The two phosphorus atoms of mevalonic acid pyrophosphate are retained in the isopentenyl pyrophosphate as shown by incubating a P^{32} doubly labelled pyrophosphate and unlabelled ATP, when the inorganic phosphate produced was alone unlabelled.

The mechanism of conversion proposed by Bloch[48] and by Lynen[54] involves phosphorylation of the tertiary hydroxyl group of mevalonic acid pyrophosphate, followed by concerted decarboxylation and elimination of the tertiary 3-orthophosphate group. Lynen[54] further proposes that in the conversion of isopentenyl pyrophosphate to farnesyl pyrophosphate, the first step is migration of the double bond into the trisubstituted position followed by cleavage of the C-O bond to yield pyrophosphate and an allylic carbonium ion which reacts with a second molecule of isopentenyl pyrophosphate to give directly geranyl pyrophosphate. This mechanism, although supported by Bloch's finding[48] that conversion of mevalonic acid to squalene in a deuterium oxide medium causes uptake of two deuterium atoms, presumably during migration of the double bond, is in accord with Arigoni's results[49] (see p.19) only if the free allylic carbonium ion is not invoked, as this would cause racemisation at the mevalonic acid 3-position. This difficulty is readily overcome by postulating either that the carbonium ion remains firmly attached to the enzyme surface until it reacts, or that the cleavage of the C-phosphate bond is simultaneous with nucleophilic attack by the double bond of a second molecule of isopentenyl pyrophosphate.

Geranyl pyrophosphate, although postulated[54] as an intermediate, has not yet been identified among the reaction products.

The recent discovery of the C_{16}-farnesol carboxylic acid (16)[57] produced by incubation of 2-C^{14}-mevalonic acid with rat liver enzyme in the absence of microsomes, is not in accord with Bloch's conclusion that the decarboxylation precedes the joining of the isoprenoid fragments. Since over 60% of the labelled mevalonic acid is converted to the C_{16}-acid, it cannot be on a side path un-connected with sterol formation. The acid, however, is only poorly converted to sterols on incubation with rat liver homogenates. To explain this, Ogilvie[57] suggests that the true intermediate is converted to (16) during the extraction process. Formation of (16) from unlabelled mevalonic acid in the presence of $C^{14}O_2$ does not cause incorporation of labelling, so the carboxylation does not take place at the farnesol stage, but must be due to incorporation of a mevalonic acid unit without prior loss of its carboxyl group.

The only stage remaining to be discussed is the conversion of farnesyl pyrophosphate (5) to squalene (1). This step requires TPNH, a metal cation (Mg^{++} or Mn^{++}), water insoluble yeast or liver fractions (eg. cell wall material) and an enzyme[54,58] The coupling takes place with exchange of two hydrogen atoms between the aqueous medium and the two carbon atoms which become linked to form the central bond of squalene. If the coupling is conducted in a medium of deuterium oxide, two deuterium atoms are incorporated. On ozonolysis of squalene so formed, the deuterium is found only in the succinic acid fragment arising


R
(20)
(22)
(21)
(23)
CH2OH
(24)
Enz O2
(1)
(29)
HO
(25)
(27)
(28)
OH
(26)
(31)
29
30
18
12
(30)
(34)
(33)
(32)
O
(35)
(36)

from the central four carbon atoms[48]. It is not yet known if the two methylene groups of the succinic acid each have one deuterium substituent, or if one methylene group has both. At least, coupling mechanisms such as (17) involving the double bonds of the farnesyl chains, can be ruled out, as they require incorporation of deuterium at positions other than the two central carbon atoms.

Although the general pathway of steroid biosynthesis is now known, much remains to be investigated. Bloch[58] has estimated that there are at least twenty additional steps, as yet unknown, between mevalonic acid and cholesterol.

Structural correllation

Such of the above results as were then available were used by Ruzicka[9-11] in his comprehensive scheme for the biogenesis of triterpenoids. Ruzicka first considers the formation of the symmetrical tetracyclosqualene (18) and the tricyclic alcohol ambrein (2) by separate proton attack on the two ends of the coiled molecule. By oxidative attack, this mechanism explains the formation of α-onocerin (19). Ruzicka's original proposal of $OH^{\oplus}$ as the initiator must be modified in view of Bloch's demonstration[31] that oxygen activated by an enzyme, rather than water, causes the cyclisation.

Stork and Burghstahler[59] point out a fundamental distinction in the cyclisation of monocyclic dienes such as (20) and acyclic trienes such as (21). Ring closure of the monocyclic diene by concerted trans attack on the cyclic double bond by

a proton and by the nucleophilic carbon atom of the second double bond should lead to a cis decalin derivative (22). Concerted cyclisation of the triene (21), on the other hand, could lead to a trans decalin (23).

On treatment of farnesic acid with mild acid in vitro, three crystalline bicyclic carboxylic acids were obtained [59]. The trans configuration of the ring junction in all three products has been established[60]: they differ only in the position of the double bond, and the orientation of the carboxyl group. One of the acids, on reduction of the carboxyl group, gave racemic drimenol (24)[61].

Both the known trans fusion of rings A, B, and C of pentacyclic triterpenoids, and the finding that there is no exchange of hydrogen between squalene and the aqueous medium during in vivo cyclisation suggest that it is a fully concerted process.

Ruzicka[10,11] lists four assumptions on which his biogenesis scheme is based.

1. The squalene molecule shall react with the four central double bonds in the trans-configuration.
2. The cyclisation shall take place with the squalene molecule already folded into the conformations of the boat and chair rings about to be formed.
3. The cyclisations shall occur in the usual trans addition manner.
4. Wagner-Meerwein rearrangements and 1:2 eliminations shall occur only when this is permitted by the stereochemistry of the system.

For the sake of simplicity, carbonium ions are shown in the diagrams, although Ruzicka throughout his papers uses the more probable carbonium ion-double bond π-complexes which also imply retention of configuration at the "carbonium ion" shown.

Both Ruzicka[10,11] and Stork[59] recognised that different cyclisation paths are necessary to arrive at the pentacyclic and euphol- and dammar-type tetracyclic triterpenoids on the one hand, and the lanosterol group and steroids on the other.

The former groups are obtained by cyclisation of squalene with the first four potential rings in the chair conformation to give the tetracyclic carbonium ion (25).

Direct addition of $OH^{\ominus}$ to (25) gives the dammarene diols[62] (26); by stereospecific migrations of hydride and methyl groups with removal of a proton from C(9), the ion (25) is converted to euphol.

Ring expansion of (25) to (27) and further cyclisation lead to the lupeol group exemplified by lupeol (29) itself.

Rather than lose a proton, the pentacyclic precursor (28) of lupeol may undergo ring expansion to (30). Removal of the C(18) proton gives directly germanicol, while by a series of hydride shifts terminated by removal of the C(12) proton, β-amyrin (31) is formed. A more extended series of stereospecific hydride and methyl shifts leads to the unusual skeletons of taraxerol, alnusenone, and ultimately friedelin (32). Taraxasterol (33) can be obtained from (30) by migration of the C(29) methyl group to the C(19) carbonium ion, and removal of a proton from C(30).

The pathway to the α-amyrin group originates in (27) or (28) which, by cyclisation or ring enlargement respectively, give (34). Methyl migration from C(19) then gives the correct skeleton and the carbonium ion properly placed for hydride migration to leave the double bond in the common 12-position (35).

Cyclisation of squalene in an alternative conformation provides the intermediate carbonium ion (36) from which lanosterol and the sterols are derived . It is of interest that formation of (36) requires that both the potential rings B and D be in the boat form in squalene before the all-trans cyclisation begins.

The diversity of known triterpenoids is caused by later oxidation at various points on the skeleton. A most common site for oxidation in pentacyclic triterpenes is the C(27) angular carbon atom which is found in all states of oxidation, methyl, alcohol, aldehyde, and, frequently, carboxylic acid. Djerassi[63] has observed that in cactus triterpenes, oxygenation of the skeleton other that at C(3) is confined to rings C, D, and E. No exception to this has yet been discovered.

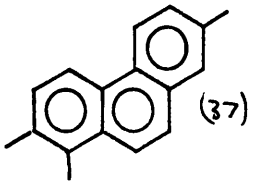
Elucidation of Structures of Some Triterpenoids

Tetracyclic triterpenoids

Lanosterol ($C_{30}H_{50}O$) and agnosterol ($C_{30}H_{48}O$).

The elucidation of the structure of lanosterol has been surveyed by Halsall[64], Barton[65], and Gascoigne[66].

The early work by Wieland, Windaus, Marker and their co-workers established the presence of a secondary hydroxyl group and two double bonds, only one of which could be hydrogenated; lanosterol is therefore tetracyclic. Oxidative fission of the reducible double bond yielded acetone, establishing the presence of a isopropylidine group. Ruzicka, Jeger, and their co-workers began work on the problem in 1944; they found that the main product on selenium dehydrogenation of the natural mixture of lanosterol and its di-hydro - and dehydro-derivatives is 1:2:8-trimethyl phenanthrene (37). The formation of this hydrocarbon is in contrast to the formation of naphthalene and picene derivatives by the dehydrogenation of pentacyclic triterpenoids. Dehydrogenation to (37) was subsequently found to be characteristic of the tetracyclic triterpenoids, and provided valuable evidence for the ring structure of lanosterol and other members of the group. The close relation of agnosterol (38) to lanosterol (6, R=H) was revealed by the oxidation of dihydro-lanosterol to dihydro-agnosterol[67]. Both agnosterol and its dihydro compound exhibit ultra-violet absorption at λ max. 243mμ, indicating the presence of a heteroannular conjugated diene. Agnosterol, like lanosterol, yields acetone on ozonolysis.



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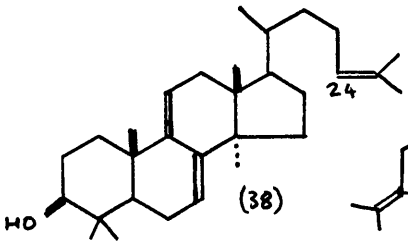

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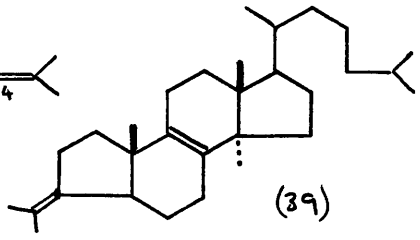

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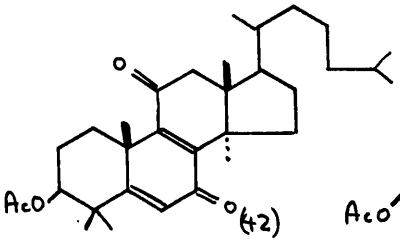

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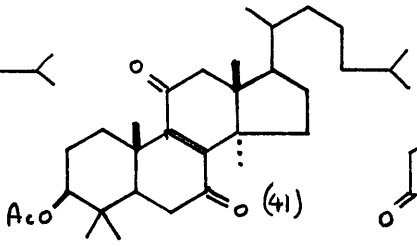

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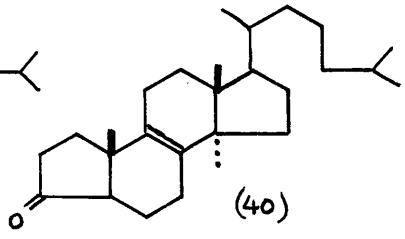

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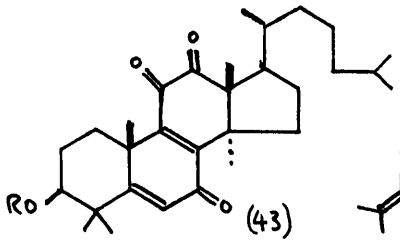

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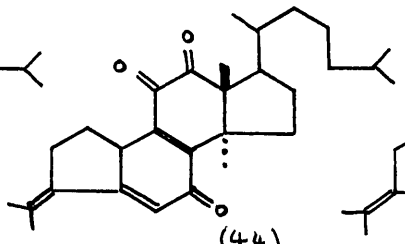

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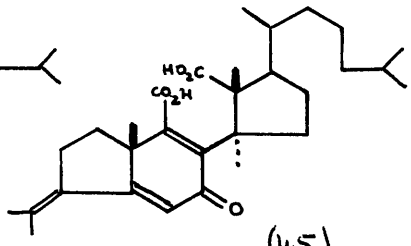

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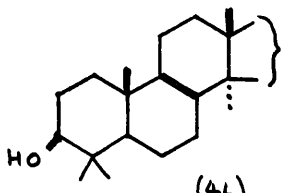

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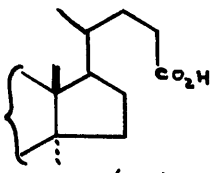

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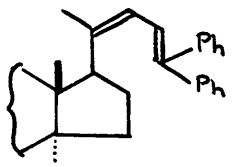


(48)

The presence of the common 3β-hydroxy-4:4-dimethyl system in ring A was established by the standard reaction sequence of phosphorous pentachloride rearrangement of 24-dihydrolanosterol to (39), cleavage of the double bond by ozone, or by hydroxylation with osmium tetroxide followed by cleavage with lead tetra-acetate yielding acetone and a trisnor ketone (40) which showed the infra-red absorption of a cyclopentanone. Oxidation of the original alcohol gave a ketone shown by its infra-red spectrum to be in a six-membered ring.

Oxidation of 24-dihydrolanosteryl acetate with chromic acid gives a yellow diketo derivative (41) containing the grouping - CO -C=C -CO - in the fully transoid arrangement[68]. Further oxidation with selenium dioxide affords successively (42) and (43) (R=Ac).

The relationship between the secondary hydroxyl group and the unsaturated system of (43) was demonstrated by the fact that the phosphorous pentachloride rearrangement of the alcohol ((43), R=H) affords a compound (44) in which the conjugation has been extended. Cleavage of the α-diketone in this compound gave a dicarboxylic acid, the ultraviolet absorption of which is consistent with the chromophore (45).

Considering the evidence thus far presented, together with the apparent absence of a replacable α-hydrogen atom in (43), the partial formula (46) for lanosterol could be deduced. The nature of the secondary octyl side chain was first demonstrated by stepwise degradation of the trisnor-acid (47), and confirmed by the isolation of 6-methyl-heptan-2-one from chromic

acid oxidation of lanostenyl acetate[69]. On degrading the diphenyl diene (48) via the methyl ketone to (49), infra red evidence suggested that the ring D of lanosterol was five-membered[70]. This was confirmed[71] by vigorous chromic acid oxidation of 3:7:11 - triacetoxy lanostane and isolation of 3:7:11 triacetoxylanan -17 -one (50).

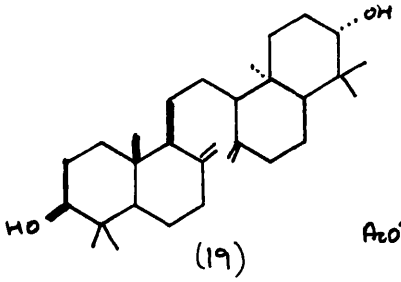
Evidence for the attachment of the side chain at the C(17) non-isoprenoid position was proved by degradative[72] and X-ray[73] studies, Barton having previously shown that the attachment was at either C(15) or C(17) rather than C(16) since the ketone group in (50) is flanked by only one CH_2 group[69].

Barton, Woodward, and their collaborators[74] have reported the conversion of cholesterol to lanosterol and agnosterol. Taken in conjunction with the total synthesis of cholesterol[75], this constitutes the formal total synthesis of lanosterol and agnosterol. The stereochemistry of lanosterol (6) was deduced from molecular rotation considerations and from the high degree of steric hindrance shown by an axial hydroxyl at C(11), and is confirmed by the X-ray analysis[73] of lanosteryl iodoacetate and by the total synthesis[74].

Onocerin ($C_{30}H_{50}O_2$).

The structure (19) has been assigned[76] to α-onocerin (α-onoceradiene diol) on the following evidence.

Ozonolysis of α-onocerin yielded a bisnordiketone which on reduction and selenium dehydrogenation yielded 1:5 dimethyl naphthalene. Selenium dehydrogenation of onocerin itself gave



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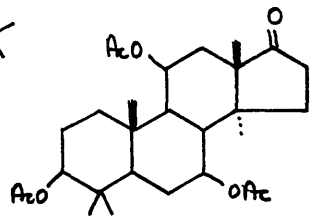

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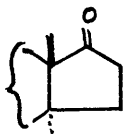

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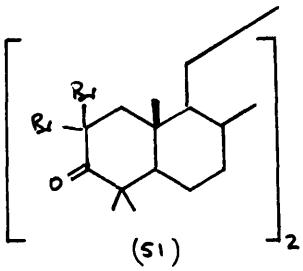

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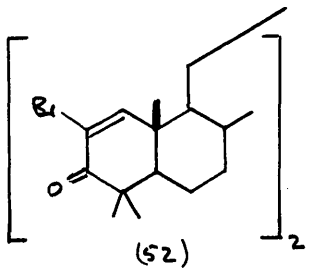

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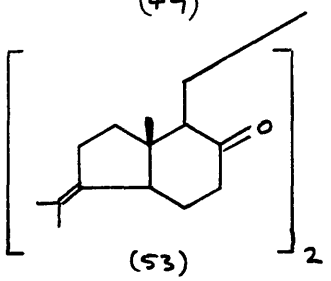

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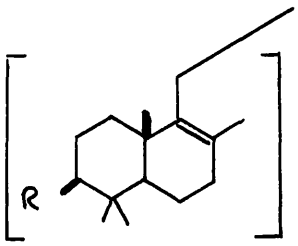

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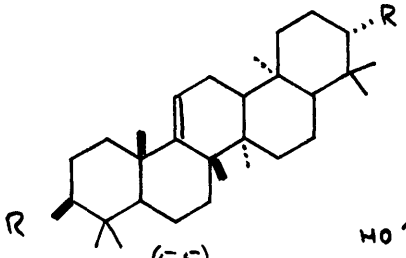

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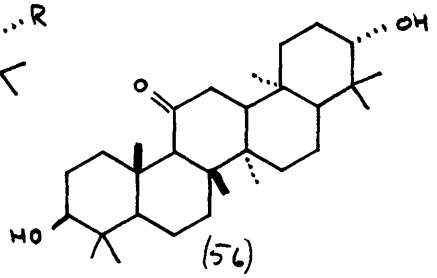

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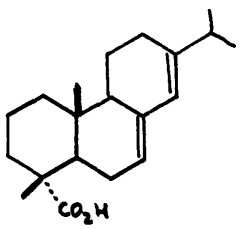

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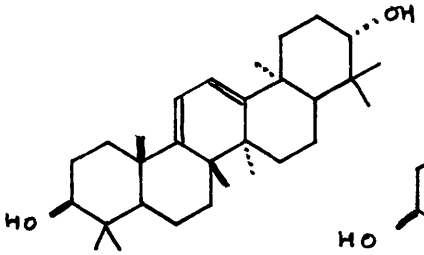

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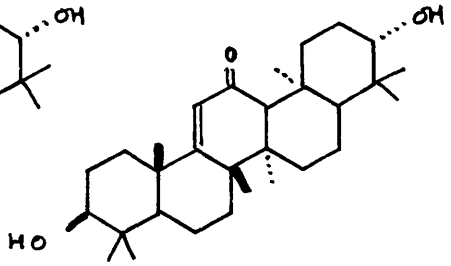


(57)

1:2:5 trimethyl naphthalene in good yield.

Hydrogenation of the vinylidene groups followed by oxidation led to a diketone shown by bromination to have four replaceable hydrogen atoms. Dehydrobromination of the tetrabromo diketone (51) afforded a bis-monobromo-enone (52) which had a characteristic ultra-violet absorption. The presence of two of the normal triterpenoid ring A groupings was shown by the formation of a bis - retro pinacol product (53) from bisnor - onocerane dione diol.

Removal of either of the hydroxyl groups of onocerin affords the same deoxy compound, and demonstrates the unique symmetry of the molecule.

Treatment of α-onocerin (19) with acid gives first β-onocerin (54, R=OH) and under more forcing conditions γ-onocerin (55, R=OH), both of which are isomeric with α-onocerin.

γ-Onocerin reacts with hydrogen peroxide in acetic acid to give a monoketone (56), saturated to tetranitromethane and showing infra red absorption characteristic of a cyclo-hexanone. The presence of only one double bond in γ-onocerin indicates that it is pentacyclic. The rate of oxidation of the double bond is similar to that of β-amyrin, and the derived ketones are similar in their resistance to mild Wolff-Kishner reduction, although both can be reduced under forcing conditions.

Bromination of dihydroxy -γ-onoceran - 11 - one (56) followed by dehydrobromination yields a conjugated enone (57) similar in its ultra-violet spectrum to the ring C enones of the amyrins. The ring C enone in the opposite sense was obtained by oxidation of γ-onocerin diacetate (55, R=OAc) with chromium

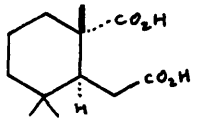
trioxide, and subsequent hydrolysis of the acetate groups. The enones obtained by the two methods were found to be identical, showing that the symmetry observed in α-onocerin is retained in the γ- series[76].

The enone, on reduction to the allylic alcohol and dehydration gives a conjugated homoannular diene (58), having an ultra-violet chromophore (λ max. 281 mμ) characteristic of the ring C homoannular dienes of the amyrins.

The symmetry of the γ-onocerane skeleton requires that the 8 and 14 methyl groups should be anti with respect to each other. The strong positive rotation of the homoannular diene ($[\alpha]_D$ +227°) is normal for ring C dienes in the α- and β-amyrin series, and implies a similar stereochemical environment, i.e. 8β- and 14α-methyl groups. The 8:14 - diketone (53) derived from α-onocerin does not invert at position 9, even with rigorous alkaline treatment, so the stereochemistry at the 9 position must be as shown (19).

The absolute configurations of the α-onocerin at positions 3, 5, and 10 have been established[77] by degradation of both abietic acid (59) of known absolute stereochemistry, and β-onoceradiene (54, R=H) to a common monocyclic dicarboxylic acid (60). The 3 - hydroxyl group was shown to be trans to the 5 - hydrogen by the ready formation of the dilactone (61).

β-Onoceradiene (54, R=H) has been synthesized[78] by electrolytic coupling of two bicyclic fragments (62) followed by dehydration.



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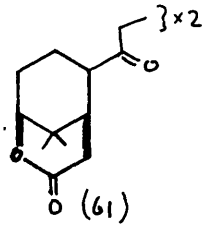

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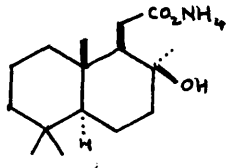

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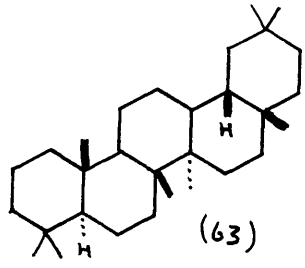

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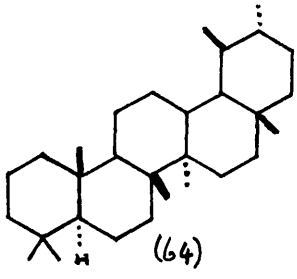

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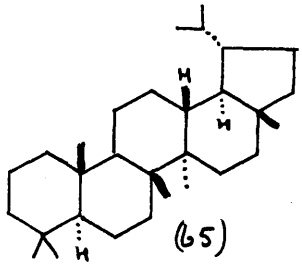

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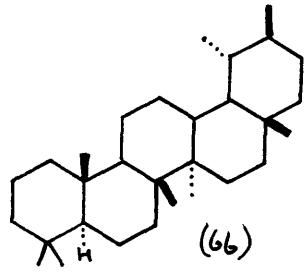

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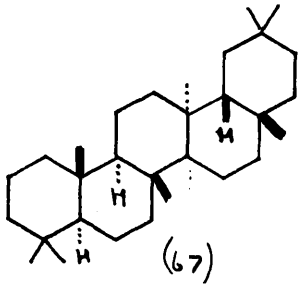

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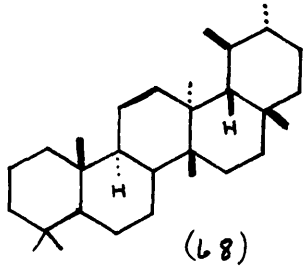

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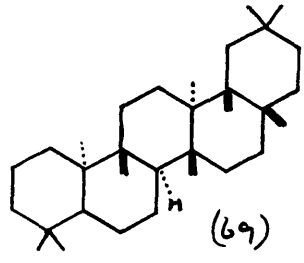

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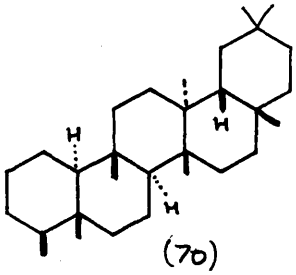

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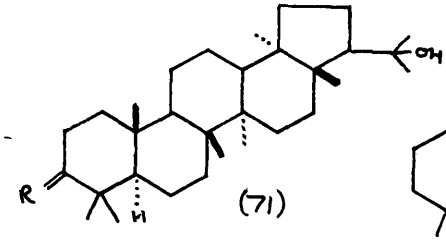

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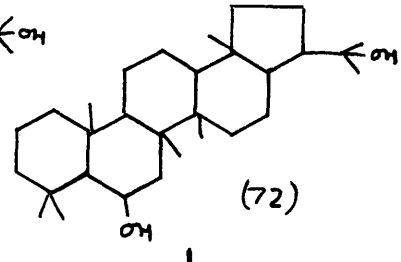

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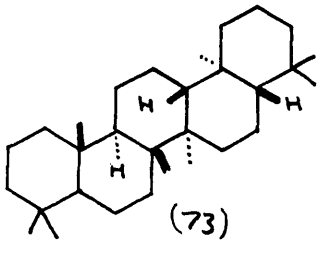

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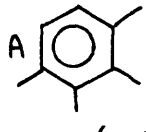

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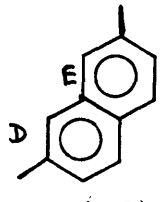

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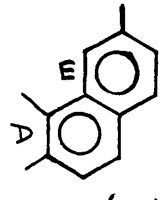

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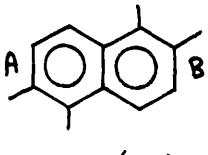

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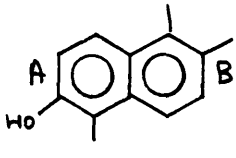

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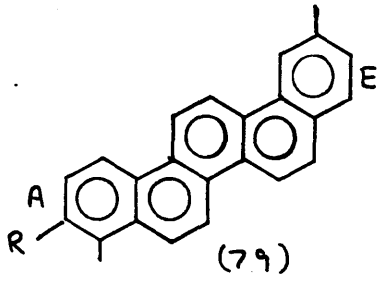


(79)

β-Onoceradiene has been converted to γ-onocerene (55, R=H), constituting the first total syntheses of these two compounds.

γ-Onocerin is the first known representative of a class of pentacyclic triterpenoids predicted by Ruzicka[11]. It's carbon skeleton and configuration in rings A to D is the same as in the major classes of pentacyclic triterpenoids; the occurrence of an oxygenated substituent at C(21) is unusual, only three other examples, machaerinic acid[79], treleasegenic acid[80], and aescigenin[81] being known.

Pentacyclic Triterpenoids.

In this review the cyclopropane compounds cycloartenol, cyclolaudenol, and cycloencalenol have not been included because of their close relationship to the tetracyclic group.

The better known pentacyclic triterpenoids, grouped according to their carbon skeletons, are listed in Tables 1 to 4.

Whereas Jeger[82], in 1950 was able to classify the pentacyclic triterpenoids of known structure into three major groups, the rapid advance in knowledge is illustrated by the fact that we now recognise nine groups - derivatives of oleanane (63), ursane (64), lupane (65), taraxastane (66), taraxerane (67), bauerane (68), alnusane (69), friedelane (70), and hopane (71, R=R'=OH). Hydroxyhopanone (71, R=O, R'=OH) and zeorin (72) belong to a family related more closely to the artefact γ-onocerane (73), than to any of the natural classes.

In elucidating the structures of pentacyclic triterpenoids, a number of techniques have found wide application. Of these, the type most important is dehydrogenation with selenium or

palladised charcoal. Unfortunately, pentacyclic triterpenoid molecules have a strong tendency to undergo rupture into two main fragments, when submitted to dehydrogenation, and only minor quantities of aromatic products characteristic of the whole carbon skeleton are formed. The main dehydrogenation products have been found to be 1:2:3:4 - tetramethyl benzene (74), 2:7 - dimethylnaphthalene (75), 1:2:7 - trimethylnaphthalene (sapotalene) (76), 1:2:5:6 - tetramethylnaphthalene (77), 1:5:6 - trimethyl-2-naphthol (78), all of which arise from rupture of the molecule, and 1:8 - dimethylpicene (79, R=H) which represents the original pentacyclic system. The more important data are summarised[86] in Table 5.

In the formulae (54) to(59) inclusive, the origin of the dehydrogenation products is indicated by lettering the rings from A to E. The formation of (74) and (77) from rings A and B of the triterpenoid skeleton is considered to take place by a retropinacolic rearrangement involving loss of the ubiquitous C(3) hydroxyl. This hydroxyl is preserved unchanged in the naphthol (78). A very important dehydrogenation product is 2-hydroxy-1:8-dimethylpicene (79, R=OH) as this preserves the pentacyclic ring system as well as the hydroxyl at C(3).

