THESIS

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DEGREE OF DOCTOR OF PHILOSOPHY

by

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STUDIES IN THE LUPANE SERIES OF TRITERPENOIDS

A C K N O W L E D G M E N T S

The author wishes to thank Dr. John McLean for his keen interest and guidance throughout the course of this work and Dr. William Lawrie for much helpful discussion of all aspects of the work.

C O N T E N T S

INTRODUCTION

The triterpenes were originally defined as a series of naturally occurring compounds containing thirty carbon atoms per molecule and divisible into six isoprene units. While this is generally correct, a number of compounds occur which contain not thirty, but thirty-one carbon atoms, but which are clearly related to the triterpenes and may be conveniently included in the general classification.

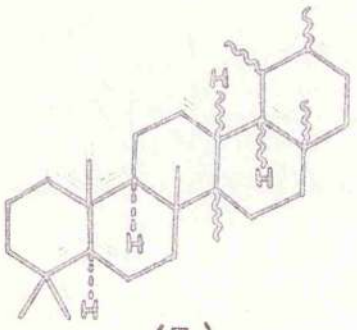
This series of compounds may be subdivided into three main groups according to the nature of their carbon skeletons....

(i) the tricyclic alcohol ambrein and the aliphatic hydrocarbon squalene, which is regarded as the biogenetical precursor of the triterpenes.

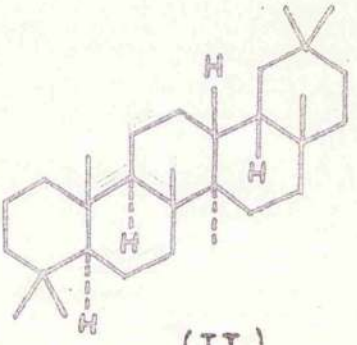
(ii) the tetracyclic triterpenes, the structures of which bear a close resemblance to the steroids.

(iii) the largest group is that of the pentacyclic triterpenes, having structures based on the four carbon skeletons of ursane (α-amyrane, I), oleanane (β-amyrane, II), lupane (III), and taraxastane (IV).

The work for this thesis is concerned solely with compounds of the lupane series of which few are known to be naturally occurring; among these are lupeol (V), betulin (VI), betulinic acid (VII), thurberogenin (VIII) and stellatogenin (IX). A new naturally occurring triterpene in this group



(I)



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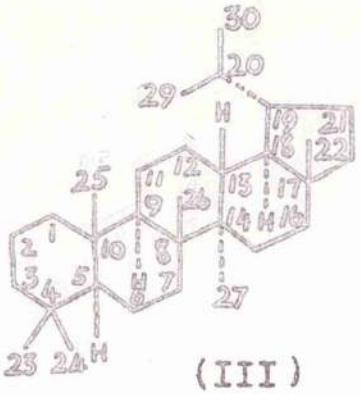

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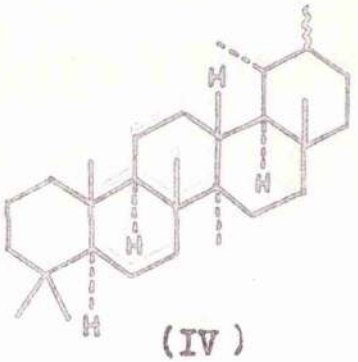

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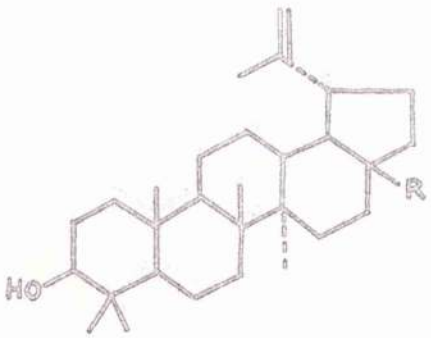
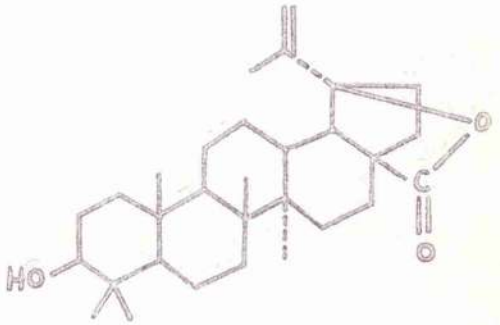


(V) R = CH_3

(VI R = CH_2OH

(VII) R = CO_2H



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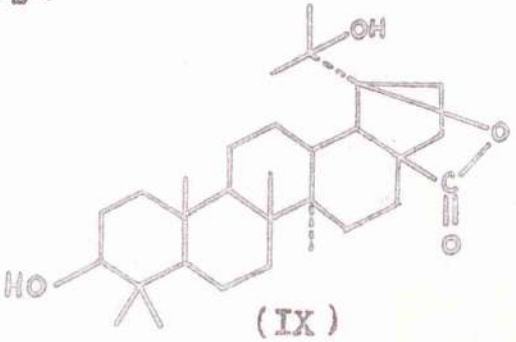


(IX)

will be described in section I of this thesis.