The oxygen function in ring A is usually present as a 3β-hydroxyl with a gem - dimethyl group at 4, (80). Less common is a 3-ketone, or a 3α-hydroxyl group. Dehydration of the 3-hydroxyl group follows one of two paths, depending on the stereochemistry at the 3 position.

Table 1. Oleanane derivatives.

Compound	-OH	C=C	$-CO_2H$	=O	References
Alcohols					
β-amyrin	3β	12			82,87
δ-amyrin	3β	13(18)			88
germanicol	3β	18			82
maniladiol	3β:16β	12			82,89,90
erythrodiol	3β:28	12			82
soyasapogenol C	3β:24	12:15			91
soyasapogenol B	3β:16:24	12			91
primulagenin A	3β:11α:28	12			82,90
longispinogenin	3β:16β:28	12			90,92
chichipegenin	3β:16β:22:28	12			93
aescigenin*	3β:22β:24:28	12			81
soyasapogenol A	3β:21:22:24	12			91
barringtogenol	2α:3β:23:28	12			94,101
A_1-barrigenol	3:15α:16β:27:28	12			95
Acids					
α-boswellic	3α	12	24		82,96
oleanolic (74)	3β	12	28		82,89
morolic	3β	12	28		97
sumaresinolic	3β:6β	12	28		82,98
echinocystic (98)	3β:16α	12	28		82,89,90
cochalic	3β:16β	12	28		99
siaresinolic	3β:19α	12	28		82,89
machaerinic	3β:21β	12	28		79

*16:21 -ether.

Compound	-OH	C=C	$-CO_2H$	=O	References
Acids (Continued)					
hederagenin	3β:23	12	28		82,96
queretaroic	3β:30	12	28		100
arjunolic	2:3:23 or 24	12	28		101
dumortierigenin*	3β:15β:22β	12	28		102
treleasegenic	3β:21β:30	12	28		80
myrtillogenic	3β:16β:28	12	29		103
entagenic	3:15:16 or 3:21:22	12	28		104
bassic	1:3:23 or 24	5(?):12	28		82
terminolic	2α:3β:6β:23	12	28		101,105
medicagenic	2β:3β	12	23:28		106
barringtogenic	2α:3β	12	23:28		94,101
Ketones and aldehydes					
oleanolic aldehyde	3β	12		28	107
glycyrrhetic acid	3β	12	30	11	82,108
machaeric acid	3β	12	28	21	79
gypsogenin	3β	12	28	23	82,96
gummosogenin	3β:16β	12		28	92
quillaic acid	3β:16α	12	28	23	82,109
lantadene A (rehmannic acid)	22β'	12	28	3	110,111
lantadene B	22β**	12	28	3	112
icterogenin	22β':24	12	28	3	113
gratiogenin	3β:19α:29 or 30	12		21	114

* lactonised to 15 -hydroxyl.
' angelate ester.
** -dimethacrylate ester.

Table 2. Ursane derivatives.

Compound	-OH	C=C	CO_2H	=O	References
α-amyrin	3β	12			115
brein	3β, 21 or 22 or 16β	12			116
uvaol	3β,28				117
β-boswellic acid	3α	12	24		118
ursolic acid	3β	12	24		117
ursonic acid		12	28	3	119
asiatic acid	2α,3β,24	12	28		106
quinovic acid	3β	12	27,28		120,121
phyllanthol	3β	13:27-cyclo			117,122

Table 3. Lupane derivatives.

Compound	-OH	C=C	CO_2H	References
lupeol	3β	20(29)		123
betulin	3β:28	20(29)		82,123
betulic acid	3β	20(29)	28	82,123
thurberogenin	3β:19	20(29)	28*	124
stellatogenin	3β:19:20		28*	125
melaleucic acid	3β	20(29)	25:28	126

* 19(28) lactones.

Table 4. Other Natural Triterpenoids.

Compound	References
taraxasterol	127
ψ-taraxasterol	127
ψ-taraxastane diol	128
taraxerol	129
taraxerene	130
calendenol	131
bauerenol	132
friedelin	133,134
cerin	135
hydroxyhopanone	83,84,85
oxyallobetul-2-ene	136

Table 5.

Triterpenoid	Dehydrogenation products.	Other products.
α- and -β amyrins	(74),(75),(76),(77),(78), (79, R=H)	(79, R=OH)
quinovic acid	(79, R=H)	$C_{26}H_{28}$ or $C_{28}H_{30}$ mp 167°
friedelin	(76), (79, R=H)	allylcyclohexene 1:2:8-trimethyl phenanthrene.
gypsogenin	(75),(76),(78),(79, R=H)	(?)$C_{27}H_{28}$ mp 117-118°
hederagenin	(74),(75),(76),(78), (79, R=H)	1:2:6-trimethyl phenanthrene.
leucotylin		1:2:5-trimethyl naphthalene.
oleanolic acid	(74),(75),(76),(78), (79, R=H)	
siaresinolic acid	(74),(76),(77),(78), (79, R=H)	$C_{25}H_{24}$ mp 143°
sumaresinolic acid	(74),(75),(76),(77), (78),(79, R=H)	$C_{25}H_{24}$ mp 143°
ursolic acid	(75),(76),(79, R=H)	
zeorin		1:2:5-trimethyl naphthalene.

Treatment of 3β-hydroxy-4:4-dimethyl triterpenoids (80) with phosphorous pentachloride in inert solvents causes dehydration with rearrangement as shown in (80) to (82). Cleavage of the double bond by conventional methods gives acetone and a C_{27} ketone showing infra red absorption at about 1740 $cm.^{-1}$ characteristic of a cyclopentanone. This method has successfully been applied to a α-amyrin (as α-amyradienone), lupanol, oleanolic acid 18-iso-lactone, and quinovic acid dimethyl ester notably by Ruzicka and his co-workers[138].

3α-hydroxy-4:4-dimethyl triterpenoids, having a 2β(axial)-hydrogen atom trans to the 3α(axial) hydroxyl group, do not rearrange on dehydration, and merely give a dimethyl cyclohexene derivative (83).

To determine the configuration of hydroxyl groups at other positions in triterpenoids, use can be made of the fact that in reduction of ketones by dissolving metals, commonly by sodium and isopropanol, the thermodynamically more stable (equatorial) alcohol is obtained[138]. Reduction of unhindered ketones by lithium aluminium hydride gives the equatorial alcohols; highly hindered ketones are reduced to the axial alcohols[139]. Hindered axial alcohols are oxidised more rapidly than unhindered equatorial ones by chromium trioxide in acetic acid[140], and are esterified, or the esters hydrolysed, less readily[87].

Mention will be made later of the use of the method of molecular ortation differences. In essence it depends on the

observation that the changes in molecular rotation of a series of derivatives of a group in a molecule are characteristic of the immediate environment of the group. The derivatives most commonly employed are alcohol, acetate, benzoate and ketone. This method has been of considerable value in determining the absolute configuration of the triterpenoids[141].

The Oleanane Group. (see Table 1.)

Since the appearance of the recent series of papers on cactus triterpenoids by Djerassi[63,106], the oleanane, or β-amyrin group, already the largest, has increased to forty three naturally occurring compounds. The remaining groups together contain only twenty six members of known structure.

The elucidation of structures in the oleanane group was done by inter-relation of the members, and degradation where there was a functional group in a suitable position for entry into the carbon skeleton. Tables of the inter-relations have been presented by Barton[86].

The ethylenic linkage present in the oleanane group of triterpenoids is resistant to hydrogenation. Its presence has been shown by titration with peracids, by the colour with tetranitromethane, and by the ready lactonisation of those members of the group which bear a carboxyl group in the 17-position. This ease of lactonisation has been employed to protect the ethylenic linkage in ring C of the skeleton while carrying out oxidative degradations in rings A and B. Such degradations have

been of importance in proving the structures of these two rings in the oleanane and ursane groups.

Oleanolic acid.

The chemistry of oleanolic acid (84) occupies a position of special importance in the formulation of triterpenoids of the oleanane group. This is because of the relative ease with which it has been possible to degrade the molecule into two halves.

Oleanolic acid $C_{30}H_{48}O_3$ was first extracted from olive leaves[142], and has since been isolated from a variety of sources. Under mild conditions acetylation of oleanolic acid gives a monoacetate. The presence of a carboxyl group is shown by methylation with diazomethane. The methyl ester cannot readily by hydrolysed, showing that the carboxyl group is hindered. Oleanolic acid gives a yellow colour with tetranitromethane showing it to be unsaturated. Titration with perbenzoic acid gives a saturated monoepoxide. The presence of a single double bond was confirmed by the formation of a saturated lactone on treating oleanolic acid with acid. To explain the ready formation of the lactone the double bond must be $\beta\gamma$-or $\gamma\delta$ - to the carboxyl group. From infra red evidence, the lactone is γ. The original double bond was shown to be resistant to catalytic hydrogenation. Oleanolic acid is thus a monohydroxy acid containing one double bond, and must therefore be pentacyclic.

The eludication of the ring structure began with the dehydrogenation studies carried out by Ruzicka and his co-workers. Selenium dehydrogenation yielded the aromatic compounds (74) to (79, R=H). The same products, with the exception of the tetramethylnaphthalene (74) were obtained by palladium dehydrogenation; it was therefore probable that oleanolic acid has a reduced picene skeleton.

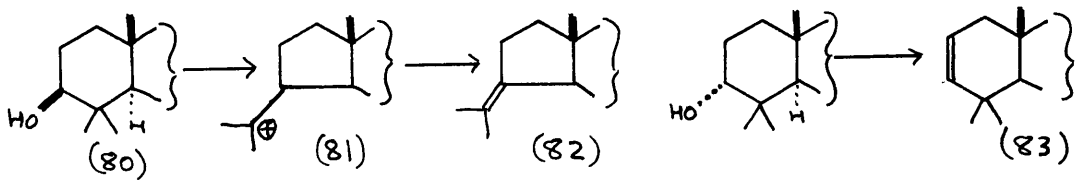
Oxidation of the acetate of oleanolic acid (84) with chromic acid leads to the formation of a lactone dicarboxylic acid (85, R=OAc, R'=H) in which ring C has been split[143]. Conversion to the 3-ketone-12-monomethyl ester (85, R=O, R'=Me) followed by pyrolysis gave a $C_{14}H_{22}O$ ketone (86). Reduction to the corresponding hydrocarbon and dehydrogenation furnished 1:6-dimethylnaphthalene (77). The other half of the molecule was found as the unsaturated methyl ester (88) which was hydrolysed and dehydrogenated to 2:7-dimethylnaphthalene (75). A hydrocarbon (79) was also produced in the reaction, and gave 2:7-dimethylnaphthalene on dehydrogenation.

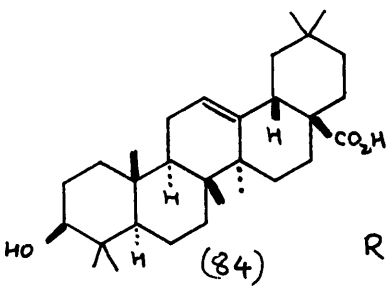
Pyrolysis of the dimethyl ester corresponding to (85, R=O) furnished, among other products a keto-acid methyl ester (90) which was reduced to the corresponding saturated acid methyl ester (91). This saturated acid was also obtained by degradation of ambrein (2). Since this in turn has been correlated with manool, which has been related to dehydroabietic acid (92) this constitutes a proof of the structure of rings A and B in oleanolic acid (84), and thus in the group of terpenoids related to oleanolic acid.

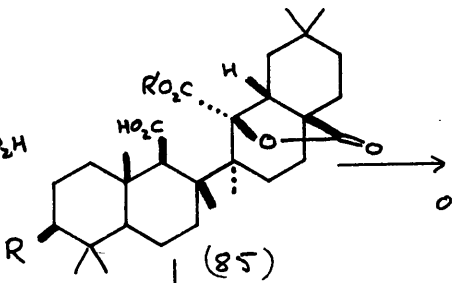
Ruzicka[144] also examined the non-ketonic fraction produced in the pyrolysis of the dimethyl ester of (85, R=O). This consisted of a mixture of mono- and di-unsaturated esters, hydrolysed by alcoholic potassium hydroxide to a mixture of acids which, on selenium dehydrogenation gave a mixture of 2:7-dimethyl naphthalene (75) and the known trimethyl tetralin (93). Palladised charcoal dehydrogenation of the mixture of esters referred to above afforded, after hydrolysis, the acid (94), which has not yet been synthesized. The ultra-violet spectrum corresponds very closely to that of 2:3:6-trimethylbenzoic acid.

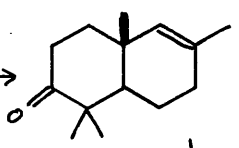
The relative positions of the ethylenic linkage and the carboxyl group were decided mainly on the basis of the following evidence. Oxidation of the methyl ester acetate of oleanolic acid (84) with selenium dioxide gave a substance analysing as $C_{33}H_{46}O_6$, for which the formula (95, R=CO_2Me) was proposed[145]. The structure was substantiated[146] by stepwise introduction of the keto groups and double bonds. In agreement, the acid produced by alkaline hydrolysis lost carbon dioxide on heating to give (95, R=H), which was capable of forming a pyridazine derivative on treatment with hydrazine. The ready formation of this derivative implies a 1:4-arrangement of the two keto groups. Further oxidation of the diene dione (95, R=CO_2Me) by chromic acid furnished an oxido compound formulated as (96), for, on drastic alkaline hydrolysis, a nor-acid containing a 1:3 diketone grouping (97) is obtained.

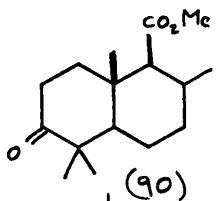
The formation of the diene-dione grouping, as in (95) by selenium dioxide oxidation is characteristic of the olean-

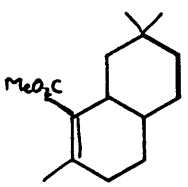

HO
H
(80)
(81)
(82)
HO
H
(83)

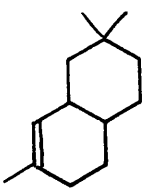

H
CO2H
H
HO
H
(84)


RO2C
H
HO2C
O
O
R
(85)


O
(86)


CO2Me
O
(90)


MeO2C
(88)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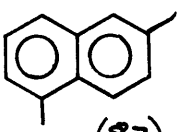
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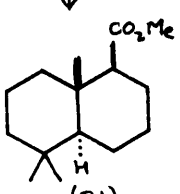
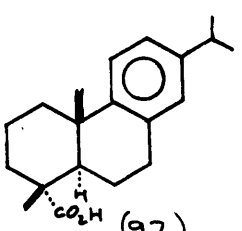
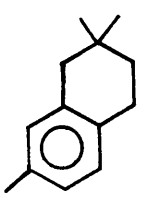

CO2Me
H
(91)


H
CO2H
(92)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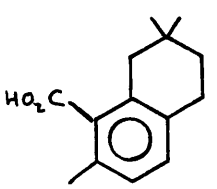
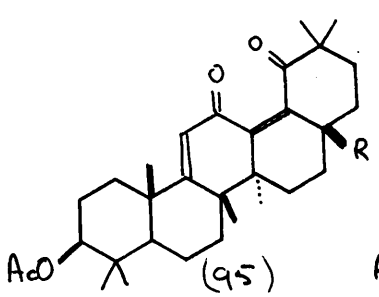
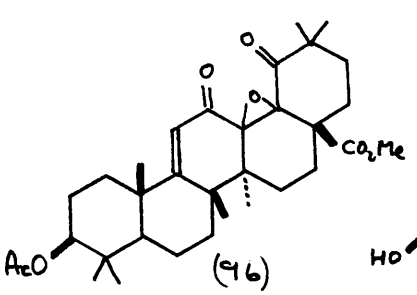
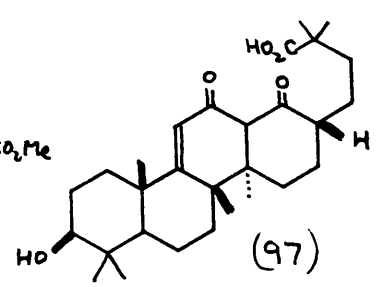
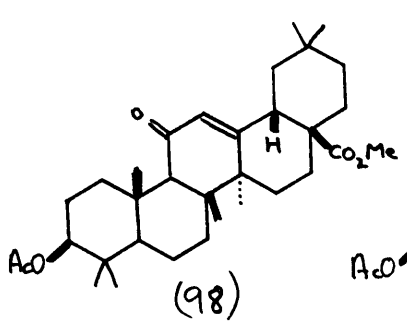
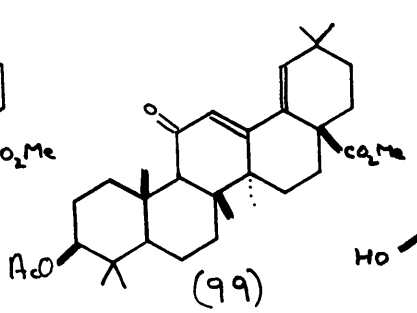

HO2C
(94)

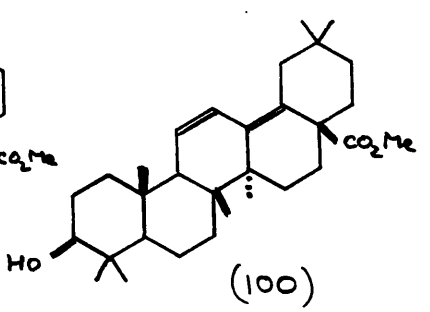

O
O
R
AcO
(95)


O
O
O
CO2Me
AcO
(96)


HO2C
O
O
H
HO
(97)


O
H
CO2Me
AcO
(98)


O
CO2Me
AcO
(99)


CO2Me
HO
(100)

13(18)-ene double bond environment, or of substances which can readily be rearranged to olean-13(18)-ene derivatives. Ursane derivatives cannot be oxidised to diene-diones of this type, and since the characteristic ultra-violet triple maximum makes its detection easy, this is a convenient way of distinguishing between ursane and oleanane skeletons.

The stereochemistry of rings A and B in oleanolic acid follow from its degradation to the ester (91) as described on p. 46, and from the retro-pinacol reaction p. 43. Barton and Holness[89] showed that the isomerisation of methyl-11-keto-oleanolate acetate (98) could be brought about by alkali as well as by acid, and that it involved inversion at C(18) by enolisation. The possibility that the isomerisation had taken place at C(9) could be excluded since it had been shown[147] that the C(11)-carboxyl group of the degradation product (85, R-AcO) is β-equatorial and hence the B/C ring junction is trans. This is supported by the observation that the dienone (99) could not be isomerised under the conditions used by Barton and Holness. Both (98) and its C(18) epimer could be reduced by sodium in alcohols to the same diene (100). This and other evidence supports the conclusion that rings D and E must be cis fused in oleanolic acid. Formation of the lactone of oleanolic acid is accompanied by inversion at C(18)[89]. The conclusions of Barton[89], and Klyne[141] enabled configurations to be assigned to all the asymmetric centres of oleanolic acid (84). An X-ray investigation of methyl oleanolate iodoacetate has also been carried out[148], and supports the structure (84).

Icterogenin.

Barton and de Mayo[113] have summarised the literature on icterogenin, the physiologically active compound isolated from Lippia rehmanni and responsible for jaundice and photo-sensitivity in sheep. They showed that icterogenins A, B and C are merely different crystalline or solvated forms, and hence rendered unnecessary these distinguishing letters.

Icterogenin (101), $C_{35}H_{52}O_6$, was shown to contain a carboxyl group, a reactive carbonyl group and a hydroxyl group. The two remaining oxygen atoms are contained in an $\alpha\beta$-unsaturated lactone or ester as was shown by the ultra violet maximum at 212 mμ. Hydrogenation using palladium saturated only the double bond conjugated with the ester carbonyl. With platinum in acetic acid, the benzene ring of the methyl ester benzoate, and the carbonyl group were also reduced. Perbenzoic acid titration of this showed that one double bond remained.

The relative positions of the carbonyl group and the free hydroxyl were shown by treatment of icterogenin and its derivatives with cold alkali, when formaldehyde was eliminated. This was shown to be analogous to the behaviour of methyl hederagonate (102) which forms methyl hedragonate (103) on similar base treatment. Furthermore, the colour given by icterogenin in the Zimmermann test was identical with that given by hederagonic acid and its derivatives. The keto group was therefore probably at C(3) and the presence of the grouping $-CH_2-CO-C-CH_2OH$ was indicated. This was subsequently confirmed.

Vigorous alkaline hydrolysis of icterogenin afforded tiglic acid, but on pyrolysis angelic acid was isolated, indicating that this acid was present in the original unsaturated ester grouping, but was isomerised by the alkali. Chromic acid oxidation of the non-volatile hydrolysis product (104) yielded an unstable diketo acid which lost carbon dioxide on warming in benzene solution to give a diketone $C_{28}H_{44}O_2$. This indicates that the secondary hydroxyl group freed by hydrolysis is β to the carboxyl group. The methyl ester of the diketo acid forms an isomeric, and more stable diketone $C_{28}H_{44}O_2$ on treatment with alkali. Neither of these diketones is identical with the compound (105) obtained from quillaic acid, and hence the angeloyloxy group must be present at C(22) rather thanC(16). Its configuration is considered to be 22β-axial because of its marked resistance to hydrolysis and the resistance of the corresponding hydroxyl group to acetylation. The diketones $C_{28}H_{44}O_2$ are presumably isomeric at C(17) which can therefore have no more than one hydrogen atom. The carboxyl group must therefore have been tertiary. This is confirmed by the resistance of the methyl ester to hydrolysis. The formation of a bromolactone (106) showed the relative positions of the carboxyl group and the double bond.

The remainder of the constitution of icterogenin (101) was demonstrated by hydrogenation of the compound $C_{30}H_{44}O_3$ obtained by elimination of formaldehyde and angelic acid from icterogenin methyl ester, which gave methyl hedragonate (103). This established the structure of icterogenin except for C(4).

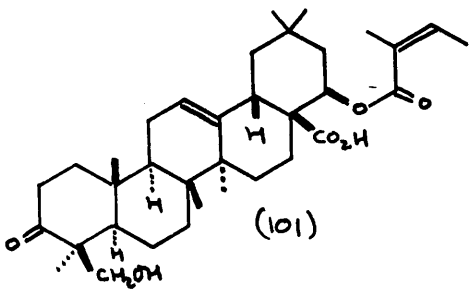
The configuration at C(4) was determined by pyrolysis of icterogenin methyl ester benzoate, hydrogenation and re-oxidation which gave a hexahydrobenzoate differing from the corresponding derivative of methyl hederagonate, although both formed methyl hedragonate on treatment with alkali. Molecular rotation differences also support a β(axial) configuration for the hydroxymethyl group at C(4).

Rehmannic acid.

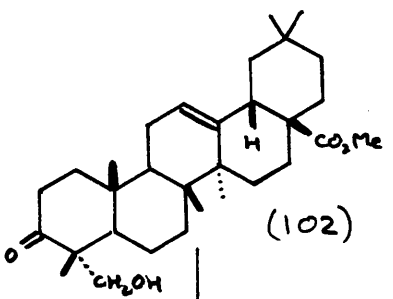
In the extraction of icterogenin from Lippia rehmanni, Barton and de Mayo[113] isolated a second substance which they named rehmannic acid (107, R=angeloyloxy). The identity of this substance with the previously known Lantadene A[149] forms the second part of this thesis[111].

Rehmannic acid[110] $C_{38}H_{52}O_5$ was shown to be a keto acid similar in infra-red spectrum to icterogenin. Pyrolysis of the methyl ester yielded angelic acid and an unsaturated compound which could be hydrogenated to methyl oleanonate. This establishes that the original ketone is at C(3) and the carboxyl at C(28) on an oleanane skeleton.

Vigorous alkaline hydrolysis of rehmannic acid afforded a hydroxy acid (107, R=OH) which on oxidation to the diketo acid readily lost carbon dioxide, indicating that the hydroxyl group had been β- to the carboxyl, and therefore at either C(16) or C(22). The hydroxyl group could not be at position 16 because the derived nor-diketone was not identical with any of the nor-diketones derived from echinocystic acid (108) in which



(101)



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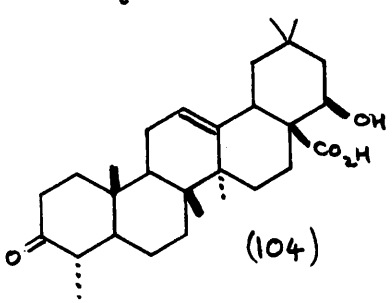

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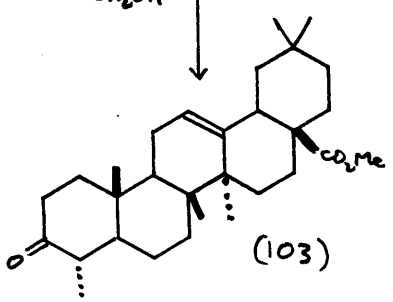

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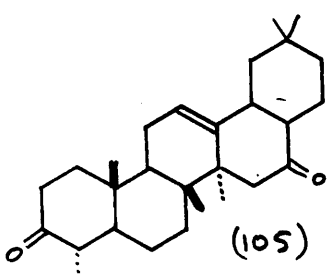

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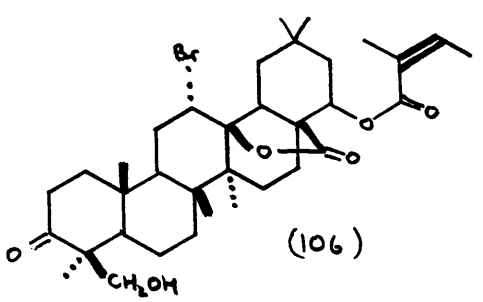

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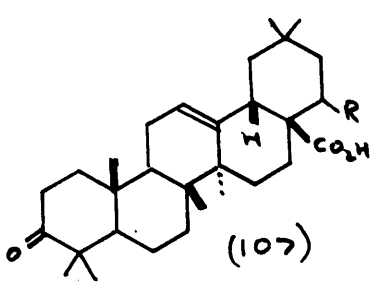
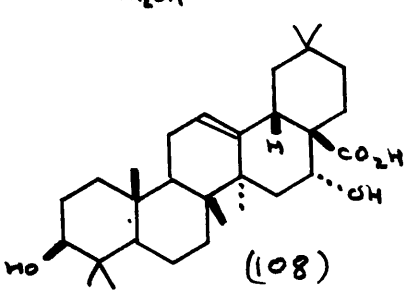


(107)



(108)

the original hydroxyl group was known to be at 16. Further, the diketo acid (107, R=O) derived from rehmannic acid gave a methyl ester different from the corresponding diketo acid methyl ester from echinocystic acid (108). The ester grouping in rehmannic acid must therefore be attached to C(22). Its configuration (β, axial) is based on comparisons of molecular rotations with the icterogenin series.

Lantadene B.

This compound, from lantana camara is devoid of the photosensitising activity of lantadene A and icterogenin. Following the work on the latter compound, its structure has been elucidated[112] as (107, R=O.CO.CH=CMe_2). After careful chromatographic purification it analysed for $C_{35}H_{52}O_5$, and is therefore isomeric with rehmannic acid. Hydrolysis yielded 22β-hydroxyoleanonic acid (107, R=OH). The steam volatile fragment was identified as ββ-dimethylacrylic acid. This acid was also eliminated on pyrolysis indicating that lantadene B is (107, R =ββ-dimethylacryloyloxy).

The ursane Group. (see Table 2.)

The reactions of the ursane group of triterpenoids are in many cases similar to those observed in the oleanane group. The ubiquitous 12 - double bond is, however, more sterically hindered and considerably less reactive than the 12 - double bond

in the oleanane series. This may be illustrated by methods of preparing the corresponding oxides. Olean -12-ene derivatives, on treatment with perbenzoic acid at room temperature give the oxide, while urs-12-ene remains unattacked. The epoxidation can be brought about by ozone. The saturated hydrocarbon, oleanane, can be obtained by Wolff-Kishner reduction of the 12-ketone; ursan-12-one, on the other hand, cannot be reduced to the hydrocarbon, nor can ketone derivatives be made. Ursane itself has only recently been prepared[150] by an indirect route involving ursan-11-one. The ursane and oleanane groups have the same constitution in rings A, B, C and D. Reactions of the groups involving only rings A and B are very similar[151].

α-Amyrin. (urs-12-en-3β-ol).

The structure of α-amyrin (109) has been established only recently[115], although it had been proposed[152], without specification of stereochemistry in ring E, in 1949.

The degradation of α-amyrin followed closely the methods used on oleanolic acid. Preliminary reactions demonstrated that α-amyrin, $C_{30}H_{50}O$, is a mono-unsaturated alcohol, and is therefore pentacyclic. The environment of the hydroxyl group (109) was shown by the standard reaction sequence following the retro-pinacol reaction (80) - (82).

Cleavage of ring C was attained by nitric acid oxidation of 3β-acetoxy-ursan-12-one to the 11:12 seco-11:12-dicarboxylic acid (110, R=AcO). Pyrolysis of the dimethyl ester of the

corresponding ketone (110, R=O) gave a mixture of mono-unsaturated keto acid methyl esters which on hydrogenation and re-oxidation furnished the keto-ester (90) identical with that derived in a similar manner from oleanolic acid. These experiments constitute a proof that rings A and B in the ursanol and oleanol skeletons are identical, and that the A/B ring fusion is trans. The non-ketonic pyrolysis products, which must be derived from rings D and E after catalytic hydrogenation, furnished a methyl ester, which gave 1:2:7-trimethylnaphthalene (76) on selenium dehydrogenation.

The size of ring C could not be decided from the above experiments, but the infra-red spectra of the 11-ketone, the 12-ketone, and the conjugated enone, 11 keto α-amyrin (cf 98), are consistent only with ring C being six-membered.

Spring and his collaborators[153] drew attention to the lack of knowledge of the stereochemistry of α-amyrin derivatives except in rings A and B. They showed that the configuration at C(9) must be the more stable one, being unaffected by treatment of the 11-ketone with alkali, and regenerated on hydrolysis of the 9(11)-en-11-yl acetate. They explained the fact that an 11-keto-urs-12-ene derivative, unlike the corresponding oleanene derivative (98) did not suffer inversion at C(18) with alkali as being due to the stability of the configuration at C(18). This they demonstrated by acid isomerisation of urs-13(18)-en-3β-yl-acetate to α-amyrin acetate proving that α-amyrin has the more stable configuration at C(18), and that, in contrast to

the oleanene series, urs-12-ene is more stable than urs-13(18)-ene. Furthermore they showed that ursa-9(11):12-dienyl acetate, ursa-11:13(18)-dienyl acetate and ursa-9(11):13(18)-dienyl acetate are all converted by acid to olea-11:13(18)-dienyl acetate. Better yields were later obtained with ursa 9(11):12-diene-3-one[154]. Thus they concluded that the configurations at C(8), C(9), C(14), and C(17) are the same as in oleanane. Jeger[155] had reached the same conclusions about the configuration of C(9) by comparison of the rotation changes in the ursane and oleanane series when the asymmetry at C(9) is destroyed, and at C(14), on the basis of similar rearrangements of the C(14) methyl group in both series. Spring's establishment that the methyl group on C(17) is β, together with the arguments[156,157] in favour of a β-hydrogen at C(18) demonstrates that the D/E junction is <u>cis</u>.

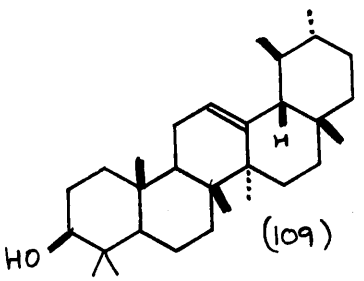
Consideration of the reason for the stability of the D/E <u>cis</u> ring junction, together with the pronounced hindrance of the 12-double bond and the 12-keto group, and the fact that ring E must differ from an oleanane skeleton, but be capable of rearranging to an oleanane type, led Spring[153] to propose a five-membered ring E with an attached isopropyl group (111).

Jeger and Ruzicka[158] pointed out that the transformation of the ursadienes to an oleadiene could equally be explained on the basis of the six-membered ring E (109) for α-amyrin, and drew attention to the analagous acid-catalysed isomerisation of lupene I. They also published details of the degradation to (113) of the methyl ester (112) obtained by pyrolysis of an α-amyrin derivative, previously reported in a thesis[159].

The methyl ester of (113) was shown to yield iodoform from the methyl group attached to C(19) and an acid which formed the trimethyl ester (114). These degradation products could not be explained on the basis of Spring's formula (111). Infra-red evidence both for[160] and against[161] Spring's formula was published.

By comparison of derivatives of the ursane series with the corresponding oleanane and 18α-oleanane compounds, Corey[162] concluded that ursane resembles the oleanane (D/E cis) rather than the 18α-oleanane (D/E trans) series in its behaviour and hence must be cis. The greater stability of the cis structure could be explained if both the methyl groups on C(19) and C(20) were equatorial (19β, 20α, 109). Isomerisation would then require these groups to be forced into the unfavourable axial configuration. It was also pointed out[162] that a five-membered ring E would inhibit the formation of ursolic lactone owing to the increased strain which would be present in a trans pentalene system. The fact that ursolic lactone is even less strained than oleanolic lactone and 18α-oleanolic lactone is shown by their infra-red absorptions[161].

Conclusive evidence for the six-membered ring E structure was provided by Jeger[115]. The α-amyrin derivative (115) was heated to 320° when scission of ring D occurred, probably by a reverse Diels Alder reaction to give a monocyclic trimethyl cyclohexene. Epoxidation and isomerisation with boron trifluoride yielded a laevorotatory trimethyl cyclohexanone (116) in



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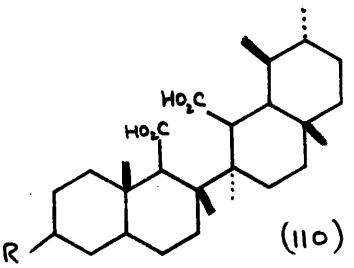

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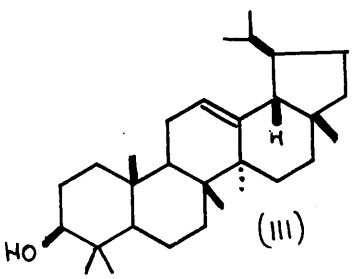

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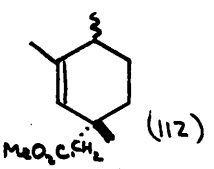

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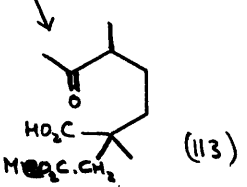

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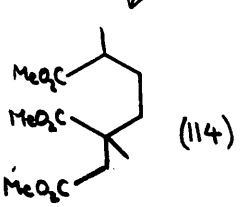

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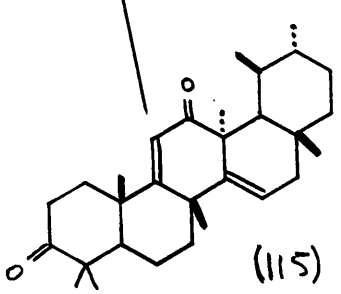
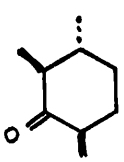


(115)



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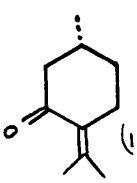


(117)

which only C(20) of the original skeleton certainly retained its original stereochemistry. Pulegone (117) of known absolute stereochemistry was converted to the same optical isomer of (116) establishing that ring E of α-amyrin is six-membered and that the 30-methyl group is α, as suggested by Corey[162] (109).

Recently Corey[163] has converted an oleanane derivative, glycyrrhetic acid to α-amyrin in such a way as to prove that both the C(29) and C(30) are in the more thermodynamically stable configurations as in (109).

Lupeol.

Interrelation of lupene and oleanane derivatives was achieved by Jones and his collaborators[164], who showed that lup-20(29)-en-3-one (118, R=O) could be isomerised by 15% sulphuric acid in acetic acid to a compound which is identical with the acid isomerisation product of olean-12-en-3-one (119). Reduction of the latter with lithium aluminum hydride afforded the known δ-amyrenol (olean-13(18)-en-3β-ol). This indicates that rings A, B, C and D in lupene and oleanane are identical, except perhaps for configuration at C(18).

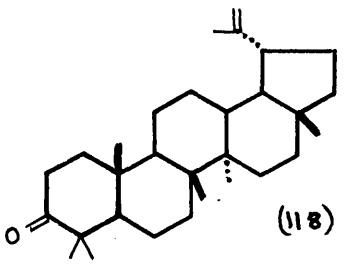
Further confirmation of this was obtained by the conversion of methyl betulonate (120) to a saturated keto-lactone (121) which was reduced by lithium aluminium hydride to a triol (122). The latter with boron trifluoride and acetic anhydride, afforded moradiol diacetate (123) demonstrating in addition, that the stereochemical configuration at C(13) in lupane and oleanane are identical.

Further study of the triol (122) showed that the hydroxyl group at C(19) is axial, that the hydrogen at C(18) is *trans* to it, and hence axial, while the hydroxymethyl at C(17) must be *cis* to it, and hence also axial. Rings D and E are therefore *trans* fused. Moradiol was found to have the hydrogen at C(13) *cis* to the hydroxymethylene group at C(17), and, this must also be true in the triol, and hence in lupeol itself. Thus lupeol could only be represented as (118, R = βOH), the configuration of the isopropenyl group not having been established.

Dehydrochlorination of lupeol hydrochloride with silver acetate in ethanol afforded lupeol, and, on heating in ionising solvents, germanicol (124). Reduction of lupeol hydrochloride however, yielded 18α-olean-3β-ol. These reactions were interpreted by Jones, on the basis of lupeol hydrochloride being 19-α-chloro-18α-olean-3β-ol, and the *trans* D/E fusion was confirmed. The above evidence shows that the isopropenyl group is *trans* to the methyl on C(17), and hence α, as in (119, R= βOH). Similar results were obtained on treatment of lupeol derivatives with formic acid.

Hydroxyhopanone.

This triterpenoid, (71), is of particular relevance to this thesis, as its carbon skeleton is the same as that proposed for zeorin (72). Hydroxyhopanone was isolated[119] from gum dammar, which is also the source of the tetracyclic triterpenoids having the squalenoid dammane skeleton.



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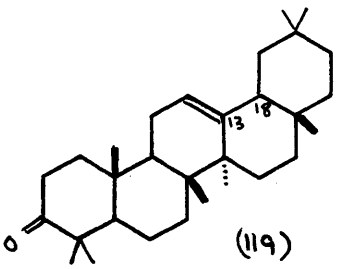

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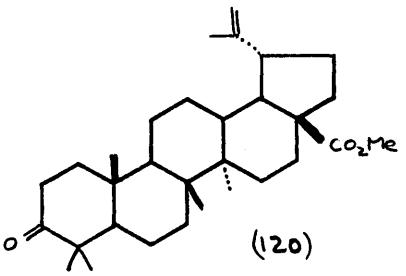

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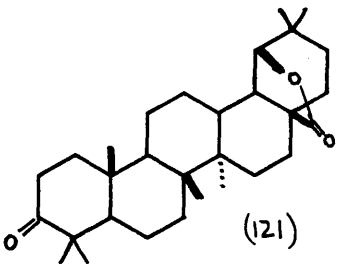

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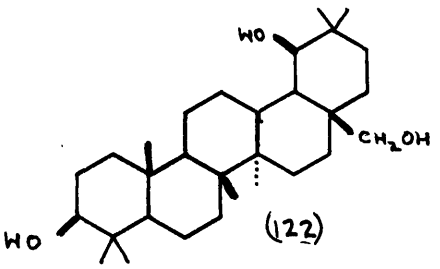

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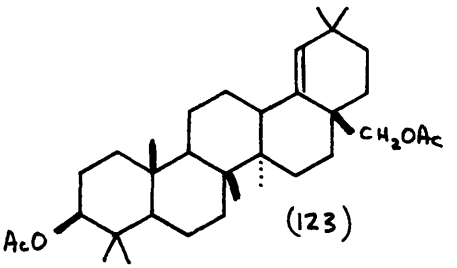

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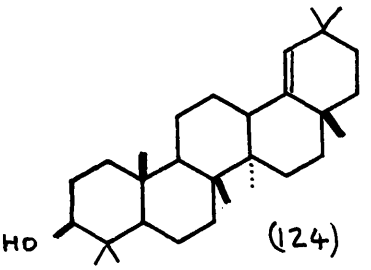


(124)

Hydroxyhopanone was found to be a saturated pentacyclic ketol, $C_{30}H_{50}O_2$. The ketone was shown to be relatively unhindered, as the oxime and dinitrophenylhydrazone could readily be made. The infra-red absorption band at 1706 $cm.^{-1}$ showed that the ketone was contained in a six-membered ring. The hydroxyl group could not easily be acetylated, but was readily dehydrated by acids, and is therefore tertiary.

The investigation of the structure of hydroxyhopanone was taken up by Jones and his collaborators[83,84,85]. The ease of reaction of the carbonyl group, and the presence of at least one α-methylene group, as shown by the Zimmermann test, made it appear probable that the ketone was at the common 3-position. Dehydration of the hindered hydroxyl group, and reduction with lithium aluminium hydride and then with catalytic hydrogen yielded a saturated alcohol (125) in which the hydroxyl group corresponded to the original ketone. Dehydration of this with phosphorus pentachloride gave the familiar retro-pinacol product (126) which on ozonolysis gave acetone. The retro-pinacol reaction is characteristic of 3β-hydroxy triterpenoids, so the position of the ketone is confirmed.

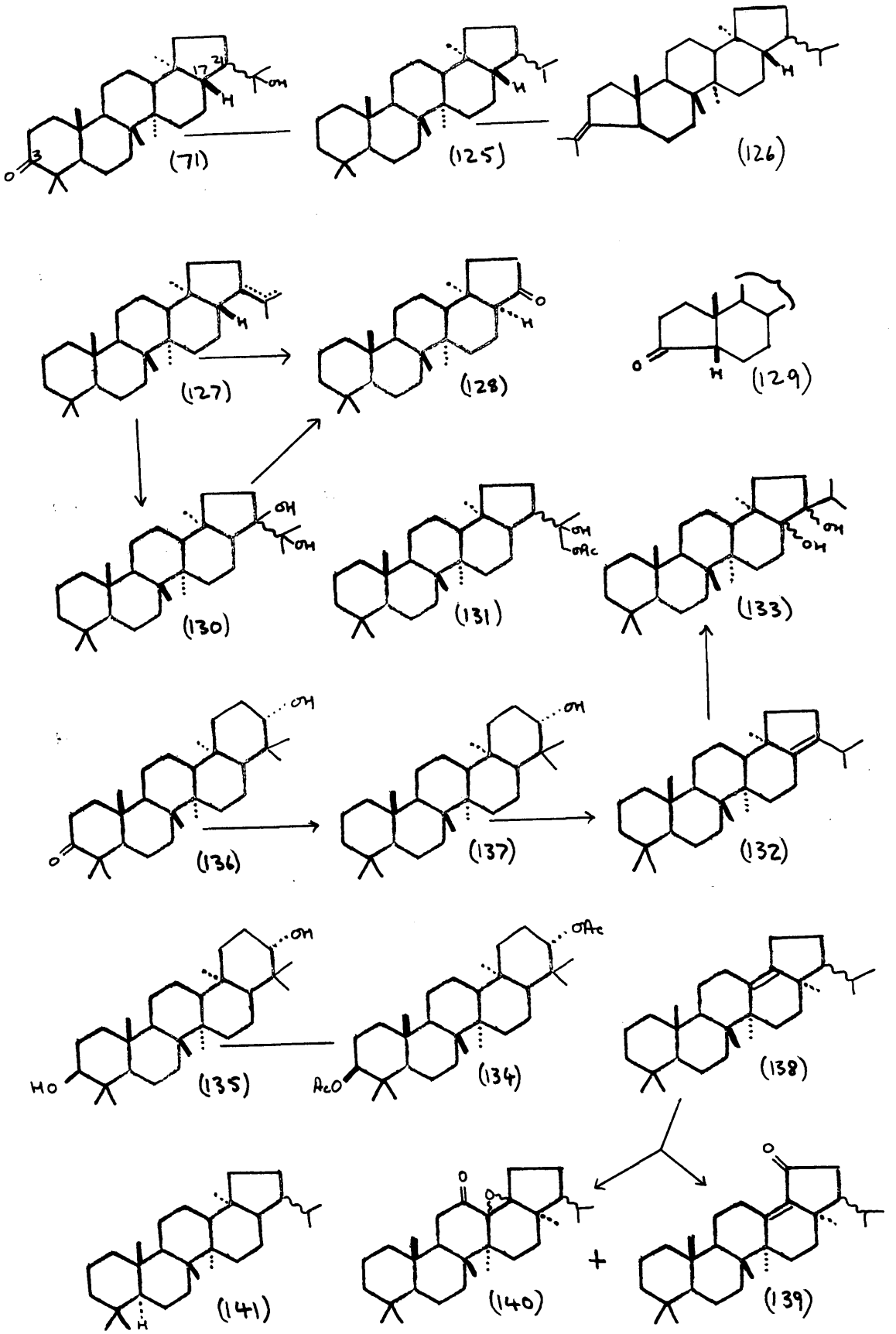
Attention was now directed to the hydroxyl group. After removal of the ketone, the hydroxyl group was dehydrated with phosphorous oxychloride to give hopene, a mixture of isopropenyl and isopropylidine compounds (127). Ozonolysis of the mixture gave both formaldehyde and acetone. Chromatography of the non-volatile fragments yielded trisnorketohopane (128) which was

shown by bromination and deuteration to have three replacable hydrogens. The infra-red spectrum of the ketone has a band at 1738 $cm.^{-1}$ characteristic of a cyclopentanone. From rotatory dispersion measurements, it was concluded that environment of the ketone is similar to that of 3-keto-A-norcholanic acid (129), but since this represents the more stable form of attachment of a five-membered to a six-membered ring, it cannot be assumed that the cis-junction is present in hydroxyhopanone itself.

Treatment of the mixture hopene (127), with osmium tetroxide, followed by acetylation yielded a diol (130) and a diol monoacetate (131) which could be separated by chromatography. The diol (130) was cleaved by lead tetraacetate to trisnorketohopane (128). These results prove the presence of a hydroxyisopropyl group attached to a five-membered ring.

Hydrogenation of hopene (127), and destruction of all remaining unsaturated material yielded a saturated hydrocarbon which is provisionally called hopane, although the configuration of the isopropyl group may not be the same as in hydroxyhopanone. Hopane has physical constants differing significantly from those of lupane and zeorinane. Hopane cannot be a stereoisomer of lupane with the isopropyl group in the β-configuration, since trisnorketohopane is not identical with the C_{27}-ketone from lupeol[123].

From the foregoing results, the structure (71) was suggested, and this was confirmed by conversion of both hydroxyhopanone and γ-onocerin to common intermediates (132) and (133)[165].


(71)
(125)
(126)
(127)
(128)
(129)
(130)
(131)
(133)
(136)
(137)
(132)
(135)
(134)
(138)
(141)
(140)
(139)

From hopene (127), the intermediates were readily prepared by simply treating with acid to give hopene I (132) and hydroxylation of this to (133).

Reduction of the known[76] 3:21-diacetoxy- γ-onoceran-11-one (134) under forcing conditions gave γ-onocerane diol (135). On refluxing this with a suspension of manganese dioxide in chloroform, oxidation to the monoketone (136) took place in good yield. Wolff-Kishner reduction then yielded γ-onoceranol (137). On dehydrating the alcohol with kieselguhr and bentonite, rearrangement took place. Isomerisation of the double bond with mild acid gave a substance (132), identical with hopene I. Hydroxylation of (132) from onocerin led to a diol (133) indistinguishable from the hopene diol.

The only remaining uncertanties in the structure of hydroxyhopanone are the configurations at C(17) and C(21). Jones[85] has shown that the D/E ring fusion is trans, and hence the 17 hydrogen is β.

Some further reactions of hydroxyhopanone are of interest because of their analogy to zeorin degradations[111]. Treatment of either hydroxyhopane or hopene I (132) with strong acid causes methyl migration and formation of hopene II (138).

Hopene II, on oxidation with sodium dichromate in acetic acid yielded two products.

The less polar, $C_{30}H_{48}O$, obtained in 40% yield had maximal ultraviolet absorbtion at 261 mμ (ε 13,900) indicative of a fully substituted αβ-unsaturated ketone with the double bond exocyclic to two rings (c.f. 4:4-dimethyl-15-oxocholest 8(14) en-3β-ol: λ max. 261 mμ, ε 14,700[27]). Its infra-red spectrum

had bands at 1697 and 1615 cm.$^{-1}$, the latter being only slightly the less intense. The band at 1697 cm.$^{-1}$ indicates a conjugated ketone in a five-membered ring, and the high intensity of the 1615 cm.$^{-1}$ band that the $\alpha\beta$-unsaturated ketone is S-cis (cisoid). These data are appropriate to structure (139).

The more polar product, $C_{30}H_{48}O_2$, obtained in 20% yield gave no colour with tetranitromethane and showed no selective light absorption between 220 and 260 mμ. Its infra-red band at 1706 cm.$^{-1}$ is characteristic of a cyclohexanone; weak bands at 910, 890, 870, and 820 cm.$^{-1}$ are characteristic of an epoxide, and indicate the structure (140).

As in the zeorinin series to be described later, hydroxylation of hopene I (132) with osmium tetroxide, followed by lead tetraacetate cleavage of the diol yielded a seco-diketone.

An interesting reaction not attempted in the zeorinin series is the oxidation of hopene I (132) with sodium dichromate in acetic acid / benzene. The main product is a conjugated cyclopentenone to which the structure (141) is ascribed. A second product, isomeric with (141), but saturated to tetranitromethane, is obtained in lower yield. Jones[111] suggests that this contains a cyclopropane ring; the ketone group is present in the five-membered ring.

Zeorin - previous work.

Zeorin was first isolated by Paterno[166,167] from the lichen Lecanora (Zeora) sordida. During the course of their extensive

investigations on the constituents of lichens, Zopf[168] and Hesse[169] reported its presence in many other lichens, but neither worker prepared any pure derivatives.

For many years the chemistry of zeorin received no attention until in 1938 and 1940, Asahina[170-172] re-isolated it, together with atranorin and leucotylin from Parmelia leucotyliza Nyl.

On the basis of evidence which will be described, Asahina showed that zeorin, $C_{30}H_{52}O_2$, is a saturated diol, and is therefore pentacyclic. He suggested that zeorin is probably a triterpenoid, since on dehydrogenation with selenium, 1:2:5-trimethyl naphthalene is formed. Leucotylin he considers to be a hydroxy zeorin because of a similarity in the dehydration reactions of the two.

On acetylation under mild conditions, zeorin forms a monoacetate which is resistant to oxidation by chromium trioxide. Zeorin can itself be oxidised to a monoketone zeorinone, which cannot be acetylated. A secondary hydroxyl group is therefore taking part in both reactions, leaving a tertiary hydroxyl group unaffected. The infra-red peak at 1706 $cm.^{-1}$ in zeorinone[173] is indicative of a keto group in a six-membered ring.

After suitable protection of the secondary hydroxyl, usually as the acetate, the tertiary hydroxyl can readily be removed by dehydrating agents. Treatment of zeorin acetate with phosphorus oxychloride in pyridine leads to a strongly dextrorotating anhydro-compound, isozeorinin acetate which was further characterised by hydrolysis to isozeorinin and by benzoylation of this.

Similar dehydration of zeorinone leads to isozeorininone. The infra-red spectrum of isozeorinin acetate shows a strong band at 884 cm.$^{-1}$ indicative of a methylidine grouping ($>C=CH_2$)[173].

On treatment of isozeorinin derivatives with concentrated hydrochloric acid in ethanol under reflux, isomerisation takes place to zeorinin derivatives in which the infra-red band at 884 cm.$^{-1}$ is no longer present. Zeorinin derivatives can also be made by direct acid catalysed dehydration of zeorin derivatives. The "anhydrozeorin acetate" prepared by Asahina and Yosioka[171] by refluxing zeorin with acetic acid is probably somewhat impure isozeorinin acetate[173].

Although Asahina and Yosioka claimed that zeorinin acetate could be hydrogenated to a deoxyzeorin acetate with a palladium catalyst, neither Barton and Bruun[173] nor the present author were able to repeat this, even on using a variety of more vigorous conditions, including high pressure. Isozeorinin acetate, on the other hand was smoothly hydrogenated to deoxyzeorin acetate. Direct hydrogenolysis of the tertiary hydroxyl group could be effected by Barton and Bruun[173], but not by the present author. Deoxyzeorin acetate was further characterised by hydrolysis to the alcohol, and by conversion of this to the benzoate. The deoxyzeorin series prepared by Barton and Bruun, and by the present author is not identical with the compounds prepared by Asahina[171]. Chromic acid oxidation of deoxyzeorin affords deoxyzeorinone.

From the above information, Barton and Bruun[173] deduced that zeorin contains the group -C Me(OH) - which first dehydrates to $C=CH_2$ and can then be isomerised by acid to a more stable double-bond position in zeorinin. Treatment of zeorinin acetate with hydrogen peroxide in acetic acid[171] or with perbenzoic acid in chloroform[171], or with ozone[174] merely causes epoxidation to the saturated zeorinin acetate oxide. The benzoate oxide was prepared in a similar manner, and, on treatment with hydrochloric acid in refluxing ethanol, was similarly dehydrated to dehydrozeorinin benzoate. This was identified as a conjugated heteroannular diene by its ultra-violet absorption maximum at 252 mμ.

The carbonyl group in zeorinone, and deoxyzeorinone cannot be situated in the usual C(3) position as in (142), because it is resistant to carbonyl reagents and to Wolff-Kishner and Clemmensen reductions, even under such vigorous conditions as those used in the preparation of lanostane[68,175].

The vigorous Clemmensen conditions caused partial isomerisation to a further isomer, neozeorininone, in which the double bond and keto group still had not moved into conjugation.

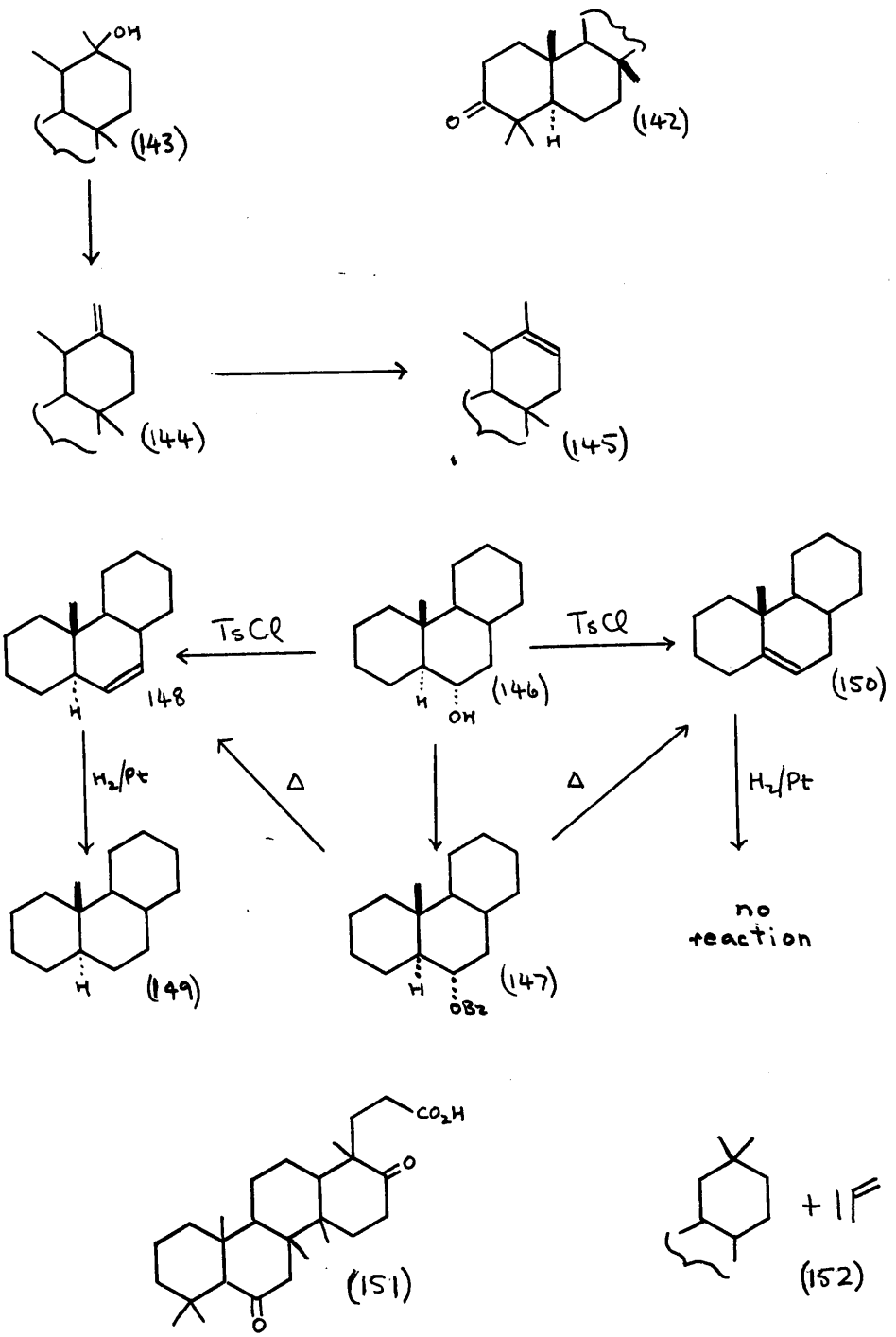
The ready acetylation of zeorin, zeorinin, isozeorinin and deoxyzeorin, together with the high degree of steric hindrance of the corresponding carbonyl compounds must be explained, on the basis of a six-membered ring, by the secondary hydroxyl group of zeorin being equatorial[87]. This was confirmed by lithium aluminium hydride reduction of deoxyzeorinone to epideoxyzeorin[89]. The epi-alcohol was smoothly oxidised back to deoxyzeorinone by chromium trioxide, but it resisted benzoylation under conditions

adequate for the benzoylation of deoxyzeorin. Added confirmation came from the reduction of zeorininone to zeorinin by sodium and n-propanol.

At this stage, Barton and Bruun noted the similarity in properties and molecular rotation differences of zeorin ($[\alpha]_D + 54^\circ$), isozeorinin ($[\alpha]_D + 78^\circ$), and zeorinin ($[\alpha]_D + 59^\circ$) on the one hand, and of ψ- taraxastane diol[128] ($[\alpha]_D$ -11°), taraxasterol[176] ($[\alpha]_D + 91^\circ$), and ψ- taraxasterol[177] ($[\alpha]_D + 47^\circ$) on the other. Thus the series (139), (140) and (141) might have been adequate partial formulae for the above mentioned relations in both series of compounds, and the parent saturated hydrocarbons could have been identical.