Some of the more important reactions involved in the structural elucidation of betulin. In 1788, Lowitz[1] observed that on heating the outer layer of the bark of the white birch tree (Betula alba) a white sublimate was obtained. This was the first recorded instance of the isolation of betulin and, over the years, various workers have attempted to assign a molecular formula to it. Vesterberg and Vesterberg[2] in 1923 reached the conclusion that it had the formula $C_{30}H_{50}O_2$ which was confirmed by Ruzicka, Brüngger and Gustus[3] in 1932 by numerous analyses of carefully dried and purified specimens.

Betulin is a very plentiful triterpene constituting up to 24 per cent of the outer bark of the Manchurian white birch (Betula alba). It is known to occur in a variety of sources, and several of its derivatives have also been shown to be naturally occurring.

The nature of the hydroxyl groups.

The presence of two hydroxyl groups was shown by the preparation of the diacetate[4,5] and several other diesters, and their unequal reactivity demonstrated by the partial hydrolysis of the diacetate to give the monoacetate.[6] This monoacetate could be oxidised with chromic acid to an

acetylaldehyde which yielded on hydrolysis the hydroxyaldehyde Further oxidation of the acetylaldehyde gave an acetylacid.[7] Both these oxidation products contained the same number of carbon atoms as the parent monoacetate and hence the more readily hydrolysed hydroxyl group in betulin is primary. When treated with sodium ethoxide the semicarbazone of the acetylaldehyde afforded lupeol, which showed that this triterpene alcohol differed from betulin only in having a methyl group in a place of a hydroxy methyl function. The acetylacid was identical to the acetate of the naturally occurring betulinic acid. Betulin monoacetate yielded an acetate phenylcarbamate which gave, on mild hydrolysis, the monophenylcarbamate. Chromic acid oxidation yielded the ketone, betulone phenylcarbamate, which on Wolff-Kishner reduction afforded desoxybetulin.[8] It follows from these reactions that the less reactive hydroxyl group is secondary.

Examination of the unsaturated centre.

The presence of at least one double bond in betulin was shown by the colouration given with tetranitromethane and the red colour displayed on application of the Liebermann-Burchard test. The existence of the ethylenic linkage was confirmed by Ruzicka, Brenner and Rey[8] who found that oxidation of betulin diacetate with perbenzoic acid gave

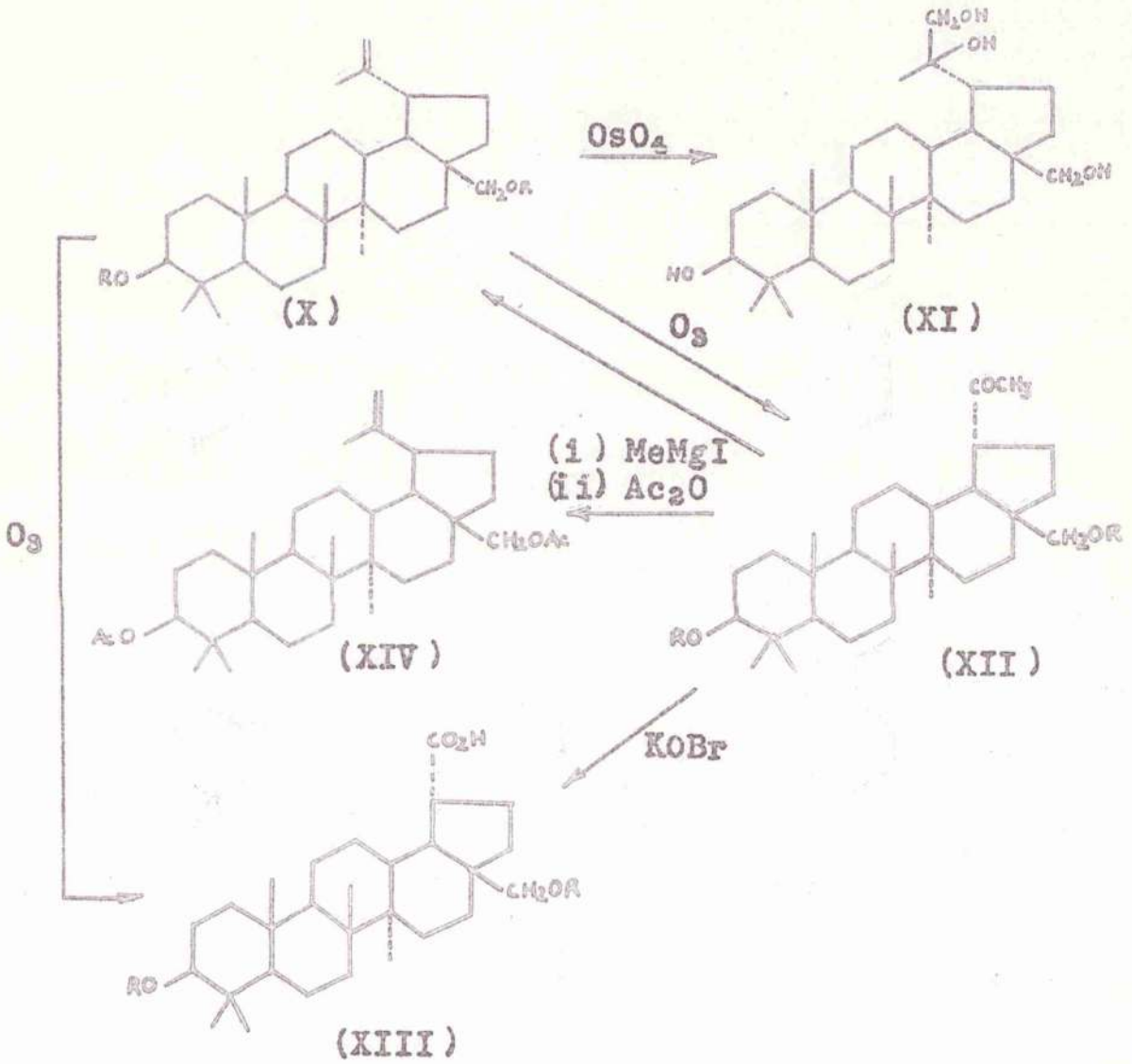
a monoxide diacetate.