Zeorinane could not be obtained by direct removal of the secondary oxygen function (as a carbonyl group). By dehydration of deoxyzeorin by toluene -p-sulphonyl chloride in boiling pyridine, a mixture of monoethylenic hydrocarbons was obtained. Hydrogenation of this gave a mixture of saturated and unsaturated hydrocarbons from which zeorinane was obtained in a pure state by destruction of unsaturated hydrocarbon with hot concentrated sulphuric acid[178]. The mixture of monoethylenic hydrocarbons could also be prepared by pyrolysis of deoxyzeorin acetate or benzoate.

Zeorinane has m.p. 186.5 - 187.5° $[\alpha]_D + 12^\circ$, closely resembling taraxastane, m.p. 186 - 188°, $[\alpha]_D + 11^\circ$, but they are not identical since there was a pronounced melting point depression on admixture. Zeorinane appears to be a new fundamental hydrocarbon of the triterpenoid series.


OH
(143)
O
H
(142)
(144)
(145)
TsCl
TsCl
148
H
(146)
OH
(150)
H₂/Pt
Δ
Δ
H₂/Pt
(149)
H
(147)
OBz
no
reaction
CO₂H
O
O
(151)
+
(152)

These indirect methods of formation of zeorinane were formulated by Barton in terms of the partial formulae (146) for deoxyzeorin to (150) for zeorinane. There is analogy for the pyrolysis reaction in the behaviour of 7-benzoyloxy groups in the cholestane series[179].

In spite of the apparent similarity of reactions in the zeorin and ψ- taraxastane diol series, there are at least two major chemical differences. Firstly, ψ-taraxasteryl acetate is readily hydrogenated[180], whereas zeorinin acetate is not. Secondly, taraxasteryl acetate is readily isomerised by hydrochloric acid in acetic acid to lupenyl - I - acetate, whereas under the same conditions, isozeorinin acetate is isomerised to zeorinin acetate, but not further.

All attempts by Barton and Bruun to move the double bond and carbonyl group of neozeorininone into conjugation and to isomerise the doubly unsaturated zeorinane mixture to a conjugated diene were unsuccessful, and these authors conclude that the two hydroxyl groups in zeorin are either not close together, or are separated by fully substituted centres.

By oxidation of zeorin with chromium trioxide in acetic acid at 55°, Rayabinin and Matyukhina[181] have recently obtained a diketo acid $C_{27}H_{42}O_2$, further characterised as the methyl ester and semicarbazone. The diketo acid, which we formulate as (151) enabled the Russian workers to deduce the presence in zeorin of an hydroxyisopropyl group attached to a five membered ring, though the assumption that it is necessarily terminal is unwarranted. These authors, however, formulate zeorinin as a ring

expanded product (152), a view in no way compatible with our results. On ozonolysis of zeorinin acetate at room temperature, they obtain "a stable ozonide", whilst in the present work, ozonolysis at -60° gave only the known zeorinin acetate oxide.

Leucotylin.

This compound, $C_{30}H_{52}O_3$, was isolated[170] together with zeorin from parmelia leucolyliza Nyl.; it has not been isolated in recent years from cladonia deformis Hoff.[170], or from nephroma articum L.[173].

Leucotylin is a triol, saturated to tetranitromethane. On mild acetylation, a diacetate is formed. The remaining hydroxyl group is readily dehydrated, and probably corresponds to the tertiary hydroxyl group of zeorin. Hydrogenation of the dehydrated diacetate leads to a deoxyleucotylin diacetate which was further characterised as the diol, deoxyleucotylin. Selenium dehydrogenation of leucotylin gives 1:2:5-trimethyl naphthalene; it is probably a hydroxyzeorin[170].

DISCUSSION

Zeorin: The Present Work

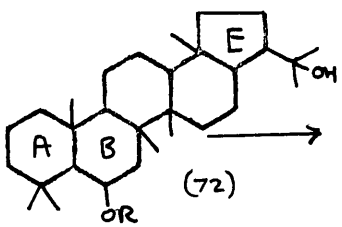
From the present study of the chemistry of zeorin, the structure (72, R=H) has been deduced[111] and it is convenient to discuss the reactions to be presented in terms of this structure.

Degradative studies

The two hydroxyl groups of zeorin are sufficiently separated in the molecule that functions derived from them have never been brought into conjugation: for example, zeorinone, the corresponding hydroxy ketone, on treatment with strong acid, dehydrates to give the rearranged, but non-conjugated enone neozeorininone[173], which cannot be isomerised by acid. The environments of the two hydroxyl groups were therefore studied separately.

The environment of the tertiary hydroxyl group.

The ready dehydration of the tertiary hydroxyl group of zeorin afforded an easy means of entry into the skeleton, and this region of the molecule was first investigated. Barton and Bruun[173] had dehydrated zeorin acetate (72, R=Ac) with phosphorus oxychloride and obtained a mixture from which they isolated isozeorinin acetate, which has an infra-red band at 887 $cm.^{-1}$ characteristic of a methylene group ($>C=CH_2$). The presence



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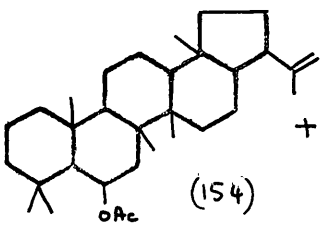

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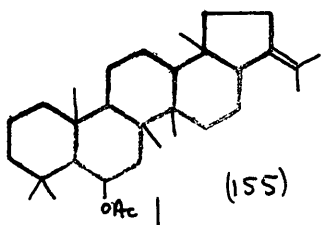

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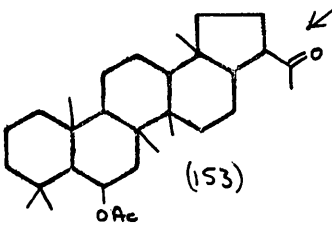

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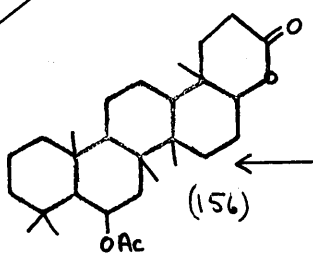

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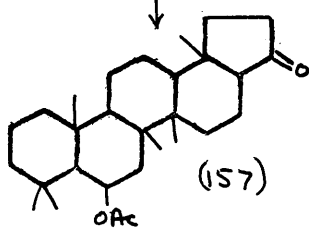

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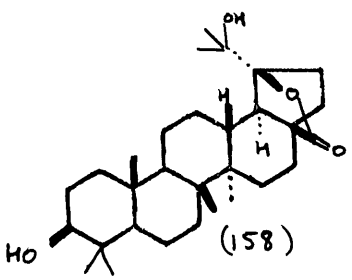

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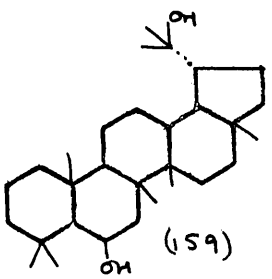

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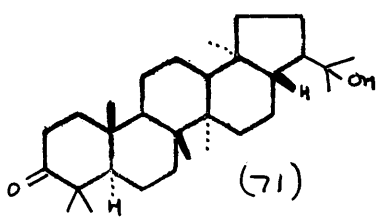

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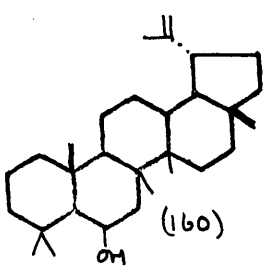

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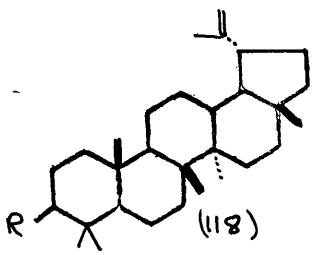

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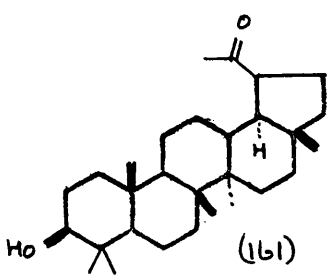

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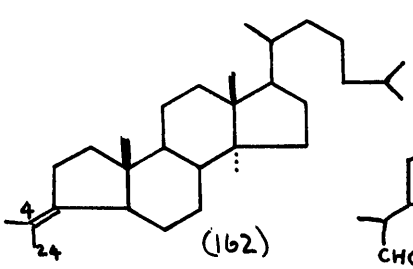

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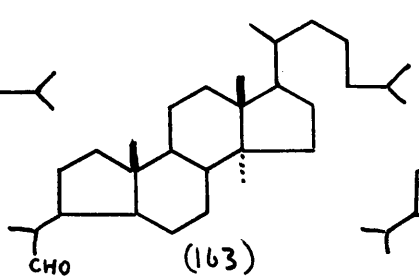

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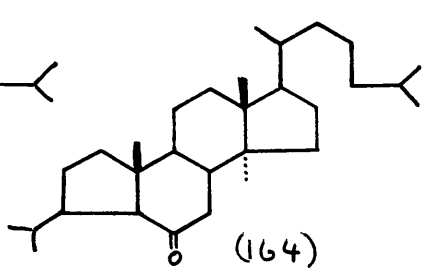


(164)

of this group was now confirmed by ozonolysis of the crude dehydration product to give a nor-ketone (153) which gave a positive Zimmermann test and readily formed a 2:4-dinitrophenylhydrazone. By bromine titration, the ketone was shown to contain at least four replaceable hydrogen atoms.

The crude dehydration product on ozonolysis yielded acetone, characterised as the 2:4-dinitrophenylhydrzaone, showing that if isozeorinin acetate is represented as (154), the isopropylidine isomer (155) must also have been present. Although the latter compound was not obtained pure, its presence was confirmed by ozonolysis of the crude dehydration product and oxidation with peroxytrifluoracetic acid, when the crystalline lactone (156) was obtained. The infra-red bands at 1735 and 1248 $cm.^{-1}$ (acetate) and 1750 $cm.^{-1}$ (δ -lactone) in carbon tetrachloride show that the lactone must be derived from a cyclopentanone (157). The structure of zeorin in this region must therefore contain a hydroxyisopropyl group attached to a five-membered ring.

At the time this work was being carried out, stellatogenin[125] (158) was the only triterpenoid in which this grouping was known to occur. More recently, the structure of hydroxyhopanone[83,84,85] (71) has been elucidated, and proved to be more closely related to zeorin.

It could readily be shown that zeorin did not have a structure (159) analagous to that of stellatogenin, since isozeorinin would then be represented by (160) which would be analagous to lupeol (118, R=OH). The behaviour of the two groupings on treatment with acid is quite dissimilar. Lupeol undergoes a skeletal rearrangement to give a six-membered ring E[164], whereas acid

isomerisation of isozeorinin gives zeorinin, in which the skeleton is unchanged, but the double bond has migrated to a tetra-substituted position in the five-membered ring, as is shown by the degradations described later.

Nor-keto isozeorinin acetate (153) readily gave a 2:4-dinitrophenylhydrazone, in contrast to the nor-ketone (161) from lupeol, which could not be converted to the oxime[182]. The difference in reactions of lupeol and isozeorinin could have been due to a difference in stereochemistry at the various positions in this region, but the likelihood of this is decreased by the fact that on neither the lupane nor oleanane skeleton in this region could there be a trans diene having the λ maximum at 252 mμ, and capable of giving on hydroxylation and dehydration, an S-trans (transoid) conjugated enone in which the ketone is in a six-membered ring, as is required by the reactions to be described later.

On the basis of the biogenetic scheme (see p. 25) the most likely alternative hypothesis for the structure of zeorin in this region is (72), analogous to that obtained by a retropinacol reaction on the regular 3β-hydroxy-4:4-dimethyl triterpane grouping, the intermediate carbonium ion capturing a hydroxyl group rather than losing a proton as in the in vitro reaction.

Since there was available a considerable quantity of iso-lanostene[183] (162), this was first considered as a model.

The double bond in zeorinin was known to be hindered, since on ozonisation at room temperature the unsaturation could be detected even after four hours, while equivalent amounts of other

unsaturated compounds could be ozonised within ten minutes. The only product isolated from ozonisation of zeorinin acetate is the epoxide. From this a diene, dehydrozeorinin λ max. 252 mμ (ε 22,030) was formed by refluxing with aqueous ethanolic hydrochloric acid.

On treatment of isolanostene (162) with hydrochloric acid under the conditions used in the preparation of zeorinin acetate, isomerisation took place. The expected analogy of the product to zeorinin was not confirmed by the behaviour of the double bonds on oxidation. Whereas zeorinin acetate on ozonisation was converted to a crystalline epoxide, the isomerised isolanostene gave, in the neutral fraction, a non crystalline product which was not purified, but had an infra-red band at 1707 $cm.^{-1}$ in carbon tetrachloride, characteristic of a cyclohexanone or acyclic ketone. The bands at 1026 and 885 $cm.^{-1}$ of the starting material had disappeared. A search for a corresponding ketonic substance in the mother liquors of the zeorinin acetate oxide showed that there was none.

The position of the double bond in the isomerised isolanostene is not clear. The infra-red band at 885 $cm.^{-1}$ suggests the presence of a methylene group $>C=CH_2$, but it is unlikely that this should be formed during, or survive, the acid treatment of isolanostene.

Reaction of the isomerised isolanostene with osmium tetroxide and working up with hydrogen sulphide leads to two products, which could be separated by chromatography on alumina. The first

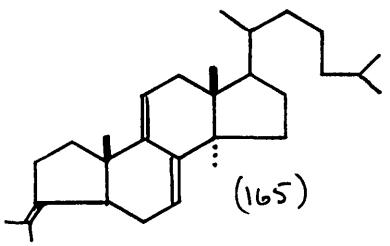
compound to be eluted was present in insufficient quantity for determination of all the necessary physical constants, but was recrystallised to constant melting point 145-147°, and has a carbonyl band at 1700 cm.$^{-1}$ (chloroform) in the infra-red.

The second compound was shown to be a diol by its infra-red spectrum (3350 cm.$^{-1}$, no carbonyl absorption in Nujol), and by its analysis. Treatment of the diol with acid caused dehydration to the carbonyl compound m.p. 145-147°.

If the double bond in the isomerised isolanostene is in the 4(23) position as a methylene group, then the compound m.p. 145-147° is the aldehyde (163). The infra-red spectrum shows no aldehyde band near 2700 cm.$^{-1}$, although this band is often weak, and may have been missed; the band observed at 1700 cm.$^{-1}$ is unusually low for an aldehyde.

If the isomerised isolanostene has a double bond at 5, then the carbonyl compound is the 6-ketone (164) which is in better agreement with the infra-red spectrum, and is the more probable assignment. This conclusion is difficult to reconcile with the observation[183] that the isolated double bond in the isolanostatriene (165) did not move into conjugation on treatment with hydrogen chloride in dry chloroform, although the ene-dione (166) with hydrochloric acid/acetic acid reflux gave the conjugated Δ^5-isomer in accordance with the present conclusions.

The partial dehydration of the 5:6-diol during the working up process is probably due to hydrochloric acid entrained in the hydrogen sulphide from the Kipp generator. This trouble could be avoided by adding a trace of pyridine to the solution of the osmium complex before passing in the hydrogen sulphide.



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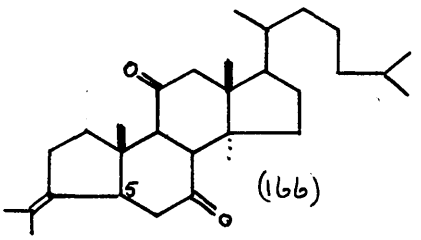

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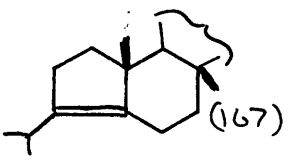

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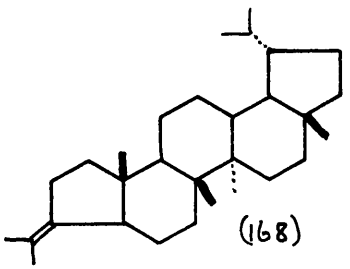

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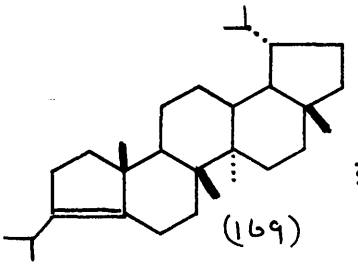

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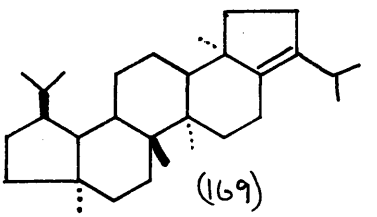

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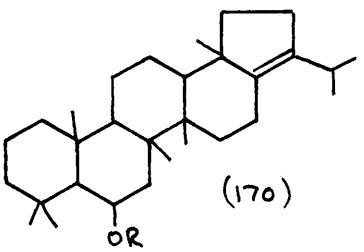

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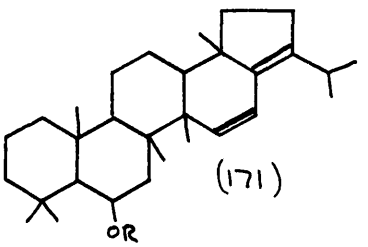

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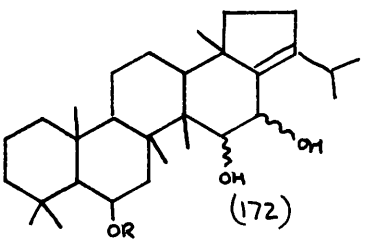

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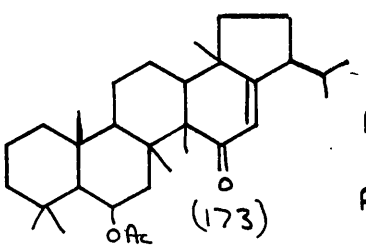

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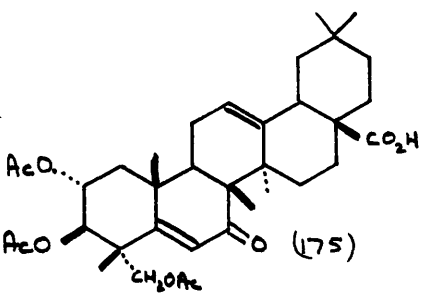

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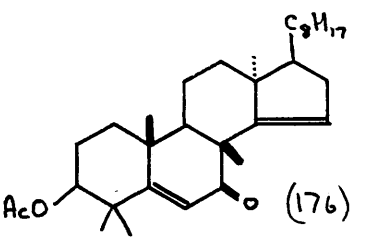

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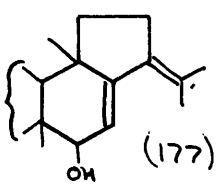

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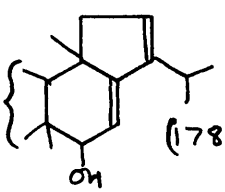

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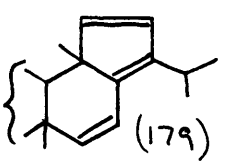

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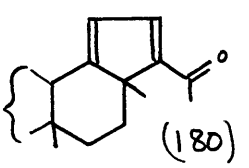

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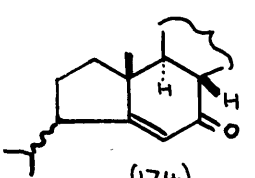

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Since it is known[184,185,186] that a 3(5)-double bond is in the position stable to mild acid in the environment (167) of pentacyclic triterpenoids, the stable position depends on the absence or presence of an 8β-methyl group. It has been noted[187,188] that introduction of a 7-double bond in the lanostanone skeleton causes irregularities in the reactions of the 3-ketone.

The difference in behaviour of the double bonds in the isomerised isolanostene and zeorinin made it necessary to select a more exact model. For this, lupanol was chosen as the starting material, since it is one of the few saturated 3β-hydroxy-4:4-dimethyl triterpenes available in quantity. Treatment of the alcohol with phosphorous pentachloride in dry petrol caused the expected dehydration with rearrangement to the known γ-lupene[184] (168). The infra-red spectrum of the crude product contained a band at 888 $cm.^{-1}$ due to the presence of some of the isopropenyl isomer of (168) which could be removed only by repeated crystallisation. Both isomers, however, on treatment with acid were converted to the stable iso-γ-lupene (169), the physical constants of which are in excellent agreement with those given by Nowak, Jeger and Ruzicka[184].

The environment of the double bond in iso-γ-lupene (169) has been established[184], and appears to be very similar to that in zeorinin. The tetranitromethane test on both is strongly positive, giving a deep brown colour. Epoxidation of iso-γ-lupene could be effected either at 0° in carbon tetrachloride with ozone or by hydrogen peroxide in acetic acid at 100°. The products from both routes were identical in infra-red spectrum, and

the mixed melting point did not depress. The melting point, 179-187° was lower, and of a wider range than that (190-191°) quoted by Ruzicka[184] who used perbenzoic acid for the epoxidation, but our value was unchanged on recrystallisation. The optical rotations are in good agreement, and both microanalyses are close to the required values.

By ozonisation of iso-γ-lupene in carbon tetrachloride at 0°, Ruzicka[184] obtained a stable ozonide. In our case, the epoxide was the only crystalline product.

On refluxing the iso-γ-lupene epoxide in aqueous ethanolic hydrochloric acid, a crystalline diene was obtained in excellent yield. The ultra-violet spectrum of this (λmax. 252mμ, ε 22,030), and strongly suggests that the environments of the two dienes are closely similar.

Hydrogenation of dehydrozeorinin in neutral solution regenerates zeorinin[171]. It is therefore probable that dehydrozeorinin contains an unhindered double bond in conjugation with the original hindered double bond of zeorinin (170, R=H), as shown in (171, R=H).

Dehydrozeorinin acetate (171, R=Ac) was reacted with exactly one equivalent of osmium tetroxide, and worked up by the hydrogen sulphide method. Instead of the expected ene-triol monoacetate (172, R=Ac) there was obtained an acetoxy conjugated enone, λmax. 230mμ (ε 10,800) I.R. . 1660 cm.$^{-1}$, 1710 + 1253 cm.$^{-1}$.

The band at 1660 cm.$^{-1}$ is characteristic of cyclohexenones; the absence of intense double bond absorption near 1610 cm.$^{-1}$ indicating that the chromophore is S-trans rather than S-cis[189].

The structure (173) is proposed for the enone. The ultra-violet spectra of alternative enone chromophores in this framework, the 2-keto-$\Delta^{3(5)}$ (λ max. 240 mμ, ε10,000)[186], and 6-keto-$\Delta^{3(5)}$ (λ max. 260 mμ, ε11,500)[190] are signigicantly different. No precise analogy for the present chromophore is known. In the lanosterol series, the enone (174)[183] has a maximum at 241 mμ (ε 9,500), but the absence of the 8β-methyl group is known to cause other irregularities (see above). The enone (175) from terminolic acid[101,105] absorbs at 233 mμ (ε 11,000), a butyrospermol derivative[191] (176) at 235 mμ (ε 14,000) while the 14α-methyl-$\Delta^{13(17)}$ isomer absorbs at 238 mμ (ε 15,000). The position of the ultra-violet maximum in this, as in other triterpenoid chromophores, appears to vary considerably with small changes in the stereochemistry.

The enone (173) was recovered unchanged after refluxing for 30 minutes in ethanolic hydrochloric acid.

By osmylation of dehydrozeorinin benzoate and cleavage of the osmate with lithium aluminium hydride, a mixture of the desired triol (172, R=H) and its monobenzoate (172, R=CO.Ph) was obtained. The products were readily separable on alumina.

Cleavage of the osmate ester from dehydrozeorinin acetate with hydrogen sulphide in the presence of pyridine led to a mixture of the enone (173) and the triol monoacetate (172, R=Ac).

Mild acid treatment of the zeorinin triol monoacetate (172, R=Ac) at room temperature in dioxan caused partial dehydration to a diene diol monoacetate (λ max. 250 mμ, ε 21,600). Under more severe conditions further dehydration took place: zeorinin triol, on refluxing in chloroform with concentrated hydrochloric

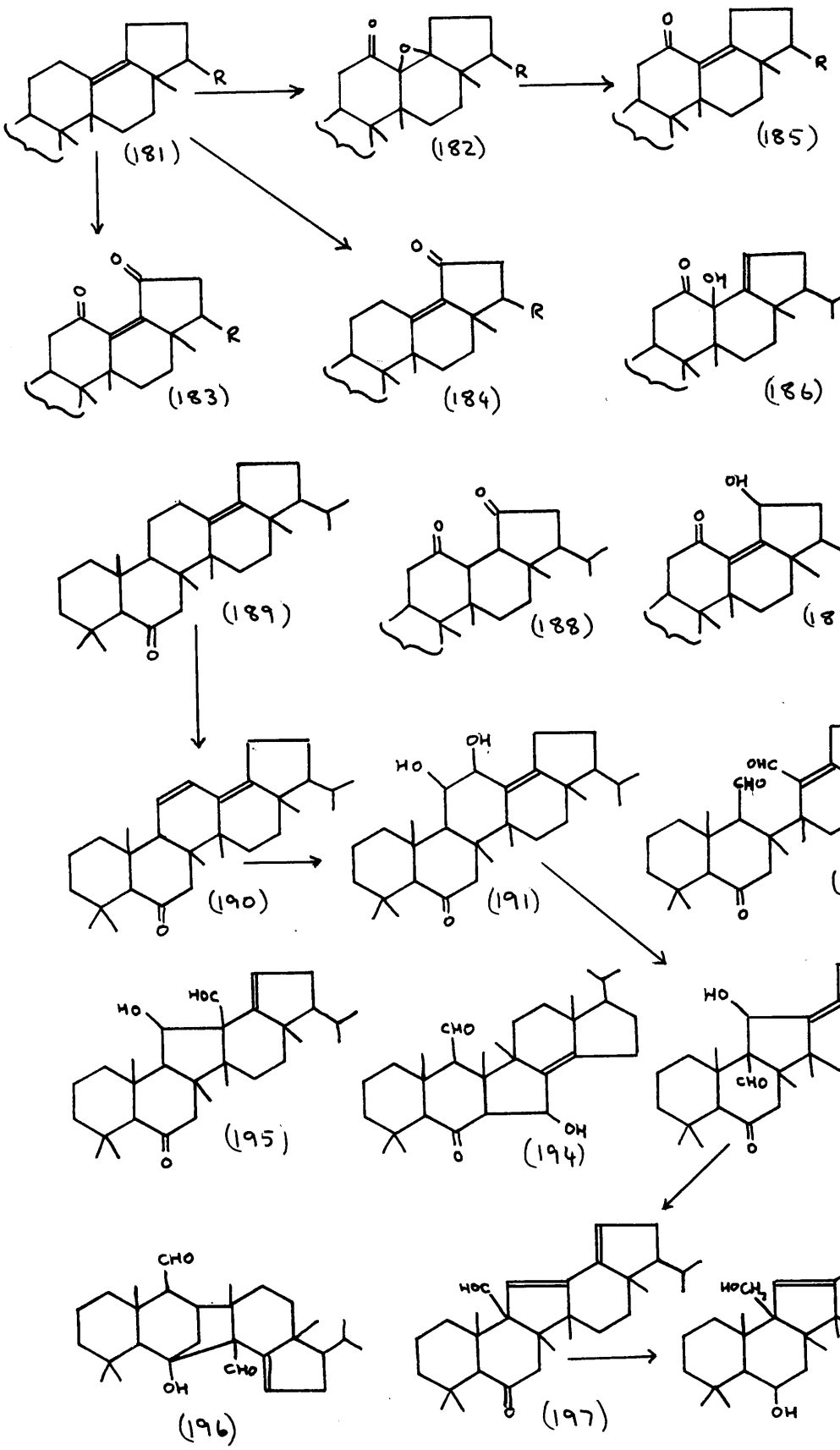
acid gave a trienol ($\lambda\lambda$ max. 295 mμ, ε 18,800, 283 mμ, ε 16,500). No dehydration to the enone (173) was observed, although dilute hydrochloric acid in dioxan at room temperature is similar to the conditions of work-up of the osmate ester in dioxan using hydrogen sulphide.

The positions of the double bonds in the dienol and triene chromophores were not investigated. Using the Fieser-Woodward rules[13], the diene (177) would be expected to absorb 249 mμ, while (178) should absorb at 239 mμ. For the triene, only the chromophore (179), which has a calculated absorption maximum at 308 mμ is near the observed value of 295 mμ. In his discussion of the rules, Fieser[13] notes that the dienone[192] (180) partly contained in a five-membered ring is of lower wavelength than the predicted value by 12 mμ. A similar effect may be occurring here, although the discrepancy lies in the opposite direction.

The neo series.

By treatment of zeorininone with hydrochloric acid in dioxan under reflux, Barton and Bruun[173] caused isomerisation to neozeorininone, $C_{30}H_{48}O$, in which the double bond is even more hindered than in zeorininone. Attempted hydrogenation with platinum oxide catalyst in acetic acid containing perchloric acid gave back the starting material. After one month with osmium tetroxide in dioxan and pyridine, neozeorininone remained unchanged.

The preparation of neozeorininone was conveniently effected by using perchloric acid in acetic acid at 100°. No isomerisation beyond neozeorininone was observed under these conditions, although


R
(181)
(182)
(185)
(183)
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OH
(186)
(189)
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(187)
(190)
HO
OH
(191)
OHC
CHO
(192)
HOC
(195)
CHO
(194)
(193)
(196)
(197)
(198)

after one hour at 100°, the yield of neozeorininone decreased slowly.

Oxidation of neozeorininone with chromium trioxide in acetic acid at room temperature leads to three products, separable by chromatography on alumina. In all three, the infra-red band due to the original cyclohexanone was unchanged.

The first compound to be eluted, $C_{30}H_{46}O_2$ has the ultra-violet (λmax. 259 mμ, ε 12,870) and infra-red (1697 composite band, 1610 cm.$^{-1}$ strong) spectra of an S-cis- $\alpha:\beta$-unsaturated cyclopentanone.

The second product, $C_{30}H_{46}O_3$ showed only the ultra-violet spectrum of a simple ketone (λmax. 288mμ, ε 108). Infra-red showed the absence of hydroxyl absorption near 3600 cm.$^{-1}$. The two atoms of oxygen introduced are as an $\alpha:\beta$-epoxy cyclohexanone (1703 cm.$^{-1}$ in the infra-red due to both carbonyl groups). The $\alpha:\beta$-epoxy ketone was reduced in excellent yield by chromous chloride[193] to the corresponding $\alpha:\beta$-unsaturated cyclohexanone (λ max. 255mμ, ε 8,400). The infra-red spectrum has bands at 1703 cm.$^{-1}$ (cyclohexanone), 1670 cm.$^{-1}$ $\alpha:\beta$-unsaturated cyclohexanone and 1612 cm.$^{-1}$ strong, which is characteristic of C=C stretch in an S-cis conjugated enone[189].

The last substance to be eluted from the column was a yellow ene-trione $C_{30}H_{44}O_3$ isolated in only two percent yield. The ultra-violet spectrum ($\lambda\lambda$ max. 375mμ, ε 460, 263mμ, ε 9,200) and infra-red spectrum (1724 cm.$^{-1}$ shoulder, 1717 cm.$^{-1}$, 1707 cm.$^{-1}$ shoulder, 1644 cm.$^{-1}$) characterise the chromophore as a 2-ene-1:4-dione.

Formation of the above derivatives requires that the double bond of neozeorininone be exocyclic to a five-membered and a six-membered ring, in each of which there is a methylene group which can be oxidised to the ketone, to give an S-cis enone. This last condition eliminates the possibility that the double bond is in the trisubstituted condition, and that rearrangement occurs during the chromium trioxide oxidation[15]. On this model the $\alpha:\beta$-unsaturated cyclopentanone cannot be formulated. The formation of the conjugated ene-dione also supports this view; the double bond position must therefore be as in (181, R=$CHMe_2$) when the various derivatives are as shown (182, R=$CHMe_2$ to 184, R=$CHMe_2$).

By chromium trioxide oxidation of α-ergostenyl acetate (181, R=C_9H_{19}), Stavely and Bollenback[194] obtained a similar series of products (182, R=C_9H_{19} to 184, R=C_9H_{19}), together with the 8:14-epoxy-15-ketone which was not isolated in the neozeorininone reaction. It is interesting that in neither case was the $\alpha:\beta$-unsaturated cyclohexanone (185) obtained in the oxidation. The ultra-violet absorption spectra are in general agreement with model compounds derived from ergosterol.

Chromophore	neoZeorinane	Ergostanyl acetate	
(183)	263mμ, ε 9,200	255mμ, ε 5,000[194]	259mμ, ε 11,300[195]
(184)	259mμ, ε12,870	259mμ, ε13,300[194]	259mμ, ε 15,700[195]
(185)	255mμ, ε 8,400	262mμ, ε 9,800[194]	262mμ, ε 10,700[195]

On heating to the melting point, neozeorinin epoxy-dione (182) was converted to a compound which did not melt below 350°.

The physical constants (see experimental) could not be reconciled with any probable structure.

The epoxy-dione, on refluxing with hydrochloric acid in ethanol, was converted to an isomer in which the epoxy group had been converted to a ketone. The ultra-violet and infra-red spectra of the product are in agreement with its formulation as a triketone with the newly formed keto group in the five-membered ring as in (188). In view of a similar allylic shift which will be discussed later, it is probable that the reaction proceeds by way of the allylic alcohol (186-188) as shown.

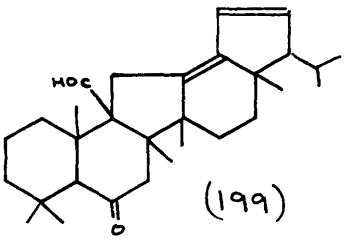
On treatment of zeorinin acetate (170, R=Ac) with perchloric acid in acetic acid under the conditions in which the ketone is converted to neozeorininone, a non-conjugated diene $C_{30}H_{48}$ was obtained instead of the expected monounsaturated acetate. This was not investigated further.

Oxidation of neozeorininone (189) with 1.2 moles of selenium dioxide in dioxan in a sealed tube at 140° gave rise to a conjugated heteroannular neozeorinadienone (190) in high yield. The newly introduced double bond is somewhat hindered, since it could not be hydrogenated by palladium in hydrochloric and acetic acids; introduction of Adams catalyst, however, caused uptake of hydrogen, giving neozeorininone.

The neo-dienone (190) was hydroxylated by osmium tetroxide to a keto-ene-diol (191) which could not be further hydroxylated. The diol readily yielded a diacetate under mild conditions, indicating that it is di-secondary.

Cleavage of the glycol (191) with lead tetraacetate in benzene was rapid and quantitative. The dialdehyde (192) was not isolated, the aldol addition product (193) being formed spontaneously. This shows no ultra-violet absorption, and has infra-red bands in carbon tetrachloride at 3353 $cm.^{-1}$ (hydrogen bonded hydroxyl) 2720 $cm.^{-1}$ (aldehyde) 1708 $cm.^{-1}$ (cyclohexanone) and 1695 $cm.^{-1}$ (hydrogen bonded aldehyde). The isolated ketone group in neozeorininone (189) absorbs at 1713 $cm.^{-1}$ in the same solvent. Three other possible formulations (194-196) of the aldol were dismissed as follows. The infra-red spectrum of the aldol shows that the aldehyde rather than the ketone is hydrogen bonded to the hydroxyl. This and the stability of the aldol to base treatment during the work up, and to solution in acetic acid during the cleavage reaction argue against (194) which would be expected readily to dehydrate to a conjugated dienone only.

Pyrolysis of the aldol at 220° in a stream of nitrogen readily caused dehydration to a conjugated diene ($\lambda max.$ 266 mμ). It was shown that the diene chromophore was not further conjugated with a carbonyl group, since on reduction of the pyrolysis product with lithium aluminum hydride in refluxing dioxan, the oily product showed only the 266 mμ maximum of the original diene. The infra-red spectrum of the reduction product confirmed the absence of a carbonyl group. Both the pyrolysis product (197) and its reduction product (198), on treatment with a drop of concentrated hydrochloric acid, rapidly changed in ultra-violet maximum from 266 mμ to 259 mμ. The formation of the diene requires that the aldol be an allylic alcohol, and the dehydration product be blocked from conjugation with the aldehyde group. This is possible only in (193); the pyrolytic dehydration probably procedes by a cyclic transition state .



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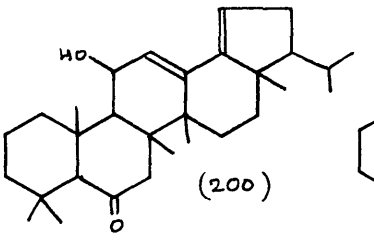

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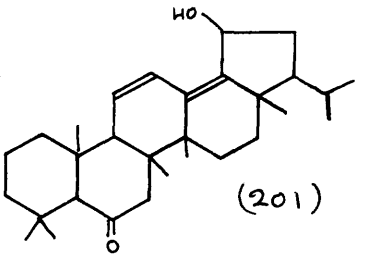

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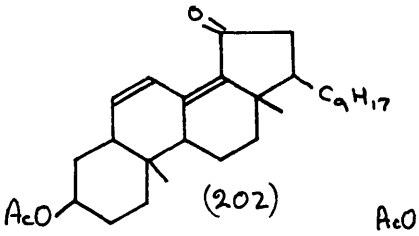

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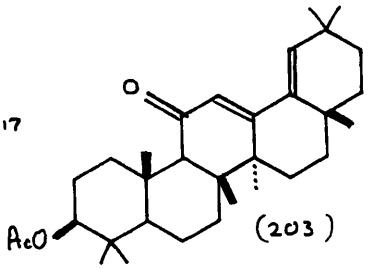

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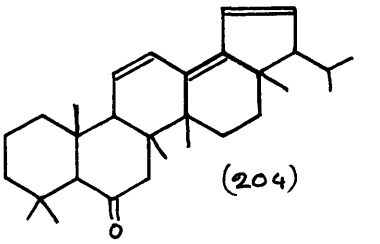

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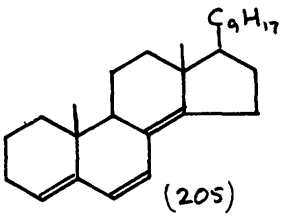

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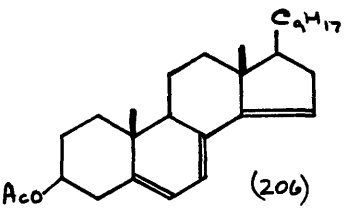

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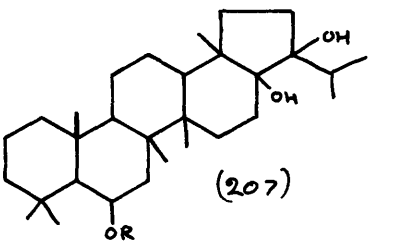

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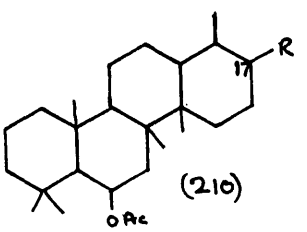

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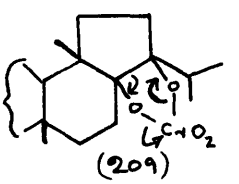
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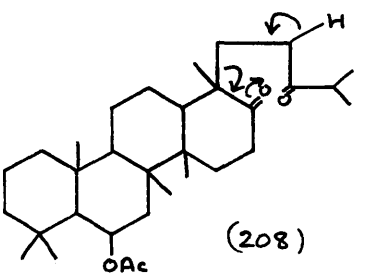

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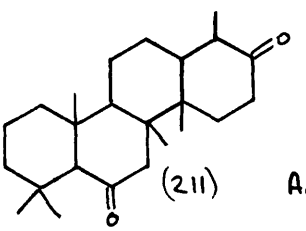

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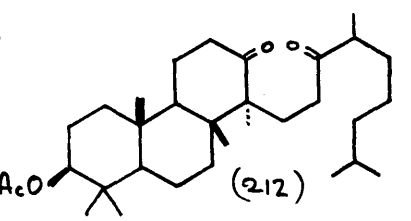

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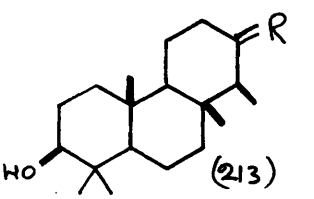

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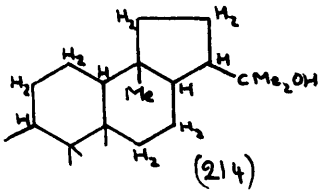

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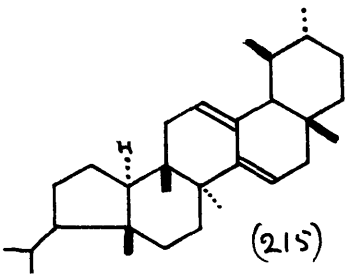


(215)

The ultra-violet spectrum of the diene (197), λ max. 266 mμ, is of considerably longer wavelength than is expected, thus ergosterol B_3, which has a 7:14-diene chromophore absorbs at 242 mμ, and is close to the value of 244 mμ predicted by the Woodward-Fieser rules[13]. The acid isomerisation product, λmax. 259 mμ, is also exceptional, since the calculated value for the diene (199) is also 244 mμ. This cannot be due to interaction through space with the aldehyde, or conjugation with the secondary ketone, since on reduction of both carbonyl groups to the diol the chromophores absorb at the same wavelength as before.

The ene-diol (191) could be dehydrated stepwise to give first a dienol, and then a triene. The first step was conveniently carried out in a concentrated dioxan solution of the ene-diol containing 5% of concentrated hydrochloric acid, when the dienol crystallised out on standing, and was not further dehydrated. The dienol readily dehydrated, even in neutral hot methanol solution, and recrystallisation could only be carried out by removal of methanol under reduced pressure.

The dienol showed an ultra-violet triple maximum 264, 254 and 246 mμ, similar to the 11:13(18)-diene of β-amyradienyl acetate[156] (260, 250, and 242 mμ), and is also remarkably similar to that of the parent diene (190), λλ max. 265, 255, and 247 mμ, and it is probable that the initial dehydration product (200) has spontaneously undergone an allylic shift to the allylic alcohol (201). In agreement with this, chromium trioxide in pyridine oxidised the dienol to a dienone λ max. 301 mμ, ε 18,000, similar to the 6:8(14)-dien-15-one chromophore λ max. 297 mμ, ε 20,400 of an ergosterol derivative[195] (202). The dienone

which would be derived from (200) is expected to absorb in the same region of the ultra-violet as the α-amyradienone chromophore[196] (203), λmax. 284mμ, ε 11,200, supporting the formulation of the dienol as (201) rather than (200).

The triene, λmax. 294mμ, ε 29,000, obtained by dehydration of both the dienol (201) and the ene-diol (191) with acid is probably (204). The exceptionally intense absorption speaks for an extended chromophore, and the position of the maximum is closer to that of the triene (205), λmax. 281mμ, ε 36,000, than to that of the alternative S-cis triene[197] (206) λ max. 319mμ, ε 16,000.

In both cases where the S-cis-diene (197) and (200) is formed, it readily isomerises to a trans-diene on treatment with acid. This is surprising in view of the stability to acid of ergosterol B_3, a 7:14-diene.

Removal of ring E.

Although it was known that ring E of zeorin is five-membered, the degree of substitution of C(20) was not known. This was determined as follows.

Treatment of zeorinin acetate (170, R=Ac) with osmium tetroxide, and cleavage of the osmate complex with hydrogen sulphide yielded a triol monoacetate (207, R=Ac), from which the triol (207, R=H) was obtained by reduction with lithium aluminium hydride. The osmate complex was also reduced directly to (207, R=H) by lithium aluminium hydride. The acetoxy glycol was recovered unchanged after treatment with acetic anhydride in pyri-

dine, indicating that the glycol is ditertiary.

Cleavage of the glycol (203, R=Ac) was rapidly effected either by lead tetraacetate, or by chromium trioxide in pyridine or in acetic acid. In all cases, the product was an acetoxy-seco-diketone (208) which showed infra-red bands at 1712 + 1250 cm.$^{-1}$ of the acetate and a band at 1700 cm.$^{-1}$ of the two unstrained keto groups. The absence of aldehyde absorption near 2700 cm.$^{-1}$ confirms that the glycol is ditertiary. The cleavage of a ditertiary glycol to a diketone by chromium trioxide is not common, but has been observed to occur with the ditertiary cis-glycol derived from β-onocerin[76]. In the absence of hydrogen on the oxygenated carbon atoms, the oxidation probably proceeds by the cyclic mechanism[111] (209).

Since the cleaved ring E is known to be five-membered, it was considered that the diketone (208) should undergo a reverse Michael reaction, as has been effected in the degradation of ring D seco diketones from β-ergostenyl acetate[198,199], cholest-14-enyl acetate[200], and isoeuphenyl acetate[201].

Purified diethylene glycol, on refluxing either alone, or with potassium hydroxide, in a stream of nitrogen, continuously evolves acetaldehyde which was identified as the 2:4-dinitrophenylhydrozone; ethylene glycol behaves similarly. These conditions, although consequently unsuitable for isolation of the volatile ketonic fragment, methyl isopropyl ketone, were excellent for obtaining the C_{24} fragment.

Addition of the acetoxy seco-dione (208) to potassium hydroxide in refluxing diethylene glycol caused evolution of

a ketonic substance in addition to the steadily evolved acetaldehyde, as judged by the rate of appearance of a precipitate on passing the gas stream through 2:4-dinitrophenylhydrzaine reagent. After forty minutes, the excess evolution of volatile ketone had ceased, and the diethylene glycol was worked up to yield an oily, water insoluble substance which could not be crystallised. Acetylation followed by chromatography yielded two diacetates $C_{28}H_{46}O_4$ (210, R=OAc) isomeric at C(17), which on reduction with lithium aluminium hydride gave the corresponding non-crystalline diols, both of which were oxidised by chromium trioxide in pyridine to the same diketone (211). The stability of (211) to acid demonstrates that the α-methyl group at C(18) is probably equatorial. Isomerisation is unlikely to have occurred during the oxidation, so it is probable that the methyl group is already equatorial in the diacetates (210, R=OAc). This is not surprising in view of the fact that the reverse Michael reaction proceeds by way of the enol (see arrows in 208), although the most stable conformation is not necessarily formed in the protonation of an enolate anion.

The diketone (211) was reduced by sodium in isopropanol to the diequatorial diol, again non-crystalline, which on acetylation gave the "C_{24} diacetate II" (210, R=equatroial OAc). The original secondary hydroxyl group of zeorin is known[173] to be equatorial. The order of elution of the two diacetates from the chromatogram is in agreement with the above more rigorous assignment of configuration; in general, equatorial acetoxy groups cause less ready elution than do the more hindered axial acetoxy groups[202].

The reduction of the initial cleavage product, the C_{24} ketol, to the two diols (210, R=OH) has analogy in reverse Michael cleavage of the isoeuphenyl acetate derivative[201] (212) by base in refluxing diethylene glycol, when the non-volatile fraction contained both the ketol (213, R=O) and its reduction product, the diol, (213, R= βH, αOH). The base catalysed reduction of ketones by alcohols, in this case diethylene glycol, is well known, however here there is yet another reducing agent present. As will be discussed presently, C(19) is liberated, presumably as formaldehyde, and this could undergo a Cannizzaro disproportionation with the C(17) ketone to give the alcohols (210, R=OH) and formic acid. The apparently complete reduction of the C(17) ketone, however, does not support this hypothesis, as at least part of the formaldehyde would be expected to be removed by the stream of nitrogen. No formaldehyde dinitrophenylhydrazone was isolated.

Since it proved difficult to separate the volatile ketone dinitrophenylhydrazone from the acetaldehyde derivative, the conditions of the reverse Michael reaction were altered.

Diphenyl ether was purified by distillation from lithium aluminium hydride, and refluxed with potassium hydroxide. A stream of nitrogen was passed through the two phase liquid system. The vapour pressure of the diphenyl ether was sufficiently high that a considerable quantity was carried in the nitrogen stream, and on bubbling it into dinitrophenylhydrazine reagent a dark red crystalline complex was formed. The same complex could be produced by addition of ethanolic diphenyl ether to the reagent.

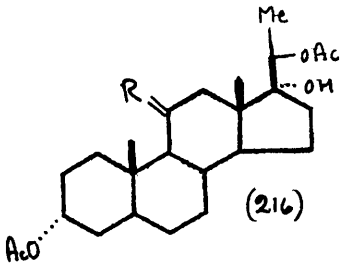
When the seco-dione (208) was introduced into the refluxing diphenyl ether and base, a volatile ketone was rapidly evolved in the nitrogen stream. The dinitrophenylhydrazone of this volatile ketone was readily separated from the diphenyl ether-dinitrophenylhydrazine complex by chromatography, and proved to be methyl isopropyl ketone dinitrophenylhydrazone. The semicarbazone was similarly prepared.

As in the case of the cleavage of the euphol derivative[201] (212), the original reaction generates an alkyl vinyl ketone which undergoes the reverse aldol reaction to give formaldehyde, which was not isolated, and the corresponding alkyl methyl ketone.

The above degradation demonstrates that the hydroxy-isopropyl group of zeorin is joined to a terminal five-membered ring. If the relationship between zeorininone (170, keto instead of OR) and neozeorininone (189) postulated above be accepted, the degradations so far described prove that zeorin must have the partial structure (214).

The isomerisation of zeorininone or zeorinone to neozeorininone (189) and not farther is, at first sight, surprising. Treatment of ursenol (109) with strong acid causes the familiar contraction of ring A, followed by a series of hydride and methyl migrations, with retention of the orientation of the migrating groups to give 1-α-amyradiene (215)[203].

The analogy of the intermediate in the zeorinone rearrangement with the ring A contracted ursene carbonium ion is, however, not complete. The presence of the 12-double bond in ursenol causes the rearranged ursene molecule 13-carbonium ion to be



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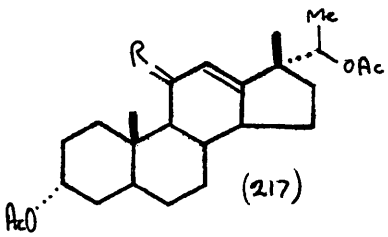

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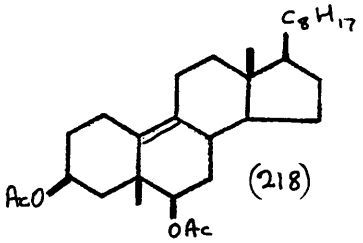

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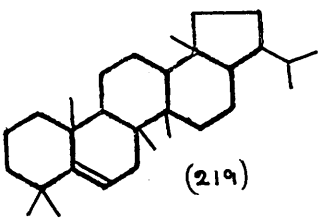
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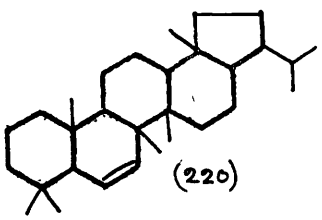
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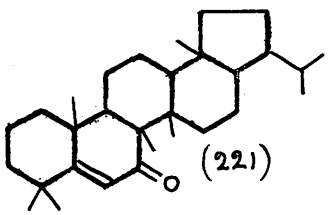

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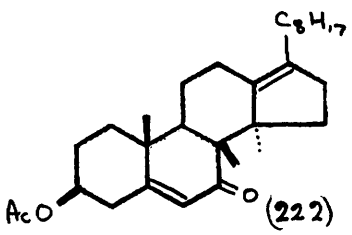

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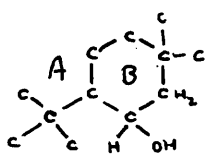

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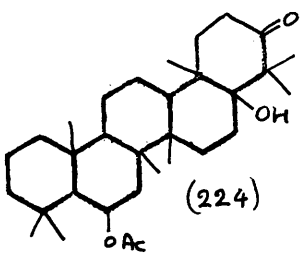

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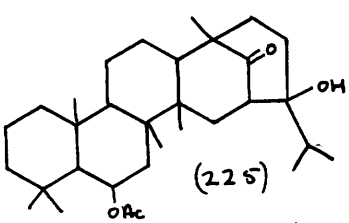

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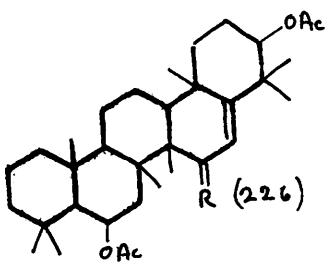

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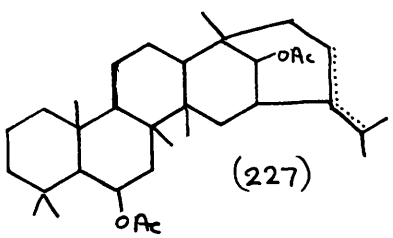

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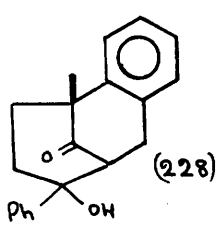

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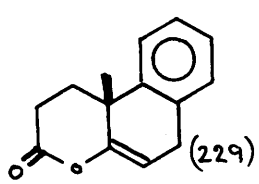

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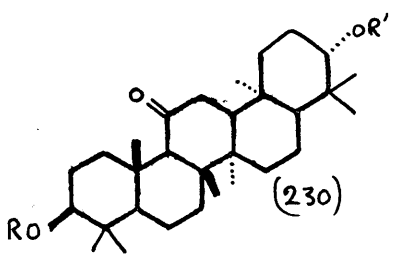

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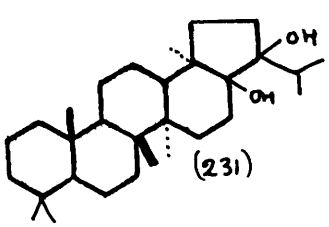

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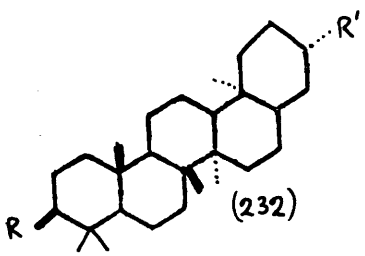

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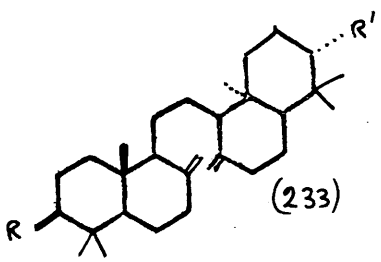


(233)

allylic, and hence more stable. If this is the cause of the difference in migrations, it implies that the rearrangement is reversible. No rearrangement in the opposite direction has been observed in vitro. The presence of the 12-double bond in ursenol may exert its influence on the reaction path merely by altering the conformation of rings B, C, and D.

It is of interest that while the tetrol diacetate (216, R=αH, βOH) and triol diacetate (216, R=H_2) rearrange to (217, R=αH, βOAc) and (217, R=H_2) respectively in acid, the corresponding keto triol diacetate (216, R=O) does not rearrange[204]. The keto group of neozeorininone (189) is similarly placed with respect to the potentially migrating C(8) methyl group and may inhibit its migration by reducing the electron availability at the methyl group which is consequently less ready to undergo migration as the anion[204].

Westphalen's diol, obtained by acid treatment of 3β:6β-diacetoxy cholestan-5α-ol has been shown[205] to be a rearrangement product (218) similar to neozeorininone.

The environment of the secondary hydroxyl group.

Previous investigation[173] indicated that the secondary hydroxyl group of zeorin cannot be in a terminal ring. In terms of the partial structure (214), the secondary hydroxyl group must be in ring B. This was confirmed in the following way.

Deoxyzeorin was dehydrated by toluene p- sulphonyl chloride in pyridine to give a mixture of mono-unsaturated hydrocarbons[173]. From hydrogenation evidence, the main component (219)

is sufficiently hindered that it cannot be reduced while the minor component (220) is readily hydrogenated using Adams catalyst.

Treatment of the mixture of unsaturated hydrocarbons with chromium trioxide in acetic acid yielded, in the neutral fraction, a conjugated enone (221) which had an infra-red band at 1650 $cm.^{-1}$ characteristic of a cyclohexenone, and showed ultra-violet absorption at 239mμ very close to that (238mμ) of a derivative[191] (222) of butyrospermyl acetate. The enone (221) was stable to vigorous treatment with bromine or selenium dioxide in acetic acid in agreement with the lack of replaceable hydrogens. Reduction of the enone with lithium aluminium hydride gave not the expected allylic alcohol, but a saturated ketone. The neutral fraction contained, as a minor constituent, a mono-unsaturated hydrocarbon $C_{30}H_{50}$ which was not investigated.

By dehydrogenation of zeorin, Asahina[171] obtained 1:2:5-trimethyl naphthalene which can most plausibly be derived from rings A and B of zeorin (72, R=H).

In order to explain the formation of the trimethyl naphthalene from rings D and E, it would be necessary to postulate removal of the tertiary hydroxyl group followed by ring expansion of the 22-carbonium ion to give a six-membered ring E. Although this path is not impossible at the higher temperature of selenium dehydrogenation, it does not occur with acid catalysts at the boiling point of acetic acid, when the product is of the zeorinin series (170).

On the assumption that the trimethyl naphthalene is de-

rived from rings A and B, the partial structure (223) can be written for this region of the zeorin molecule. In agreement with this, the rotatory dispersion of zeorinone has been found[206] to be of a type characteristic of 6-keto-pentacyclic triterpenoids.

In view of the considerable body of evidence indicating that triterpenoids are formed by cyclisation of squalene, it is reasonable to combine the partial structures (214) and (223) in the modified γ-onocerane skeleton as already implied in formula (72, R=H).

Attempted interrelations of zeorin with known triterpenoids.

Correlation of zeorin (72, R=H) with triterpenoids of known structure which contain six cyclohexane rings would involve either expansion of ring E of zeorin to a six-membered ring, or contraction of a six-membered ring of a known triterpenoid. Both methods were attempted but neither was successful.

Since it was known that expansion of ring E did not occur during acid dehydration of the tertiary hydroxyl group of zeorin (72, R=H), and that this cannot be due to steteochemistry of the tertiary hydroxyl group, enlargement of this ring could be brought about only through a ring cleavage product. For this, the seco-dione (208) appeared to be promising; aldol formation could proceed by reaction of an anion either at C(4) or at C(6) with the other ketone group to give respectively the γ-onocerane skeleton (224) which was the object of the synthesis, or the

abnormal skeleton (225). Treatment of the acetoxy seco-dione (208) with ethanolic potassium hydroxide gave an acetoxy-ketol in low yield. Considerable improvement was obtained by addition of water to the solution, when the acetoxy ketol crystallised slowly, disturbing the equilibrium which otherwise lies 1:4 in favour of the acetoxy seco-dione.

Dehydration of the tertiary hydroxyl group of the acetoxy ketol was attempted using thionyl chloride in pyridine, and phosphorous oxychloride in pyridine under various conditions, but in no case was a double bond formed, and only the starting material or its precursor, the acetoxy dione (208), could be isolated.

In order to prevent reversal of the cyclisation, the acetoxy ketol was reduced with lithium aluminium hydride and reacetylated to give a mixture readily separable on alumina into an anhydro-diacetate $C_{34}H_{54}O_4$ and a saturated triacetate.

The anhydro-diacetate was oxidised by chromium trioxide under the conditions used in the preparation of the ring B enone (221). If the anhydro-diacetate were (226, R=H_2) it was anticipated that the product would be an enone (226, R=O) with ultraviolet absorption in the region 230-240 mμ. In the event, there was no neutral product, and the acidic fraction absorbed weakly (ε 1,000) at 266mμ. The anhydro-diacetate is therefore probably (227), the position of the double bond being uncertain.

Attempted cyclisation of the acetoxy seco-dione (208) with hydrochloric acid in acetic acid[207] and with boron trifluoride etherate yielded only the starting material or the acetoxy ketol (225).

The formation of the abnormal skeleton (225) finds analogy in the formation of (228) by treatment of the model compound (229) with phenyl magnesium bromide[208]. The intermediate ketone undergoes an aldol condensation by the only path open to it without formation of a cyclobutane ring. In the case of the seco-dione (208) derived from zeorin, the direction of cyclisation is presumably defined by the more ready formation of the secondary C(16) anion rather than the tertiary C(22) anion. The enol lactone (cf. 229) derived from cholesterol, on treatment with methyl magnesium iodide, cyclyses to give cholest-4-enone. The intermediate diketone cyclyses to give the cholestane skeleton rather than a bridged ring system analegous to (228) because of the ready formation of the primary C(4) anion.

Attention was now directed to an alternative method of correlation involving conversion of 3:22-dihydroxy-γ-onoceran-11-one[76] (230 R=R'=H) and zeorin to the common intermediate (231).

The 11-keto group of (230, R=R'=H) could not be removed by the usual Huang Minlon method, but by using more forcing conditions, and prolonging the reaction to six days, the product, dihydroxy-γ-onocerane (232, R=R'=H) was formed in good yield, and was acetylated to the diacetate.

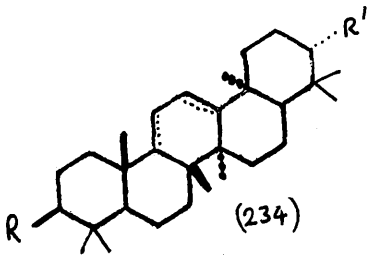
3:22-Diacetoxy-γ-onocerane (232, R=R'=OAc) in aqueous dioxan was treated with one equivalent of aqueous potassium hydroxide in an attempt to cause half hydrolysis[76] to the monoacetate. Owing to the low solubility of the diacetate in dioxan, the conditions used in the half hydrolysis of the diacetate of

α-onocerin[76] (19), could not be followed. Using a larger quantity of dioxan it was possible to dissolve the diacetate completely, but on addition of the aqueous potassium hydroxide, if formed a lower layer which corroded the glass of the flask, but left the diacetate virtually untouched, with formation of a small amount of the diol monoacetate (232, R=OH, R'=OAc), which was oxidised to the ketol acetate (232, R=O, R'=OAc) and reduced to γ-onoceranol (232, R=H, R'=OH). In the absence of water, potassium hydroxide did not dissolve in the dioxan.

It was now considered advisable to carry out the half hydrolysis at the α-onocerin stage, before conversion to the γ-onocerin series. A disadvantage of this is the necessity of working with mixture of 9(11)- and 12-double bond isomers and their derivatives after cyclisation to the γ-series, until the ring C functional groups, and hence the asymmetry, has been removed.

α-Onocerin diacetate (233, R=R'=OAc) was converted to the acetoxy ketone[76] (233, R=O, R'=OAc). Cyclisation of this with acid yielded the γ-onocerin acetoxy-ketone mixture (234, R=O, R'=OAc) in 10% yield under the most favourable conditions.

In the γ-onocerin series, conversion of the ring C double bond to the corresponding ketone is effected by ozone. Ozonisation of the γ-onocerin acetoxy ketone mixture (234, R=O, R'=OAc) led only to acidic material and the starting material. That the keto group was involved in the conversion to acids was shown when lupanone, under the same conditions of ozonisation, was also converted to acidic material.



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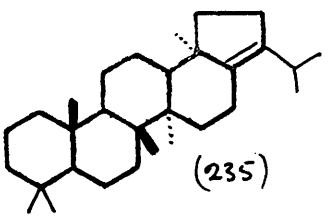

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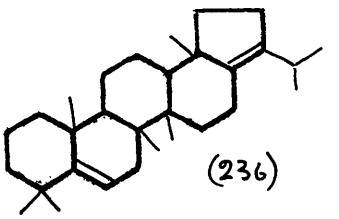

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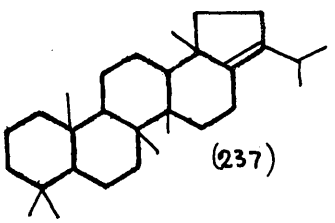

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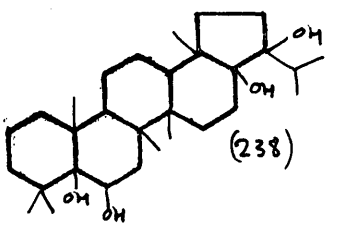

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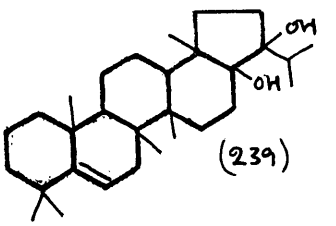

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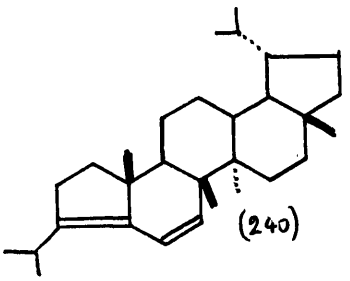

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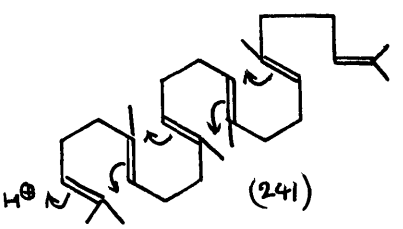

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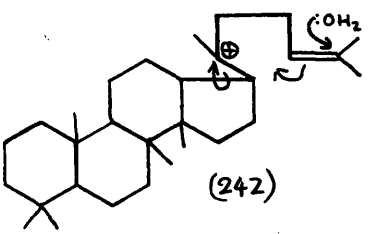

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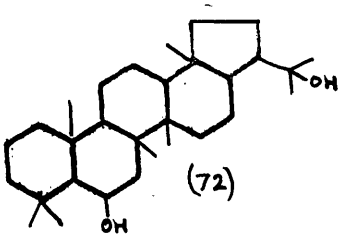

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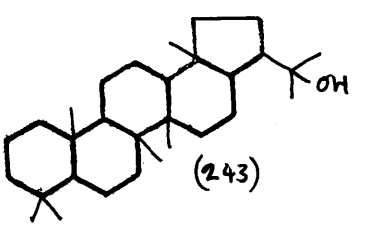

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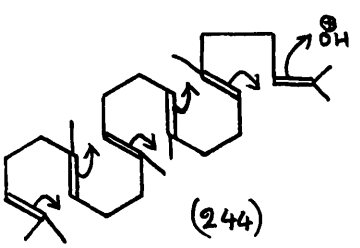

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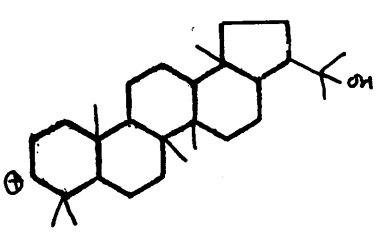


In order to avoid this side reaction, γ-onocerin acetoxy ketone mixture (234, R=O, R'=OAc) was reduced by the simple Wolff-Kishner method to the mixture of γ-onoceranols (234, R=H, R'=OH) which, on ozonisation and reduction by the prolonged forcing Wolff-Kishner method previously described, yielded γ-onoceranol (232, R=H, R'=OH), identical with that obtained by the other route.

Up to this stage, all the substances described had been fully characterised, and the I. R. spectra are completely in accord with the formulations. There remained three stages to be completed to arrive at the desired diol (231), and there was available only 17 mg. of pure γ-onoceranol, so characterisation of all the stages was not attempted.

Dehydration of γ-onoceranol (232, R=H, R'=OH) with phosphorus pentachloride caused the familiar retro-pinacol reaction to give the ring contracted product which was crystalline, but not characterised. Treatment of this with hydrochloric acid in ethanol caused double bond migration, as in the lupanol case[184], to give (235), which was characterised. A total of 10 mg. of this hydrocarbon was obtained crystalline. Hydroxylation of this with osmium tetroxide yielded a crystalline product, presumably the diol (231) in insufficient yield for characterisation. The m.p., 205-209°, is in fair agreement with the value 218-219° found by Jeger and his co-workers[165] in their synthesis of the diol (231) from α-onocerin. The crucial step in their synthesis is the half oxidation of γ-onocerane diol with manganese dioxide in refluxing chloroform, which gives the

corresponding ketol (232, R=O, R'=OH) in 10% yield, the remainder being starting material which can be recycled.

Since the diol (231) had been fully characterised by Jeger, no attempt was made to repeat our synthesis on a larger scale.

The conversion of zeorin to the intermediate diol (231) also presented difficulties which were not surmounted in the time at our disposal.

Zeorinin (170, R=H) was dehydrated by pyrolysis of the acetate to yield a mixture of di-unsaturated hydrocarbons. Hydrogenation of this mixture gave the diene (236) which cannot by hydrogenated under normal conditions, together with the mono-unsaturated hydrocarbon (237). It was hoped that hydroxylation of this mixture with osmium tetroxide would give a mixture of the desired diol (231) and the tetrol (238). In fact, the tri-substituted double bond of (236) was resistant to hydroxylation, so the product was a mixture of the diol (231) and the ene-diol (239) which could not be separated by chromatography. Ozonisation of the mixture in an attempt to convert (239) to an acid gave, as the only isolable product, a saturated compound containing three oxygen atoms. Since the infra-red spectrum showed the absence of ketone, it is likely that this is an epoxy-diol.

Stereochemistry.

No further attempt at correlation of the two series was made. Since they were not correlated, little can be said about the stereochemistry of zeorin. The virtual identity of the ultra-violet spectra of dehydrozeorinin (171, R=H) and of the

γ-lupane diene (240) make it probable that the environments of the chromophores are similar, and that rings D and E of zeorin have the stereochemistry shown, or its optical antipode.

Analysis of molecular rotation differences in the series zeorinin (170), zeorinane triol (207), the seco-dione (208) and dehydrozeorinin (171), and the corresponding differences in the γ-lupane series[111,184], oleanolic lactone series[186], and γ-onocerane series[165] (see table 6) indicate that the environment and absolute configuration of rings D and E are similar to those of the ring contracted rings A and B of the common pentacyclic triterpenes. Jones[85] has arrived at a similar conclusion by examination of molecular rotations in the hopanone and zeorinone series. He suggests that C(13), C(14), and C(18) probably have the same stereochemistry in zeorin and hydroxyhopane, and points out that the observed differences are compatible with variations at C(17) and C(21).

Table 6.

Skeleton	A	B	C	D	B-A	B-C	B-D
Zeorin[111]	+252°	+141°	---	+336°	-111°	---	-195°
Zeorin acetate[111]	+328°	+266°	+160°	+349°	-62°	+106°	-189°
γ-lupane[111]	+53°	-101°	---	+122°	-154°	---	-223°
oleanolic lactone[186]	+206°	+61°	-84°	---	-145°	+145°	---
γ-onocerane[165]	+201°	+53°	---	---	-148°	---	---
hopanol acetate[85]	---	+113°	-32°	---	---	+145°	---

The Biogenesis of Zeorin.

The structure (72, R=H) for zeorin is in accord with the biogenetic scheme outlined on p. 25. A number of different routes of cyclisation can be envisaged.

In the preferred scheme, protonation takes place at C(3) of (241) as in the formation of ambrein (2) and cyclisation proceeds in the usual manner through the first four potential rings A to D, giving the tetracyclic carbonium ion (242). Ring expansion of this, followed by cyclisation of the remaining potential ring in the Markownikoff sense to (243), and hydroxylation would give (72). The cyclisations could also be completely concerted (241 to 243).

Since it appears that cyclisation of squalene is most commonly initiated by a positive, oxygen-containing species, the alternative cyclisation depicted by (244) must be considered. In this scheme there is initial oxidative attack at the terminal double bond in a non-Markownikoff sense, followed by cyclisation through to the far end of the squalene chain. This leaves a carbonium ion or equivalent at C(3) which must be removed reductively. The first scheme, (241), therefore appears to be the simpler. The introduction of the secondary hydroxyl group at C(6) probably occurs later, and is not involved in the cyclisation.

In general, triterpenes have a marker at the site of initiation of cyclisation, usually a C(3) oxygen function and at or near the site of the final cyclisation. It is noteworthy

that zeorin and taraxerene[130], both lichen triterpenes, do not have markers at the points at which cyclisation begins.

It is conceivable that the five-membered ring of zeorin may have been a modification of a six-membered ring originally of the 3β-hydroxy-4:4-dimethyl type. The in vitro equivalent of this reaction is well known (see p.43).

Lantadene A

The poisoning of cattle by Lantana camara is sufficiently serious to be of economic importance in Australia, India, and South Africa[208]. From South African lantana, Louw[209] isolated two C_{35} triterpenoid esters which he named lantadene A and Lantadene B; the former he showed to be physiologically active in cattle.

In an examination of South African lantana, Barton[112] isolated only lantadene B, and showed it to have the constitution (107, R=[illegible].CO.CH C.Me_2). It is thus closely related to icterogenin[113] (101) and rehmannic acid[110] (107, R=angeloyloxy).

An investigation of Australian Lantana camara was now undertaken[111]. In the batch used, lantadene A was shown to be the major triterpenoid constituent. A very minor quantity of lantadene B was found to be present by chromatography of the methyl esters.

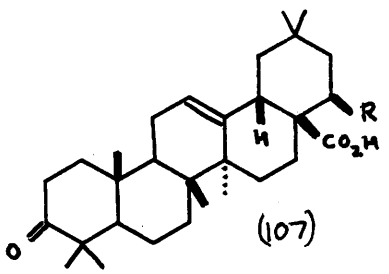
The authenticity of the isolated lantadene A was confirmed by comparison of the triterpenoid and its 2:4-dinitrophenylhydrazone derivative with the original compounds obtained by Luow[209], and kindly forwarded to us by Dr. O. Jeger of Zurich.

From an examination of the physical constants of lantadene A and its derivatives (see table 7), it appeared likely that it is identical with rehmannic acid. The mixed melting points of the two compounds and their corresponding derivatives did not depress, and the infra-red spectra were virtually identical. Although these criteria are normally sufficient to establish identity, it had been noted that, in this series, mixed melting point depressions are not always obtained between closely related compounds[112]. The infra-red spectra of lantadene A and lantadene B differ only slightly in the fingerprint region and not noticeably in the carbonyl region. It was therefore considered advisable to repeat on lantadene A the degradations which had already been carried out on rehmannic acid[110].

Hydrolysis of lantadene A with ethanolic potassium hydroxide yielded a crystalline hydroxyketone carboxylic acid proved by mixed melting point and rotation to be 22β-hydroxyoleanonic acid (107, R=OH). The identity was confirmed by methylation to give methyl -22β-hydroxyoleanonate identified by rotation and mixed melting point with authentic material obtained from lantadene B.

Pyrolysis of lantadene A methyl ester yielded a volatile acid identified as angelic acid. The triterpenoid fraction was an oil separated by chromatography into a colourless oil and crystalline starting material, lantadene A methyl ester.

Hydrogenation of the oily fractions and acetylation yielded methyl oleanolate acetate (245, R=Ac). Hydrolysis of this gave the crystalline alcohol ester (245, R=H) which was converted



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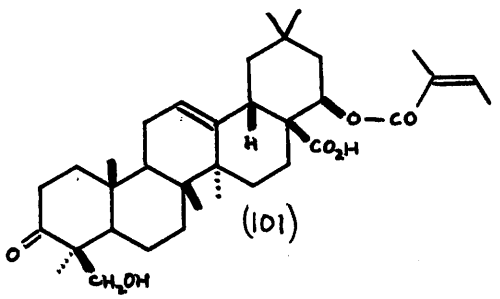

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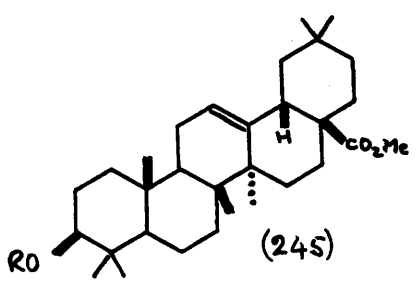

(245)

to the benzoate (245, R=CO.Ph). The three derivatives were compared with authentic compounds, and showed satisfactory agreement of rotations and no depression of the appropriate mixed melting points.

The results confirm beyond doubt that lantadene A and rehmannic acid are the same compound (107, R=angeloyloxy). The acetate of lantadene A obtained by Louw[209] is probably a mixed anhydride of the same type as had been prepared from lantadene B[112,209].

Table 7.

	Lantadene A				Rehmannic acid		Lantadene B	
	Louw		this investigation					
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
Acid	276-280°	+81°	282-286°	+89°	282-287°	+84°	293-294°	+85°
	282-287°*							
Me ester	125°	--	137-139°	+86°	140°	+86°	234-236°	+89°
2:4-DNP	268°	+35°	271-272°	+35°	273-274°	+27°	264-265°	+48°

*after several recrystallisations

The triterpenoid content of Lantana camara appears to vary considerably. A specimen of South African origin which was later examined yielded only traces of crystalline triterpenoid. In a second batch of Australian Lantana camara, neither lantadene A nor lantadene B could be isolated. There was obtained a triter-

penoid acid which was given the name lantadene C. Methylation yielded a monomethyl ester. The microanalyses of the acid and its methyl ester are consistent either with $C_{35}H_{52}O_5$ or $C_{30}H_{46}$ or $_{48}O_4$. The first formula is also that of lantadenes A and B.

Attempts to obtain a volatile acid fragment by hydrolysis, as was done successfully with rehmannic acid[110] (lantadene A) yielded, on titration, less than 10% of the expected volatile acid. The triterpenoid product of the base and acid treatment was no langer lantadene C, but an acid, lantadene D, probably isomeric with lantadene C. Lantadene D could also be obtained by acid treatment of the glycosidic fraction of the crude Lantana camara extract.

The infra-red spectra of lantadenes C and D showed the presence of a hydroxyl and carbonyl group in both. The methyl ester could readily be made, but attempted acetylation and benzoylation of lantadene D methyl ester yielded only starting material. A band at 885 $cm.^{-1}$, present in the infra-red spectrum of lantadene C, and possibly due to an unsaturated methylene group, is no longer present in lantadene D. The Zimmermann test on both lantadenes C and D is negative.

EXPERIMENTAL

Zeorin

Rotations were determined in chloroform solution. Ultra-violet absorption spectra were taken in 95% ethyl alcohol solution with the Unicam SP 500 spectrophotometer. Intra-red spectra were kindly determined by Dr. G. Eglinton and his associates, and microanalyses by Mr. J. Cameron.

22-Keto-23-norisozeorinin acetate (153).

The mixture of unsaturated compounds obtained from the dehydration of zeorin acetate (700mg.) with phosphorous oxychloride in pyridine, was dissolved in chloroform, cooled to -60°, and ozone passed in till the solution no longer gave a colour with tetranitromethane. The product was extracted, washed, and chromatographed on alumina. Elution with benzene: petrol (7:3) gave keto-norisozeorinin acetate (250mg.); crystallised from methanol, m.p. 232-238° (dec.), $[\alpha]_D$ +44°, (c, 0.93). Found C78.89%, H 10.53%; $C_{31}H_{50}O_3$ requires C79.10%, H 10.71%. Zimmermann test is positive. 2:4-Dinitrophenylhydrazone m.p. 239-240°, found N 8.6%; $C_{37}H_{54}O_6N_4$ requires N 8.61%.

Bromination of keto-norisozeorinin acetate.

The nor-ketone (38.6mg.) was dissolved in acetic acid (10ml.) containing approximately 100 mg. of bromine. A solution of pregnenolone (45.3mg.), and a blank, were made up similarly. The absorption of bromine was followed by titration; the nor-ketone took up 4.4 molecules of bromine and pregnenolone 4.12 molecules indicating that the nor-ketone has four hydrogen atoms

α- to the ketone.

Isolation of acetone dinitrophenylhydrazone.

Zeorin acetate (500mg.) was dehydrated and ozonised as before. Water (2ml.) was added and the solution refluxed on steam for 5 minutes. Distillation of the solution into 2:4 dinitrophenylhydrazine reagent, extraction with chloroform and filtration through a bentonite-kieselguhr column yielded acetone dinitrophenylhydrazone (30mg.) m.p. 120-123° undepressed on mixing with an authentic specimen.

Zeorin trisnor lactone acetate (156).

Zeorin acetate (3g.) was dehydrated and ozonised as before, the product, filtered through alumina in benzene, was an oil (1.75g.).

The oil (1.75g.) in methylene dichloride (30ml.) with sodium phosphate (1.5g. anhydrous) and peroxytrifluoracetic acid (approximately 1g.) was refluxed for 15 minutes. The solution was worked up as before, hydrolysed, re-acetylated, and chromatographed on alumina. Elution with methanol:acetic acid (9:1) gave a solid (350mg.) crystallised from aqueous methanol m.p. 282-285° (dec.); $[\alpha]_D$ +43°(c,1.00); found C75.75%, H 10.05%; $C_{29}H_{46}O_4$ requires C75.95%, H 10.1%. I.R. in CCl_4 1735 1248 cm.$^{-1}$- OAc, 1750 cm.$^{-1}$. δ-lactone.

Zeorinin acetate oxide.

Zeorinin acetate (300mg.) in chloroform was ozonised at -60°. The crude product was filtered through alumina in benzene:

ether (9:1). Crystallisation from ethanol yielded zeorinin acetate oxide m.p. 245-251°, $[\alpha]_D$ +77° (c,0.44) undepressed in m.p. on mixing with authentic material.[173]

isoLanost-5-ene.

Isolanost-3-ene (3g.) in chloroform (50ml.) was treated with dry hydrogen chloride for 1 hour. The product crystallised from methanol as needles m.p. 87°, depressed on mixing with starting material, $[\alpha]_D$ +73° (c, 2.06) found C 87.32%, H 12.56%, $C_{30}H_{52}$ requires C 87.30%, H 12.70%.

Ozonisation of isolanost-5-ene.

Isolanost-5-ene (207mg.) m.p. 87° in chloroform at -60° was ozonised for 65 minutes, when the tetranitromethane test became negative. Activated zinc (300mg.) and acetic acid (4ml.) were added, and the solution warmed to room temperature. Extraction and washing gave an oil. The infra-red spectrum in carbon tetrachloride showed a single carbonyl peak at 1707 $cm.^{-1}$. Peaks at 1026 and 885 $cm.^{-1}$, present in the starting material, were now absent.

isoLanostane-5ξ:6ξ-cis diol.

iso-Lanost-5-ene (327mg.) in dioxan was left 12 days in the dark with osmium tetroxide (300mg, 1.5 equivalents). Working up by H_2S and chromatography, and crystallisation from methanol yielded iso-lanostane-5ξ:6ξ-diol as plates m.p. 146-146.5°

$[\alpha]_D$ +39° (c,1.73), found C 80.84%, H 11.99%; $C_{30}H_{50}O_2$ requires C81.02%, H 11.79%, I.R. (nujol) 3350 cm.$^{-1}$ -OH, no carbonyl absorption. Earlier fractions from the chromatogram yielded a small quantity of a second compound m.p. 143-144° from methanol. Tetranitromethane test negative. This proved to be the 6-ketone (164); see below.

isoLanostan-6-one(164).

isoLanostane diol (10mg.) in chloroform (10ml.) with concentrated hydrochloric acid (1 drop) was refluxed for 10 minutes. The product was extracted and crystallised from methanol I.R. ($CHCl_3$) 1720 cm.$^{-1}$, m.p. 145-147° depressed on mixing with the diol, undepressed on mixing with the second byproduct m.p.143-144° from the osmylation.

γ-Lup-3(5)-ene.

Lupanol (354mg.) in dry petrol (40ml.) with phosphorous pentachloride (400mg.) was left at 0°C for one hour. The product was extracted, filtered through alumina (10g. activity III) and crystallised from methanol as thick needles m.p. 174-184°, Beilstein's test negative. The I.R. (nujol) peak at 888 cm.$^{-1}$ is reduced on crystallisation indicating, the removal of the isopropenyl isomer. Ruzicka, Jeger and Huber, Helv. Chim. Acta, 1945, 28, 942, obtain m.p. 212-213° corr. for the pure isopropylidene compound. Isomerisation of the mixture of hydrocarbons (200mg.) by refluxing in ethanol (300ml.) with concentrated hydrochloric acid (50ml.) for 35 minutes, and crystallisation from methanol, gave γ-lup-3(5)-ene m.p. 135-136° $[\alpha]_D$ +13°

(c, 2.875) as shining plates, yield 130mg. after four recrystallisations. Found C 87.95%, H 12.17%; $C_{30}H_{50}$ requires C 87.73%, H 12.27%.

γ-Lupane-3:5-epoxide.

1) γ-Lup-3(5)-ene (163mg.) in methylene dichloride (10ml.) at 0° was treated with ozone until the solution gave no colour with tetranitromethane (2½ hrs.). The non-crystalline product in petrol was chromatographed on alumina (8g. activity III). Petrol eluted the epoxide crystallised from acetone as plates (28mg.) m.p. 179-187°, $[\alpha]_D$ +5° (c, 1.24), found C 84.40%, H 11.77%; $C_{30}H_{50}O$ requires C, 84.44%, H 11.81%. The I.R. shows the absence of carbonyl absorption.

2) γ-Lup-3(5)-ene (282mg.) in acetic acid (50ml.) at 100° was mixed with hydrogen peroxide (1 ml. of 30%). Tests with tetranitromethane showed that the double bond was absent after 5 to 7 minutes. Extraction and chromatography yielded the epoxide (24mg.) as the only crystalline product, m.p. 179-187° undepressed on mixing with the product obtained by the other route.

γ-Lupane-3(5):6-diene.

γ-Lupane-3:5 epoxide (24mg.) in ethanol (20ml.) with concentrated hydrochloric acid (2ml.) was refluxed for 1½ hours at 82° with the minimum amount of chloroform (0.5ml.) to prevent the separation of an oil. Extraction and filtration in petrol through alumina (1g. activity I) yielded the diene as plates from acetone, m.p. 111-112° $[\alpha]_D$ +30° (c, 0.58, 2 dm. tube).

Found C 87.78%, H 11.72%, $C_{30}H_{48}$ requires C 88.16%, H 11.84%. The U-V spectrum has a single maximum at 252 mμ (ε21,500).

Dehydrozeorinin (171, R=H).

Dehydrozeorinin benzoate (171, R=CO.Ph) (268mg.) in dioxan (15ml.) was refluxed with lithium aluminium hydride (300mg.) for 5 mins. Extraction with ethyl acetate yielded dehydrozeorinin m.p. 174-183° as short blades from methanol. $[\alpha]_D$+79° (c, 1.707, 2 dm.). Found C 84.82%, H 11.23%; $C_{30}H_{48}O$ requires C 84.84%, H 11.39%. Asahina and Yosioka (Ber. 1940, 73, 742) prepared dehydrozeorinin m.p. 183-185.5° by acid dehydration of zeorinin oxide.

Acetylation of dehydrozeorinin (171, R=H) (100mg.) in pyridine (5ml.) and acetic anhydride (5 ml.) on the steam bath for 6 hours gave the acetate (171, R=Ac) as prisms m.p. 213-222°, capillary m.p. 222-223°, $[\alpha]_D$+75° (c, 1.885, 2 dm.). Found C 82.16%, H 10.80%, $C_{32}H_{50}O$ requires C 82.34%, H 10.80%.

Dehydrozeorinin acetate was also prepared by acid catalysed dehydration of zeorinin acetate oxide. Asahina and Yosioka[171] obtained dehydrozeorinin acetate as prisms m.p. 223-227°.

6-Acetoxy-zeorin-17en-15-one (173).

Dehydrozeorinin acetate, (171, R=Ac) (894mg.) and osmium tetroxide (485mg., 1.00 equivalent) in dioxan solution (25ml.) were left for four days at room temperature in the dark. Hydrogen sulphide was passed in from a Kipp generator and the osmium trisulphide filtered off. Chromatography of the product in carbon tetrachloride on alumina (17g. activity III) and elution with 2% to 50% ether in benzene yielded the conjugated enone (173),

crystallised from aqueous methanol as needles m.p. 141-142°, $[\alpha]_D$ +76° (c, 0.658, 2 dm.). Found C 76.85%, H 10.30%; $C_{32}H_{50}O_3$, H_2O requires C 76.75%, H 10.47%. I.R. (nujol) 1710+1253 cm.$^{-1}$ - acetate, 1660 cm.$^{-1}$ -conjugated enone. U-v. λmax. 230 mμ (ε10,800).

The enone was recovered unchanged (mixed m.p. and u.-v.) after refluxing for 30 minutes in ethanol (5ml.) containing hydrochloric acid (1 drop).

Zeorinin-6:15:16-triol (172, R=H) and its 6-monobenzoate (172,R=CO.Ph

Dehydrozeorinin benzoate (171, R=CO.Ph) (187mg.) in dioxan (8ml.) was left with osmium tetroxide (95mg. 1.1 equivalents) in the dark for 5 days. Addition of lithium aluminium hydride (200mg.) in ether (3ml.) and extraction with ethyl acetate yielded a crystalline solid. Chromatography on alumina (6g. neutral deactivated) yielded two products.

1) 2% to 10% benzene in carbon tetrachloride eluted zeorinin-6:15:16-triol-6-monobenzoate (172, R=CO.Ph) crystallised from methanol or petrol as needles (7mg.) m.p. 227-241°, $[\alpha]_D$ +55° (c, 0.571, 2 dm.). Found C 78.82%, H 10.08%; $C_{37}H_{54}O_4$ requires C 78.96%, H 9.67%. U.-v. $\lambda\lambda$ max. 274 mμ (ε 905), 232 mμ (ε14,000). I.R. (nujol) 3380 cm.$^{-1}$ -hydroxyl, 1687+1288+1597+1584+714 cm.$^{-1}$ - benzoate. I.R. (carbon tetrachloride) 1703 cm.$^{-1}$. The carbonyl absorption is at a lower frequency than usual (1719 cm.$^{-1}$).

2) Benzene to 10% ether in benzene eluted from the column zeorinin-6:15:16-triol (172, R=H), recrystallised from methanol as prisms (43mg.) m.p. 235-252°, $[\alpha]_D$ +35° (c, 1.36, 2 dm.).

Found C 78.32%, H 11.40%; $C_{30}H_{50}O_3$ requires C 78.55%, H 10.99%. I.R. (nujol) 3555+3415+3340+ $cm.^{-1}$ -hydroxyl, no carbonyl absorption. The tetranitromethane test gives no colour.

Zeorinin-6:15:16-triol-6-monoacetate (172, R=Ac).

Dehydrozeorinin acetate (171, R=Ac) (338mg.) in dioxan/pyridine (10:1, 6 ml.) with osmium tetroxide (189mg., 1.03 equivalents) was left for 10 days at room temperature. Working up by addition of H_2S, evaporation of the solvents, addition of ethyl acetate and filtration yielded a colourless solution. Evaporation and chromatography on alumina (9g. acitivity III) yielded, in order of elution, dehydrozeorinin acetate (171, R=Ac) (30mg.), the enone acetate (173) (53mg.), and the triol monoacetate (172, R=Ac) (173mg.), m.p. 201-212° ex petrol, $[\alpha]_D$ +59° (c, 1.19, 2 dm.). Found C 76.45%, H 10.01%; $C_{32}H_{52}O_4$ requires C 76.75%, H 10.47%. The ultra-violet spectrum showed only end absorption.

Acid dehydration of the triol monoacetate (172, R=Ac).

The zeorinin triol monoacetate (172, R=Ac) (21mg.) in dioxan (5ml.) and concentrated hydrochloric acid (0.10ml.) was left at room temperature for three days. On working up by addition of water and extraction, the diene diol monoacetate was obtained as needles, recrystallised from methanol containing one drop of pyridine to 213-222°. U.-v. λ max. 250 mμ (ε 21,600). Found C 79.63%, H 10.55%; $C_{32}H_{50}O_3$ requires C 79.62%, H 10.44%. On refluxing the diene diol monoacetate with concentrated hydrochloric acid in chloroform, the ultra-violet maximum at 250 mμ disappeared

while a maximum at 286mμ appeared.

Acid dehydration of the triol (172, R=H).

Zeorinin triol (172, R=H) (10 mg.) in chloroform (3ml.) and ethanolic hydrogen chloride (2ml.) was refluxed for 30 minutes. Removal of the chloroform and addition of more ethanol yielded, on cooling, needles of the trienol (cf. 179) recrystallised from chloroform-petrol to m.p. 320-326°, $[\alpha]_D$ -17° (c, 0.376, 2 dm.). U.-v. $\lambda\lambda$ max. 295mμ (ε18,800) and 283mμ (ε16,500). Found C 84.66%, H 10.43%; $C_{30}H_{46}O$ requires C 85.24%, H 10.97%.

Neozeorininone (cf. 181).

Zeorinone (1.29g.) in acetic acid (100ml.) at 100° was mixed with Analar perchloric acid (6.5ml.) (72%). The solution became dark red within two minutes, and the neo-ketone began to crystallise out after seven minutes. After 40 minutes on the steam bath, the solution was poured into water, extracted, and the product crystallised from petrol as prisms (1.05g.) m.p. 225-234° undepressed on mixing with authentic neozeorininone m.p. 225-230° (literature m.p. 238-240° presumably not taken on the Kofler.)

Chromium trioxide oxidation of neozeorininone.

Neozeorininone (1.620g.) in acetic acid (1 litre Analar) with chromium trioxide in acetic acid (20ml. 1.08 N i.e. 3.0 [O] per molecule) was left overnight at room temperature. Dilution, extraction and reduction with sulphur dioxide yielded colourless crystals. Chromatography in carbon tetrachloride on

alumina (35g. activity V) yielded three substances.

1) Carbon tetrachloride eluted from the column a conjugated enone (cf. 185) crystallised from ethanol as plates (853mg.) m.p. 278-282°, $[\alpha]_D$+39° (c, 1.67). Found C 82.28%, H 10.81%; $C_{30}H_{46}O_2$ requires C 82.13%, H 10.57%. U.-v. λ max. 259 mμ(ε12,870). I.R.(chloroform) 1697 cm.$^{-1}$.(cyclohexanone cyclopentenone) and 1610 cm.$^{-1}$ (strong band characteristic of an S-cis enone).

2) Carbon tetrachloride to 50% benzene in carbon tetrachloride eluted an epoxy ketone (cf. 182) crystallised from petrol as prisms (398 mg.) m.p. in both cases 272-350° with recrystallisation at 220°. This was unchanged on repeated crystallisation. $[\alpha]_D$+91° (c, I.05). U.-v. 288 mμ(ε108). Found C 79.13%, H 10.11% $C_{30}H_{46}O_3$ requires C 79.25%, H 10.20%. I.R. (nujol) 1703 cm.$^{-1}$ cyclohexanone. The tetranitromethane test is negative.

The wide melting range of the epoxy compound is due to conversion to a higher melting compound. The epoxy-ketone (10mg.) was heated to 280° for five minutes in a Woods metal bath. The substance appeared to recrystallise with partial sublimation. The product crystallised from methanol as felted needles (5mg.), m.p. greater than 350°. Found C 79.93%, H 10.59%. The u.-v. shows no significant absorption. I.R. (nujol) 1718 cm.$^{-1}$, 1697 cm.$^{-1}$+1677 cm.$^{-1}$ shoulder. The tetranitromethane test is negative.

3) 1% to 25% ether in benzene eluted from the column a conjugated ene-dione (cf.183) crystallised from methanol as yellow plates (27mg.) m.p. 284-290° $[\alpha]_D$ +31° (c, 0.89, 2 dm.).

Found C 79.58%, H 9.69%; $C_{30}H_{44}O_3$ requires C 79.60%, H 9.80%. U.-v. λmax. 375mμ (ε460), 263mμ (ε9,200). I.R. (nujol) 1707 cm.$^{-1}$ (broad) 1630 cm.$^{-1}$ I.R. (carbon tetrachloride) 1724 cm.$^{-1}$ shoulder, 1717 cm.$^{-1}$, 1707 cm.$^{-1}$ shoulder, 1644 cm.$^{-1}$.

neoZeorinin-6:12-dione.

The epoxy-dione (cf. 182) (106mg., 0.233 m. mole) in acetone (15ml.) was mixed at room temperature with ethanolic chromous chloride reagent[193] (3ml. 0.63N). The solution became green immediately. On addition of further 6 ml. of the reagent a permanent blue colour was visible. After fifty minutes, the product was worked up by dilution and extraction. Chromatography on alumina showed it to be homogeneous. Crystallisation from petrol yielded neozeorinin-6:12-dione as needles (95mg.) m.p. 266-268° with a sharp change from needles to prisms at 235-236°. $[\alpha]_D$ +53° (c, 1.20, 2 dm.). Found C 81.94%, H 10.19%; $C_{30}H_{46}O_2$ requires C 82.13%, H 10.57%. U.-v. λmax. 255 mμ (ε8,400). I.R. (nujol) 1700 cm.$^{-1}$ (cyclohexanone) 1670 cm.$^{-1}$ (cyclohexenone) 1603 cm.$^{-1}$ (high intensity C=C stretch of an S-cis enone).

neoZeorinane-6:12:19-trione.

The epoxydione (cf. 182) (150mg.) in ethanol (18ml.) and concentrated hydrochloric acid (4 ml.) was refluxed for 20 hours. Evaporation and crystallisation from ethanol yielded the triketone (188) as hexagonal plates m.p. 280-285° (depressed to 265-268° on admixture with the starting material m.p. 274-350°). $[\alpha]_D$ 55°, (c, 1.75, 2 dm.). Found C 79.42%, H 10.35%;

$C_{30}H_{46}O_3$ requires C 79.24%, H 10.20%. The u.-v. spectrum shows only the isolated ketone peak at 295 mμ(ε, 150). I.R.(nujol) 3350 cm.$^{-1}$ very weak, 1740 1703 cm.$^{-1}$ cyclopentanone and cyclohexanone.

neoZeorinane diene.

Zeorinin acetate (805mg.) in acetic acid (50ml.) at 100° was treated with perchloric acid (2 ml. of 72%). After 4 minutes white crystal clusters appeared. The product crystallised from ethanol as needles m.p. 177-179°, $[\alpha]_D$ + 50° (c, 2.13), found C 88.16%, H 11.92%, $C_{30}H_{48}$ requires C 88.16%, H 11.84%, U.-v. λ max. 206 mμ(ε20,000).

neoZeorina-11:13(18)-dien-6-one (190).

neoZeorininone (189) (424mg., 1.00 m mole) and selenium dioxide (133mg., 1.20 m.mole) in dioxan (2 ml.) in a sealed tube were kept at 140° for 1 hour. The crude product was dissolved in chloroform and the insoluble black selenium filtered off. Chromatography of the product on alumina (15g. activity I) and elution with carbon tetrachloride to 50% benzene in carbon tetrachloride yielded the dienone, crystallised from ethanol as plates m.p. 255-256°, $[\alpha]_D$ +30°, (c, 1.34, 2 dm.). Found C 85.51%, 85.11%, H 10.76%, 11.21%; $C_{30}H_{46}O$ requires C 85.24%, H 10.97%. U.-v. λ λ max. 265 mμ(ε20,600), 255 mμ(ε29,000), and 247 mμ(ε 26,100). I.R. (nujol) 1703 cm.$^{-1}$ (cyclohexanone), 858+800+753 cm.$^{-1}$.

Attempted hydrogenation of the dienone (31mg.) in cyclo-

hexane/dioxan/acetic acid (1:5:2, 10ml.) with palladium black (10mg.), caused no uptake of hydrogen, even after addition of concentrated hydrochloric acid (1 drop).

On introduction of Adam's catalyst, hydrogenation took place. The product crystallised from ethanol as rods m.p. 232-237°, undepressed on mixing with authentic neozeorininone, m.p. 233-237°. The I.R. spectra (nujol) were also identical.

11:12-cis-Dihydroxy-neozeorinin-6-one(191).

neoZeorina-11:13(18)-dien-6-one (110mg.) and osmium tetroxide (200mg.) in pyridine (1ml.) and dioxan (5ml.) were left in the dark at room temperature for twelve days. Working up by hydrogen sulphide and crystallisation from petrol yielded the diol (191) as needles m.p. 214-221°, $[\alpha]_D$+33° (1.24, 2 dm.). Found C 78.80%, H 10.99%; $C_{30}H_{48}O_3$ requires C 78.89%, H 10.59%. The u.-v. spectrum shows only end absorption. Chromatography indicated that the product was homogeneous.

neoZeorininone was recovered unchanged in good yield after treatment for one and a half months with osmium tetroxide under the same conditions.

Cleavage of the diol (191).

Titration of the diol (191) (23.3mg., 0.102 m.mole) and of cholestane 5α-6α-diol (20.7mg., 0.100 m.mole) with lead tetra-acetate as described by Criegee (Ber., 1931, 64, 264) indicated the cleavage in less than five minutes of respectively 1.00 and 0.99 glycols.

The product (193) of cleavage of dihydroxy-neozeorininone was worked up by extraction, and crystallised from petrol as needles m.p. 173-181°, $[\alpha]_D$ -119° (c, 1.14, 2 dm.). Found C 78.71%, H 10.29%. $C_{30}H_{48}O_2$ requires C 78.89%, H 10.59%. The only maximum in the u.-v. spectrum is at 300mμ ε160. I.R. (carbon tetrachloride dilute solution, all frequencies ±1 cm.$^{-1}$) 3553 cm.$^{-1}$ H- bonded hydroxyl, 2720 cm.$^{-1}$ (aldehyde), 1708+ 1695 cm.$^{-1}$ (aldehyde (?)+cyclohexanone).

The isolated 16-ketone in neozeorininone absorbs at 1713 cm.$^{-1}$

Pyrolysis of the neo-aldehyde (193).

The aldehyde (193) (36mg.) was heated in a stream of nitrogen at 0.15 mm. pressure and the vapour passed through a tube at 220°. The product (197) condensed and crystallised just outside the heated zone. Chromatography on alumina (2g., activity III) and elution with 3% benzene in carbon tetrachloride to pure benzene yielded prisms (17mg.) from methanol, m.p. 172-177°, $[\alpha]_D$-3° (C, 0.94, 2 dm.). Found C 85.45%, H 10.99%; $C_{30}H_{46}O$ requires C 85.24%, H 10.97% u.-v. λ max. 266mμ (ε13,100) I.R. (nujol) 2706 cm.$^{-1}$ (aldehyde) 1700 cm.$^{-1}$ 805 cm.$^{-1}$ (strong).

Reduction of the pyrolysis product (197) (75mg.) in dioxan (4ml.) with lithium aluminium hydride (3ml. of saturated solution in ether) by evaporation until the solution boiled at 100°, followed by dilution with water and extraction, yielded a non-crystalline solid (198), u.-v. λ max. 265 mμ (ε12,000).

Chromatography on alumina (2g. deactivated) did not produce any crystalline fraction. The major portion, eluted with carbon

tetrachloride had λ max. 261 mμ (ε11,000). I.R. (carbon tetrachloride) 3580 cm.$^{-1}$-hydroxyl, no significant carbonyl absorption.

On refluxing either the original amorphous solid, or the chromatographed product above with 1 drop of conc. HCl in the 5 ml. of spectroscopic ethanol solution, the position of the u.-v. maximum was changed to 259mμ (ε13,000) and 259mμ (ε13,500) respectively. The original aldehyde (λmax. 266 mμ, ε13,100), on similar acid treatment, showed a similar shift to 259 mμ (ε13,800).

Dehydration of 11:12-dihydroxy neozeorininone.

The diol (46mg.) in dioxan (1 ml.) with concentrated hydrochloric acid (1 drop) was left at room temperature for two hours, when crystallisation of the product as colourless needles appeared to be complete. Recrystallisation from methylene dichloride/methanol in the cold under reduced pressure yielded the partial dehydration product, the dienolone (201), m.p. 180-199° with recrystallisation to rectangles at 185-195°, $[\alpha]_D$ +25°, (c 0.965, 2 dm.). Found C 81.59%, H 10.86%, $C_{30}H_{46}O_2$ requires C 82.13%, H 10.57%. U.-v. $\lambda\lambda$ max. 264 mμ (ε 8,900), 254 mμ (ε 13,600), and 246 mμ (ε 12,000) I.R. (nujol) 3350 cm.$^{-1}$ (weak), + 1697 cm.$^{-1}$, + 1135+1086+804 cm.$^{-1}$.

neoZeorinane-1(2):9(10):11(12)-triene-16-one (204).

On refluxing the dienolone (201) in ethanol alone, or more rapidly on addition of concentrated hydrochloric acid, further dehydration took place giving neozeorinane-11:13(18):19-trien-6-one as colourless needles from petrol m.p. 244-247°, $[\alpha]_D$ +259°

(c, 0.809, 2 dm.). Found C 85.58%, H 10.24%, $C_{30}H_{44}O$ requires C 85.65%, H 10.54%. U.-v. λmax. 284mμ (ε29,000). I.R. (nujol) 1703 cm.$^{-1}$ (cyclohexanone), 1630 (weak but sharp)+ 906+790+721 cm.$^{-1}$.

Zeorinane triol monoacetate (207, R=Ac).

Zeorinin acetate (817mg.) in dioxan (7ml.) with osmium tetroxide (643mg., 1.3 equivalents) was left at room temperature in the dark for 14 days. Working up by H_2S gave the triol monoacetate which was shown to be homogeneous by careful chromatography on alumina, and crystallised from petrol, m.p. 248-254°, $[\alpha]_D$+53° (c, 2.37); found C.76.71%, H 10.71; $C_{32}H_{54}O_4$ requires C 76.44%, H 10.83%. The triol monoacetate was recovered unchanged in 80% yield after refluxing with HCl/chloroform under conditions which converted iso lanostane-5:6-diol to the ketone. Attempted acetylation of the triol monoacetate (207, R=Ac) with acetic anhydride in pyridine at room temperature for three days yielded only starting material.

Zeorinane-triol (207, R=H).

The triol monoacetate (50mg.) in dioxan (4ml.) with lithium aluminium hydride (50mg.) was refluxed for 30 minutes. Extraction, washing and crystallisation from petrol yielded the triol (37mg.) m.p. 255-270°, not improved by repeated crystallisation from methanol or petrol. $[\alpha]_D$+31° (c, 2.40). Found C 78.09%, H 11.52%, $C_{30}H_{52}O_3$ requires C 78.02%, H 11.38%. I.R. (nujol) peaks at 3350 cm.$^{-1}$ and 3430 cm.$^{-1}$, no carbonyl absorption.

The triol was also obtained by direct reduction of the osmate ester from zeorinin acetate.

6-Acetoxy-17:21-diketo-A-secozeorinane (208).

1) The triol monoacetate (207, R=Ac) (25.1 mg.) in Analar benzene, and lead tetra-acetate in Analar acetic acid (3.00ml., .0500$\bar{N}$) was diluted with Analar benzene to 10.00ml. Withdrawal of 2.00 ml. aliquots after 10 and 20 minutes and titration with iodide/thiosulphate in the presence of sodium acetate buffer showed that oxidation was complete (10 mins. 1.12[O] permmolecule, 20 mins. 1.10[O]). Extraction yielded the acetoxy seco-dione (208) m.p. 124-125° undepressed on mixing with the product obtained by the other routes.

2) Zeorinane triol monoacetate (343mg.) in benzene (25ml.) with lead tetra-acetate (500mg.,1.4 equivalents) in acetic acid (20ml.) was left at room temperature for 10 minutes. Addition of ethylene glycol (5 drops), dilution with water and extraction yielded the acetoxy-seco-dione, crystallised from methanol as felted needles (315mg.) m.p. 125-128°, $[\alpha]_D$+32° (c, 3.33), found C 76.68%, H 10.58%. $C_{32}H_{52}O_4$ requires C 76.75%, H 10.47%. I.R. (nujol) 1712+1250 cm.$^{-1}$ -acetate 1700 cm.$^{-1}$ 3- and 5- ketones.

3) Zeorinane triol monoacetate (30mg.) in acetic acid (20ml.) with chromium trioxide in acetic acid (5ml. .056$\bar{N}$) was allowed to stand at room temperature for 80 minutes. Dilution with water and extraction yielded the acetoxy seco-dione m.p. 122-124° undepressed on mixing with the lead tetra-acetate product.

4) Zeorinane triol monoacetate (70mg.) in pyridine (4ml.) was mixed with chromium trioxide (100mg.) in pyridine. After 1 day at room temperature the product was worked up by sulphur dioxide and extraction, crystallised from methanol as the felted needles characteristic of the acetoxy seco-dione $[\alpha]_D$+32° (c, 1.24) m.p. 124-126°, undepressed on mixing with an authentic sample.

The triol monoacetate was recovered unchanged in good yield after attempted cleavage with excess periodic acid in dioxan at room temperature for two days.

Reverse Michael reaction on zeorinane-6-acetoxy-17:21-seco-17:21-dione (208).

Diethylene glycol (20ml., twice distilled from lithium aluminium hydride) was refluxed, and a stream of nitrogen bubbled through. The evloved gas was passed into 2:4-dinitrophenyl hydrazine solution in dilute sulphuric acid, when a crystalline derivative rapidly formed. Crystallisation from methanol yielded acetaldehyde dinitrophenylhydrazone m.p. 163-165° undepressed on mixing with an authentic specimen. Addition of potassium hydroxide (1g.) to the refluxing solution did not decrease the evolution of acetaldehyde. After refluxing for 24 hrs., acetaldehyde was still being given off at approximately the same rate.

Diphenyl ether (15ml.), which had been distilled from lithium aluminium hydride, was refluxed in a stream of nitrogen. On passing the gas into a solution of dinitrophenylhydrazine, dark red needles formed at the rate of about 10 mg. per hour.

Addition of potassium hydroxide (1g.) did not retard the formation of the red compound. The same compound could be prepared in good yield by mixing an alcoholic solution of the pure diphenyl ether with aqueous dinitrophenylhydrazine reagent.

The acetoxy-seco-dione (118mg.) was added to the refluxing mixture of diphenyl ether and potassium hydroxide, when the nitrogen stream immediately gave a light yellow precipitate on passing it into the dinitrophenylhydrazine reagent. After 45 minutes, the evolution of carbonyl compound ceased. The product was extracted and chromatographed on bentonite: kieselguhr (300 mg., 1:1 by volume) in carbon tetrachloride. The dark red diphenyl ether adduct passed straight through the column. Benzene eluted methyl isopropyl ketone dinitrophenylhydrazone (14 mg., 21% of theoretical yield) m.p. 121-122°, undepressed on mixing with an authentic specimen. Found C 50.17%, H 5.35%, N 20.9%; $C_{11}H_{14}O_4N_4$ requires C 49.62%, H 5.30%, N 21.0%.

The ketone evolved from 127 mg. of the acetoxy-seco-dione was passed into semicarbazide hydrochloride solution. Extraction with chloroform and crystallisation from petrol yielded methyl isopropyl ketone semicarbazone as needles (2mg., 5% of theoretical yield) m.p. 112-113°, undepressed on mixing with authentic material m.p. 114-115°.

Diethylene glucol (15ml.) and potassium hydroxide (0.5g) were refluxed in a stream of nitrogen. The acetoxy-seco-dione (742mg.) in an open tube was dropped into the solution, and refluxing continued for 40 minutes. The solution was cooled under nitrogen, diluted and extracted with ethyl acetate giving

a red gum. Chromatography on alumina (12g. neutral deactivated) yielded no crystalline fraction.

Acetylation of the combined hydroxylic fractions (eluted with 15% benzene in carbon tetrachloride to ether, 413mg.) with acetic anhydride and pyridine for three days, followed by chromatography on alumina (15g. activity III) yielded two isomeric diacetates.

1) Carbon tetrachloride to 25% benzene in carbon tetrachloride eluted the "C_{24}-diacetate I" (cf.210) crystallised from methanol as plates (30mg.) m.p. 194-195°, $[\alpha]_D$ +83° (c, 1.24, 2 dm.). Found C 75.27%, H 10.25%; $C_{28}H_{46}O_4$ requires C 75.29%, H 10.38%. I.R. (carbon tetrachloride) 1727 cm.$^{-1}$ -acetate; no cyclohexanone or hydroxyl.

2) Benzene eluted the "C_{24}-diacetate II" (cf. 210) crystallised from methanol or petrol as needles m.p. 213-215°, $[\alpha]_D$ + 41° (c, 1.16, 2 dm.). Found C 75.55%, H 10.43%, $C_{28}H_{46}O_4$ requires C 75.29%, H 10.38%. I.R. (carbon tetrachloride) 1727 cm.$^{-1}$ -acetate; no cyclohexanone or hydroxyl.

The "C_{24}-dione" (211).

"C_{24}-diacetate II" (25mg.) in dioxan (1 ml.) with lithium aluminium hydride (40mg.) in ether (1 ml.) was heated until all the ether had been removed, then refluxed for 15 minutes. The product, worked up by extraction and washing as usual, was the non-crystalline diol.

Oxidation of the diol (25mg.) with chromium trioxide (30mg.) in pyridine (2ml.), and working up merely by addition of ether,

filtration and washing with water, yielded the "C_{24}-dione" (211) (15mg.) as rods from methanol m.p. 212-216°, $[\alpha]_D$+15° (c, 0.347, 2 dm.). Found C 80.34%, H 10.74%; $C_{24}H_{38}O_2$ requires C 80.39%, H 10.68%.

Similar treatment of "C_{24}-diacetate I" (27mg.) yielded the dione (12mg.) m.p. 211-217° undepressed on mixing with "C_{24}-dione" above. The I.R. spectra (nujol) were identical in the fingerprint region. The carbonyl region shows a single strong band at 1703 $cm.^{-1}$.

On treatment of the "C_{24}-dione" with concentrated hydrochloric acid (4 drops) and chloroform (4ml.) at the reflux temperature for 30 minutes, the starting material was recovered unchanged, m.p. 208-214°, undepressed on mixing with "C_{24}-dione".

Reduction of the "C_{24}-dione" (211).

The "C_{24}-dione" (10mg., from "C_{24}-diacetate I") in dry benzene (3ml.) and dry isopropanol (2.5ml.) was refluxed while sodium (1.0g.) was added in small pieces during 4 hours. Extraction with ethyl acetate and acetylation with acetic anhydride and pyridine for 4 days at room temperature yielded the "C_{24}-diacetate II", crystallised from methanol as needles (8mg.), $[\alpha]_D$ +37° (c, 0.314, 2 dm.), m.p. 215-217°, recrystallised at 216-220°, second m.p. 221-223°. The product was undepressed on mixing with an authentic specimen which showed the same double m.p. The I.R. spectrum was also identical with that of "C_{24}-diacetate II".

Zeorinane enone (221).

Zeorin acetate (4.168g.) was dehydrated with phosphorous oxychloride and hydrogenated as described[173], yielding impure deoxyzeorin acetate. On reduction of the ester with lithium aluminium hydride and dehydration of the secondary alcohol with toluene p-sulphonyl chloride and pyridine as before, a mixture of unsaturated hydrocarbons was obtained as a white, readily crystalline solid. (1.737g.).

Oxidation of the unsaturated hydrocarbon mixture (1.737g.) with chromium trioxide (2g.) in refluxing acetic (80ml.) for 20 minutes, followed by chromatography and crystallisation from methanol, yielded zeorinane enone, (195mg.) m.p. 186-7°, $[\alpha]_D$ -51° (c, 1.51), λ max. 239 mμ (ε 12,100), I.R. (nujol) peaks at 1650, 1610, and 1292 cm.$^{-1}$ no. -OH. Found C 85.18%, H 11.50%, $C_{30}H_{48}O$ requires C 84.84%, H 11.39%.

The first two fractions of the chromatogram yielded a minor product (35mg.) from ethanol m.p. 162-163°, $[\alpha]_D$ -42° (c, 2.09, 2 dm.). Found C 88.01%, H 12.30%; $C_{30}H_{50}$ requires C 87.73%, H 12.27% . U.-v. no absorption in the region 330-210 mμ.

Zeorinane 7-ketone.

Zeorinane enone (221) (8mg.) in dry ether (2ml.) with lithium aluminium hydride (10mg.) was allowed to stand overnight at room temperature. The product was extracted with ethyl acetate and benzene, washed quickly with dilute hydrochloric acid and with water, and the product crystallised from methanol as needles (5mg.) m.p. 155-158°. Rotation and analysis were not taken. The product has no selective absorption in the u.-v. region apart from the saturated ketone band (ε 75) at

285mμ. The I.R. spectrum in nujol shows 1700 cm.$^{-1}$ (cyclohexanone); no hydroxyl.

Zeorinane enone attempted bromination.

Zeorinane enone (221) (21.2mg.) 0.050 m-mole) in Analar acetic acid (5.00ml.) was mixed with a solution of bromine and hydrogen bromide in acetic acid (20ml. .0123 $\overline{N}Br_2$. i.e. 0.123 m.mole) and left for 36 hours at room temperature. On extraction and crystallisation from methanol, starting material was recovered in 80% yield, m.p. 187°, undepressed on mixing with zeorinane enone m.p. 187.5°. The Beilstein test showed the absence of bromine.

Zeorinane enone stability to selenium dioxide.

Zeorinane enone (221) (155mg.) in acetic acid (3ml.) with selenium dioxide (100mg.) was refluxed for 16 hrs. The product (6mg.) crystallised from methanol m.p. 186-186.5° undepressed on mixing with the starting material.

Zeorinane enone attempted borohydride reduction.

The enone (221) (24mg.) and sodium borohydride (30mg.) in dioxan:methanol (5ml., 1:1) was left over night at room temperature. The product crystallised from methanol [α]$_D$ -49° (c, 1.70) proved to be starting material m.p. 183-185°, mixed m.p. with starting material undepressed.

The ring-A-expanded keto diol monoacetate.

The seco-dione (208) (112mg.) was dissolved in warm ethanol (2ml.) and ethanolic potassium hydroxide (20ml. of 5%) added. Water (approx. 5ml.) was added with shaking so that a permanent precipitate was just avoided. The solution was seeded with the cyclised product (0.1mg. finely powdered) and allowed to stand at room temperature for four hours, when the product crystallised out as short needles (90mg.). A second crop (8mg.) of the same substance later separated. The product was crystallised from petrol m.p. 184-187°, $[\alpha]$ + 60° (c, 2.14), found C 76.94%, H 10.21%, $C_{32}H_{54}O_4$ requires C 76.75%, H 10.47%. I.R. (nujol) 3450 $cm.^{-1}$ -OH, 1727+1252 $cm.^{-1}$ -OAc, 1700 $cm.^{-1}$ cyclohexanone. Attempted cyclisation using potassium tert-butoxide at room temperature for 1 hr. and 16 hrs., and at the reflux temperature for 15 mins., 1½ hrs., and 16 hrs., led only to tar formation.

Attempted dehydration of the cyclised product with thionyl chloride in dry pyridine at 0° and at room temperature for varying lengths of time (30 mins. to 18 hrs.) yielded only the starting material or the acetoxy seco dione precursor (appropriate mixed m.p.s undepressed.)

Attempted dehydration of the cyclised product (27mg.) with phosphorous oxychloride (3 drops) in dry pyridine (3ml.) also yielded only the starting material (13mg.) m.p. 179-184° undepressed on mixing with authentic material.

Lithium aluminium hydrode reduction of the cyclised product.

The cyclised product (903mg.) was refluxed for 40 mins. with lithium aluminium hydride (1g.) in dioxan (20ml.). Extraction, acetylation (3 days at room temperature0 and chromatography on alumina (30g. activity III) yielded two products, both colourless to tetranitromethane. 1) Petrol eluted an anhydro-diacetate crystallised from methanol as thick plates (65mg.) m.p. 209-215°, $[\alpha]_D$+ 95° (c, 1.59), found C 77.19%, H 10.33%, acetyl $CO.CH_3$ 15.70%; the pentacyclic monounsaturated diacetate $C_{34}H_{54}O_4$ requires C 77.52%, H 10.33%, $COCH_3$ 16.70%; the tetracyclic monounsaturated diacetate $C_{34}H_{56}O_4$ requires C 77.22%, H 10.67%, COMe 16.69%; I.R. (nujol) 1727+1232 $cm.^{-1}$ -OAc, no -OH.

The anhydro-diacetate (17.7mg.) in acetic acid with chromium trioxide (1 ml. 1.07 $\overline{N}$. i.e. 6[O]per molecule) was heated on steam for 35 minutes as in the preparation of the ring D enone (221). The non-crystalline product was found to contain no neutral fraction; and had u.-v. λ max. 266$m\mu$ (ε1,015). 2) The benzene eluate from the column yielded a tri-acetate as needles from methanol m.p. 160-163°, $[\alpha]_D$ 46° (c, 2.107), found C 73.72%, H 10.39%, COMe 22.16%, the pentacyclic triacetate $C_{36}H_{58}O_6$ requires C 73.68%, H 9.96%, COMe 22.0%, the tetracyclic triacetate requires C 73.43%, H 10.27%, COMe 22.0%. I.R. (nujol) no -OH, 1720 1244 $cm.^{-1}$ OAc.

γ-Onocerane-3:21-diol (232, R=R'=OH).

3:21-diacetoxy-γ-onoceran-11-one (230, R=R'=Ac) (788mg.) was reduced under forcing Wolff-Kishner conditions with anhydrous hydrazine and sodium in diethylene glycol solution. The solution was kept at 180° for 3 days, and at 210° for 3 days. It was found that two days at each temperature were not sufficient. Extraction and crystallisation from chloroform petrol yielded γ-onocerane diol (620mg.) as plates m.p. 380° (sealed capillary) $[\alpha]_D$+35°, (c, 0.917, 2 dm.), found C 81.26%, H 11.58%, $C_{30}H_{52}O_2$ requires C 81.02%, H 11.79%. The u.-v. spectrum shows no selective absorption.

Acetylation of the diol (204mg.) with acetic anhydride and pyridine at 100° for 30 minutes yielded the diacetate (232, R=R'=OAc) (190mg.), crystallised as small plates from methanol, m.p. 347-348° (evac. capillary), $[\alpha]_D$+35°, (c, 0.825, 2 dm.), found C 77.29, H 10.37; $C_{34}H_{56}O_4$ requires C 77.22%, H 10.37%; I.R. (nujol) 1723+1254 cm.$^{-1}$ -acetate.

γ-Onocerane-3:21-dione (232, R=R'=O).

Oxidation of the diol (232, R=R'=OH) (143mg.) with chromium trioxide (300mg.) in pyridine (7ml.) yielded γ-onocerane-3:21-dione, crystallised as blades (86mg.) from petrol, m.p. 332-333°, $[\alpha]_D$+72° (c, 1.74), found C 81.50%, H 11.03%, $C_{30}H_{48}O_2$ requires C 81.76%, H 10.98%. I.R. (nujol) 1700 cm.$^{-1}$ cyclohexanone.

γ-Onocerane (232, R=R'=H).

Sodium (200mg.) was dissolved in diethylene glycol (10ml.), and hydrazine (2ml.) and γ-onocerane-3:21-dione (225mg.) were then added and the solution kept at 180° overnight. On diluting the cold solution the hydrocarbon readily precipitated and was filtered off. Filtration of the product in petrol through alumina (5g. activity I) followed by crystallisation from ethanol yielded γ-onocerane as rods m.p. 302-305°, $[\alpha]_D$ +40°, (c, 0.287, 2 dm.) on the second recrystallisation; m.p. 302-305°, $[\alpha]_D$ +29°, (c, 0.330, 2 dm.) (!) on the third recrystallisation. Found C 87.31%, H 12.60%, $C_{30}H_{52}$ requires C 87.30%, H 12.70%.

Half hydrolysis of 3:21-diacetoxy-γ-onocerane.

Diacetoxy-γ-onocerane (189mg.) in dioxan (50ml.) was refluxed with potassium hydroxide in 40% aqueous dioxan (6.66ml. 0.503 $\overline{N}$ i.e. 90% of the theoretical for the hydrolysis of one ester group). The product was poured into water, filtered off, and chromatographed on alumina (10g. activity I) in chloroform: carbon tetrachloride (1:9). Starting material (160mg.) was recovered (mixed m.p. and I.R.). Later fractions from the column (20% to 50% ether in chloroform) yielded impure γ-onocerane-3:21-diol monoacetate (232, R=OH, R'=OAc) (20mg.), m.p. 320° (sublimed), which was not purified. The flask was found to be corroded, accounting for the small extent of hydrolysis.

22α-Acetoxy-γ-onoceran-3-one (232, R=O, R'=OAc).

The impure γ-onocerane-3:21-diol monoacetate (20mg.) was oxidised by chromium trioxide in pyridine to the 3-acetoxy-21-ketone, needle clusters from petrol, m.p. 281-283° (sublimed), $[\alpha]_D$+48° (c, 1.11), found C 78.00%, 77.85%, H 11.15%, 11.11%, $C_{32}H_{52}O_3$, $\frac{1}{2}CH_3OH$ requires C 77.99%, H 10.86%. I.R. (nujol) 3455 cm.$^{-1}$ - methanol (?), 1721+1253 cm.$^{-1}$-acetate, 1700 cm.$^{-1}$-cyclohexanone.

γ-Onoceranol (232, R=H, R'=OH).

Reduction of the 21-acetoxy-3-ketone (15mg.) with hydrazine (1 ml.) and sodium ethoxide (300mg.) in ethanol (5ml.) at 190° for 20 hrs. in a sealed tube, and chromatography of the product yielded γ-onoceranol as plates (10mg.) from methanol, m.p. 284-292°, $[\alpha]_D$+17° (c, 0.93), found C 84.21%, H 12.20%, $C_{30}H_{52}O$ requires C 84.05%, H 12.23%.

21α-Acetoxy-γ-onocer-9(11)-en-3-one and 21α-acetoxy-γ-onocer-12-en-3-one mixture (234, R=O, R'=OAc).

21α-Acetoxy-α-onoceradien-3-one (2.014g.) in Analar acetic acid (50ml.) was heated to 100° and perchloric acid (2.0ml. 72%) added. After 8 minutes, the red solution was poured into water, extracted, and the γ-onocerin acetoxy-ketone mixture crystallised as plates (205mg.) from petrol, m.p. 284-287°. Found C 79.33%, H 10.71%; $C_{30}H_{50}O_3$ requires C 79.62%, H 10.44%.

On treating α-onocerin acetoxy ketone (2.010g.) as before, but leaving the acid solution on the steam bath for only 2 minutes,

the product was 21α-acetoxy-β-onoceradiene-3-one, (120mg.), needles from petrol m.p. 169-172°, $[\alpha]_D$ +148° (c, 1.545). Found C 79.57%, H 10.81% $C_{32}H_{50}O_3$ requires C 79.62%, H 10.44%. The tetranitromethane test gave a strong yellow colour, in contrast to the faint colour given by compounds of the γ-onocerin series.

Ozonisation of the γ-onocerin acetoxy ketone mixture (234, R=O, R'=OAc).

The mixture (106mg.) of 21α-acetoxy-γ-onocer-9(11)-en-3-one with its 12-double bond isomer in methylene dichloride (8ml.) and carbon tetrachloride (8ml.) was treated with ozone for 5 hrs. at room temperature. Chromatography of the product on alumina (2g. activity III) in benzene and elution with benzene to ether yielded the non-acidic portion (37m.g.) which crystallised as plates from methanol; m.p.s of the various fractions were about 297-310°, tetranitromethane test positive.

Ozonisation of lupanone.

Lupan-3-one (121mg.) in methylene dichloride (5ml.) and carbon tetrachloride (10ml.) was treated with ozone for 5 hours at room temperature. The product (120mg.) in benzene was filtered through alumina (2.5g. activity III). Benzene to ether eluted a total of 14 mg. of non-crystalline material. Lupanone itself is readily eluted with benzene under these conditions.

γ-Onocer-9(11)-en-3β-ol and γ-onocer-12-en-3β-ol mixture (234, R=H, R'=OH).

γ-Onocerin acetoxy ketone mixture (96mg.) with sodium ethoxide (80mg.) and hydrazine (1 ml.) in ethanol (4ml.) was left at 200° for 18 hrs. in a sealed tube. The product, a mixture of γ-onocer-9(11)-en-3β-ol and the Δ^{12} isomer, was extracted and crystallised from ethanol as small plates (71mg.) m.p. 283-284° (constant) $[\alpha]_D$ +78° (c, 1.355). Found C 83.93%, H 12.05%; $C_{30}H_{50}O$ requires C 84.44%, H 11.81%.

Acetylation of the γ-onocerenol mixture (60mg.) with acetic anhydride (4ml.) and pyridine (4ml.) at 100° for 1 hr., followed by chromatography, yielded the acetate (234, R=H, R'=OAc) as plates from petrol, m.p. 264-268°, $[\alpha]_D$ +88°, (c, 1.163). Found C 81.85%, H 11.03%; $C_{32}H_{52}O_2$ requires C 81.99%, H 11.18%. I.R. (nujol) 1723+1252 $cm.^{-1}$-acetate.

3β-Acetoxy-γ-onoceran-11-one and 3β-acetoxy-γ-onoceran-12-one mixture.

γ-Onocerenyl acetate mixture (200mg.) in Analar carbon tetrachloride (20ml.) was ozonised for 6 hrs. at room temperature. The product was filtered through alumina (5g. activity III) in benzene, and crystallised from methanol as plates, m.p. 265-285°, $[\alpha]_D$ +36° (c, 1.475), $[\alpha]_D$ +32° (c, 1.005). Found C 79.32%, H 10.83%; $C_{32}H_{52}O_3$ requires C 79.28%, H 10.81%, I.R. (nujol) 1727+1254 $cm.^{-1}$ -acetate, 1697 $cm.^{-1}$ -cyclohexanone. The tetranitromethane test is negative.

γ-Onoceranol (232, R=H, R'=OH).

The acetoxy-γ-onoceranone mixture (150mg.) was treated by the forcing Wolff-Kishner method for a total of 6 days, as described in the preparation of γ-onocerane-3:21-diol on p.140. The product crystallised from petrol as needles (45mg.) m.p. 294-298°, $[\alpha]_D$ +34° (c, 2.54); $[\alpha]_D$ +38° (c, 1.775). Found C 84.22%, H 12.15%; $C_{30}H_{52}O$ requires C 84.04%, H 12.23%, I.R. (nujol) 3350 $cm.^{-1}$-hydroxyl; no carbonyl absorption. The product did not depress on mixing with γ-onoceranol obtained by the other route via 3β-acetoxy-γ-onoceran-21-one.

Ring contraction of γ-onoceranol.

γ-Onoceranol (17mg.) in Analar benzene (5ml.) was cooled to 0° and phosphorous pentachloride (20mg.) added. After 35 mins., ice (5g.) was added and the benzene solution washed thoroughly with sodium bicarbonate solution and water as usual, but not with acid. The product in sodium-dried petrol was filtered through alumina (1g. activity I) and crystallised from ethanol as needles (9mg.) m.p. 147-154° (not recrystallised to constant m.p.).

Acid isomerisation of the ring A-contracted γ-onocerene.

The retro-pinacol product (8.8mg.) in ethanol (5ml.) with concentrated hydrochloric acid (1 ml.) was refluxed for 10 mins. Extraction and crystallisation from ethanol yielded plates (4mg.) m.p. 151-155° (constant), $[\alpha]_D$ +75° ± 10° (c, 0.148, 2 dm.).

The product (235) depressed (to m.p. 142-147°)on mixing with the starting material m.p. 147-154° above.

Hydroxylation of the hydrocarbon (235).

The hydrocarbon (10mg.) from two dehydrations and isomerisations, in dioxan (4ml.) was left in the dark for 17 days with osmium tetroxide (20mg.). Addition of lithium aluminium hydride (40mg.) in ether (0.5ml.) and extraction with ethyl acetate yielded a gum (9mg.). Chromatography of this in petrol on alumina (0.3g. activity I), and elution with ether gave blades from acetone (0.5mg.) m.p. 199-207° (recrystallised once), m.p. 205-209° (recrystallised twice), m.p. 204-207° (recrystallised a third time as needles from petrol). The product m.p. 205-209° was depressed to 194-202° on mixing with the zeorinane-3:5-diol m.p. 248-251° described on p.148. On repeating the mixed m.p. with the extremely small sample of the third recrystallisation material, m.p. 204-207°, the mixture melted at 204-228°.

α-Onoceradienone (233, R=H, R'=O).

α-Onoceradienol (2.2g.) in pyridine (30ml.) was left at room temperature overnight with chromium trioxide (2g.) in pyridine (3ml.). The product was worked up by sulphur dioxide and extraction. The ketone crystallised from petrol as needles (1.73g.) m.p. 193-195°, $[\alpha]_D$ +14° (c, 1.482). Found C 84.75%, H 11.63%; $C_{30}H_{48}O$ requires C 84.84%, H 11.39%. I.R.(nujol) 1705 cm.$^{-1}$ -cyclohexanone, 1643 cm.$^{-1}$ $C=CH_2$. The tetranitromethane test and Zimmermann test are both strongly positive.

Cyclisation of α-onoceradienone.

1) Perchloric acid (1.0ml.72%) was added to a solution of α-onoceradienone (393mg.) in Analar acetic acid (25ml.) at 100°. After 20 mins., the solution was poured into water, worked up by extraction, and crystallised from methanol as the small insoluble plates (42mg.) characteristic of the γ-series.

2) α-Onoceradienone (625mg.) in Analar benzene (5ml.) and concentrated sulphuric acid: Analar acetic acid (15.85, 50ml.) was kept at room temperature overnight as described by Barton and Overton[76]. Crystallisation from petrol yielded needles (24mg.) m.p. 166-169°, $[\alpha]_D$+182°, (c, 0.695, 2 dm.). Found C 81.77%, H 10.76%. Tetranitromethane test strongly positive. The high rotation and strong tetranitromethane colour reaction are characteristic of the β-onocerin series; the product is probably β-onoceradienone.

Since the yield is so poor, this route was not further studied.

Attempted preparation of zeorinane 17:22-diol (231).

Zeorinin acetate (219mg.) was pyrolysed at 540°/1mm. under the conditions described by Barton and de Mayo[113]. Titration of the acetic acid produced showed that 90% elimination had taken place in the first pass, and a further 7% on the second pass. Hydrogenation on Adams catalyst of the triterpenoid product (75.4mg.) in n-butanol:cyclohexane (2:1, 5ml.) containing hydrochloric acid (1 drop) showed the uptake of 1.46 ml. of hydrogen (39% of 1 double bond). A second quantity of

the pyrolysis product took up 43% of 1 double bond.

The hydrogenated mixture of hydrocarbons (267mg.) in dioxan (4ml.) with osmium tetroxide (450mg.) in dioxan (5ml.) was left in the dark for 14 days. The osmate ester was decomposed with excess lithium aluminium hydride and worked up by extraction. The product gave a strong yellow colour with tetranitromethane. Ozonisation for 30 minutes at -70° in methylene dichloride did not diminish the tetranitromethane colour, but treatment with ozone for 1½ hrs. at 25° gave a white tar, colourless with tetranitromethane. Chromatography on alumina, and elution with carbon tetrachloride to benzene gave needles from methanol m.p. 243-251° $[\alpha]_D$ -24° (c, 0.622) (constant, 2.4mg. at the fourth recrystallisation). Found C 78.31%, H 10.62%; $C_{30}H_{50}O_3$ requires C 78.55%, H 10.99%; $C_{30}H_{52}O_2$ requires C 81.02%, H 11.79%. The tetranitromethane test is negative.

The Lantadenes.

Rotations were determined in $CHCl_3$ solution. Ultraviolet absorption spectra were taken in EtOH solution with the Unicam S. P. 500 Spectrophotometer. Infra-red spectra were kindly determined by Dr. G. Eglinton and his associates. Silica gel for chromatography was obtained from Messrs. Hopkin and Williams Ltd. Light petroleum of b. p. 40-60° was used throughout unless stated to the contrary. The m.p.s of lantadene A and rehmannic acid were determined as follows: the sample was inserted at

220° and heated at about 1 degree per 3.5 sec. to 260° and then at one degree per 15 sec. until melted.

Examination of Australian "Lantana camara".

The material examined (C.S.I.R.O. sample number 5384) was worked up essentially according to the directions of Louw[210]. The powdered leaves and terminal branchlets (1.7kg.) were extracted with methanol (7 l.) at room temperature (occasional stirring) for 2 days. The green solution was filtered and concentrated *in vacuo* to 3 l. Chlorophyll was removed by stirring with charcoal (3 x 25 g.), and the resultant brown solution evaporated to dryness, on a steam bath, *in vacuo*. The residue was dissolved in hot methanol (200ml.), diluted with benzene (1.5 l.), and left for 2 days at room temperature. The benzene solution was decanted from precipitated tar (water-soluble glycosides), and evaporated to dryness, on a steam bath, *in vacuo*. Benzene (3 x 100 ml.) was added and removed in the same way to ensure that no methanol remained. The residue was taken up in benzene (200ml.) and chromatographed over silica gel (230g.). Elution with benzene-ether with proportions of ether increasing up to 100% gave 13 fractions of crystalline (from benzene or methanol) material with m.p.s ranging from 224-244° to 267-270°. The highest-melting fractions were combined and recrystallised from chloroform-methanol to constant m.p., to furnish lantadene A (prisms; 1.5g.), m.p. 282-286°, $[\alpha]_D$ +89° (c, 1.41), $\lambda\lambda$ max. 209 and 280 mμ (ε 13,900 and 130 respectively) (Found: C, 75.9; H, 9.45. $C_{35}H_{22}O_5$ requires C, 76.05; H, 9.5%). The homogeneity

of this material was established by extensive crystallisation and by further chromatography over silica gel. Lantadene A crystallises in two forms, prisms (see above) and needles. The two forms have identical physical constants and can be interconverted by seeding.

Lantadene A was undepressed on admixture with authentic lantadene A, m.p. 276-280°, provided by Dr. Louw, and with rehmannic acid, m.p. 282-287°. Since the m.p.s vary so much, it is recommended that all comparative m.p.s should be taken simultaneously. Lantadene A was, however, slightly depressed in m.p. on admixture with lantadene B. Authentic lantadene A, lantadene A as described in the present work, and rehmannic acid, all had identical infra-red spectra in Nujol.

Methylation with diazomethane gave "lantadene A methyl ester", m.p. (solvated needles from methanol) 137-139°, $[\alpha]_D$ +86° (c, 2.15) (Found: C, 75.7; H, 10.15. $C_{36}H_{54}O_5$, $\frac{1}{2}CH_4O$ requires C, 75.25; H, 9.7%). A non-solvated form, m.p. 149-150°, was obtained by crystallisation from benzene-light petroleum; further recrystallisation from methanol gave the solvated form.

In order to confirm the homogeneity of lantadene A, material (m.p. 280-282°) (256mg.) was methylated with diazomethane and chromatographed over alumina (20g.) in light petroleum. Elution with light petroleum-benzene mixtures up to 100% benzene content (19 fractions) gave only lantadene A methyl ester. Elution with 1:9 ether-benzene furnished lantadene B methyl ester (m.p. and mixed m.p.; 3mg.). Lantadene A methyl ester was undepressed in m.p. (solvated form) on admixture with methyl

rehmannate.

Lantadene A was converted into its 2:4-dinitrophenylhydrazone in the usual way. Recrystallised from methanol this had m.p. 271-272°, $[\alpha]_D$+35° (c, 1.07) (Found: C, 67.45; H, 7.55; N, 7.3. Calc. for $C_{41}H_{56}O_8N_4$: C, 67.2; H, 7.7; N, 7.65%). It was undepressed in m.p. on admixture with authentic material, m.p. 268°, $[\alpha]_D$+35° (c, 0.23), from Dr. Louw and with rehmannic acid 2:4-dinitrophenylhydrazone, m.p. 273-274°, $[\alpha]_D$+27° (c,0.34), The authentic 2:4-dinitrophenylhydrazone, that from lantadene A as described in the present work, and the corresponding rehmannic acid derivative, had identical infra-red spectra in Nujol. Lantadene B-2:4-dinitrophenylhydrazone[112] had $[\alpha]_D$48° (c,0.42).

Pyrolysis of Lantadene A Methyl Ester.

The methyl ester (89.2mg.) was pyrolysed at 530°/0.15 mm. under the conditions used by Barton and de Mayo[110]. The acid eliminated (0.87 mol.) was identified as angelic acid by m.p. and mixed m.p. (liquefied on admixture with tiglic acid). The neutral portion of the pyrolysate was chromatographed over alumina (4g.) in light petroleum. Elution with 1:1 benzene-light petroleum gave oils (46.6mg.). Elution with benzene afforded unchanged lantadene A methyl ester (m.p. and mixed m.p.). The oily fractions, on hydrogenation in "AnalR" acetic acid over platinum for 20 hr. (uptake, 2 mols. of hydrogen), and acetylation with pyridine-acetic anhydride overnight at room temperature, gave methyl oleanolate acetate (m.p., mixed m.p., and rotation). The identity was confirmed by alkaline hydrolysis

to methyl oleanolate (m.p., mixed m.p., and rotation) and by benzoylation (pyridine-benzoyl chloride over night at room temperature) to methyl oleanolate benzoate (m.p., mixed m.p., and rotation).

Hydrolysis of Lantadene A.

Lantadene A (127mg.) was refluxed for 8 hrs. with ethanolic potassium hydroxide (4%; 10ml.). Chromatography of the product over silica gel (5g.) in 1:1 benzene-light petroleum (b.p. 60-80°) gave (13 fractions), on elution with 1:1 benzene-ether, 22 β-hydroxyoleanonic acid (m.p. and mixed m.p.). The identity was confirmed by methylation to give methyl 22β -hydroxyoleanonate (m.p., mixed m.p., and rotation).

Lantadene C.

The bag of Lantana camara from which lantadene C was obtained was one of the same batch of six (C.S.I.R.O. No. 5384) from which lantadene A was extracted, as described above. The leaves and branchlets (2.4kg.) were treated exactly as described previously for the lantadene A extraction. Chromatography of the benzene soluble fraction on silica, and elution with 50% ether in benzene yielded a partly crystalline fraction (total weight 17.88g.) which on recrystallisation three times from chloroform/benzene and three times from chloroform/ethanol gave pure lantadene C (2.5g., 0.1% yield) m.p. 281-286°, $[\alpha]_D$ +93° (c, 0.43 in chloroform). Found C, 76.27%, 76.09%, 75.61%, 75.84%, H, 9.58%, 9.56%, 9.26%, 9.34%; average C, 75.95%, H, 9.46%;

$C_{35}H_{54}O_5$ requires C 75.77%, H 9.81%, $C_{35}H_{52}O_5$ requires C 76.04%, H 9.48%, $C_{30}H_{48}O_4$ requires C 76.22%, H 10.24%, $C_{30}H_{46}O_4$ requires C 76.55%, H 9.85%. I.R. (nujol) 3350+1692+1645+885 cm.$^{-1}$. U.-v. $\lambda\lambda$ max. 279mμ(ε 101)and 208mμ(ε 3,390). The Zimmermann test is negative. Lantadene C depressed in melting point on mixing with authentic lantadene A and with lantadene B.

Lantadene C methyl ester.

Lantadene C (54mg.) in dioxan (2ml.) was left for 1 hour at room temperature with excess etheral diazomethane. The solvents were removed under reduced pressure with heating, and the product crystallised from chloroform petrol to constant m.p. 197-202°, $[\alpha]_D$ +80° (c, 2.2 in chloroform). Found C 75.91%, H 10.13%; $C_{36}H_{58}O_5$ requires C 75.74%, H 10.24%, $C_{36}H_{56}O_5$ requires C 76.01%, H 9.92%, $C_{31}H_{50}O_4$ requires C 76.50%, H 10.36%.

Lantadene D.

The methanol-soluble, benzene-insoluble glycosidic fraction of the Lantana camara extract was dissolved in methanol (1 litre) and hydrochloric acid (250ml. concentrated and 750ml. of water) added. The mixture was refluxed for five hours, and on cooling was found to consist of a dark brown solution over a black granular solid (200g.). This was filtered off, extracted with chloroform, and the chloroform partly removed. Addition of methanol and further evaporation yielded a brown crystalline solid. Charcoaling and recrystallisation from chloroform/methanol yielded pure lantadene D m.p. 322-329°, $[\alpha]_D$ +106°, (c, 0.18 in chloroform). Found C 76.01%, H 9.57%. I.R. (nujol) 3300+1722+

1644+1122 cm.$^{-1}$. U.-v. - no strong absorption.

Lantadene D methyl ester.

Lantadene D (60mg.) was methylated with diazomethane as above to give the methyl ester crystallised from chloroform methanol to constant m.p. 169-170° $[\alpha]_D$+88°, (c, 1.67 in chloroform). Found C 77.00%, H 9.94%, $C_{31}H_{48}O_4$ requires C 76.81%, H 9.98%. Lantadene D methyl ester was recovered unchanged on attempted acetylation and benzoylation.

Hydrolysis of lantadene C.

Lantadene C (289mg.) was refluxed for 6 hours with ethanolic potassium hydroxide (15ml.,4%). The solution was evaporated to about 3 ml., water (15ml.) added and the solution re-evaporated to 3ml. On cooling, a white sticky solid separated, and was filtered off and washed with water. The combined filtrates were acidified with dilute sulphuric acid and distilled. The distillate was collected in 10 ml. fractions and titrated with N/100 sodium hydroxide solution using phenolphthalein indicator. Successive distillates gave the following titration results (in ml. of N/100 NaOH): 2.8, 0.75, 0.30, 0.25: total 4.10 ml. N/100. The quantity of acid expected on the basis of one volatile acid molecule per triterpenoid molecule is 55 ml. The acid evolved is therefore negligible.

In case the volatile acid had remained in the white solid described above, this was now acidified and distilled, but the first 10 ml. of distillate consumed only 0.15 ml. of N/100 sodium hydroxide. A volatile acid was therefore not hydrolysed under

these conditions, from the lantadene C molecule.

The white solid was chromatographed and crystallised from methanol to m.p. 318-324°, undepressed on mixing with lantadene D m.p. 322-327°. Methylation of the product and chromatography yielded lantadene D methyl ester m.p. 165-166°, $[\alpha]_D$ +86° (c, 0.42 in chloroform). The melting point was undepressed on mixing with authentic material.

Examination of South African "Lantana camara".

The powdered leaves and branchlets (1.52 kg.) were extracted and processed as described above. However, even repeated chromatography afforded no crystalline ketonic (to the Zimmermann reagent) fraction.

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