Hydrogenation of the diacetate readily yielded dihydrobetulin diacetate. These two compounds were fully saturated showing the presence of one double bond and that betulin resembled the amyrins in that it consisted of a pentacyclic system.

Investigation of the carbon skeleton.

A number of dehydrogenation experiments were carried out by a variety of workers[9,10,11,12] who obtained most of the characteristic dehydrogenation products derived from the amyrins, namely 1,2,3,4-tetramethylbenzene, 2,7-dimethylnaphthalene, 1,2,7-trimethylnaphthalene, 1,5,6-trimethyl-2-hydroxynaphthalene, and 1,8-dimethylpicene. On the now accepted formula for betulin (X, R = H) it is necessary to postulate enlargement of ring E preceding the formation of products normally derived from rings D and E. It seems possible that some of the products obtained may have arisen from oleanolic acid which is known to occur with betulin in birch bark.[11]

A dihydroxydihydrobetulin (XI) was formed when betulin (X, R = H) was treated with osmic acid. Periodic acid oxidation of this tetrol afforded a dihydroxyketone (XII, R = H) containing one less carbon atom.[13] Osonolysis of betulin



diacetate (X, R = Ac) gave the diacetate of this norketone (XII, R = Ac) with formaldehyde as a by-product, thus indicating the presence of an exocyclic methylene group in betulin. The dihydroxyketone (XII, R = H) was shown to be a methyl ketone by the oxidation of its diacetate to an acid (XIII, R = Ac), which could also be obtained by direct

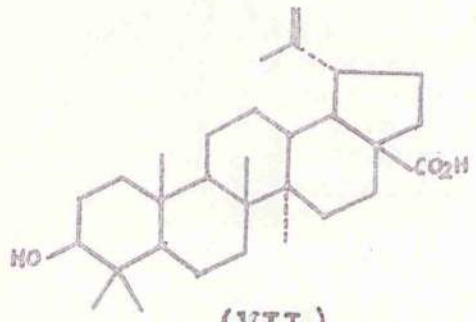
ozonolysis of betulin diacetate.[14]

These further results indicated that the double bond in betulin is present as an isopropenyl side chain, a conclusion which was confirmed by the reformation of betulin diacetate when the diacetate of the hydroxyketone (XII, R = Ac) was treated with methyl magnesium iodide and the product dehydrated with acetic anhydride. The product was accompanied by an isomer, probably the C_{19}- epimer (XIV).

Conversion of betulin to moradiol.[15,16]

Treatment of methyl betulonate (XV) with sulphuric acid gave a saturated ketolactone (XVII) which was also formed by chromic acid oxidation of the lactone (XVI, R = H) obtainable from betulinic acid (VII). Reduction of the ketolactone with lithium aluminium hydride afforded a triol(XVIII, R = H) which under mild acetylating conditions gave a diacetate (XVIII, R = Ac). The triacetate can be obtained by more vigorous acetylation. Dehydration of the triol diacetate (XVIII, R = Ac) with phosphorus oxychloride gave moradiol diacetate (XIX, R = Ac), the structure of which is well established by its relationship with oleanolic acid.

That the hydroxyl group in the triol diacetate (XVIII, R = Ac) is a hindered secondary group, and not tertiary,



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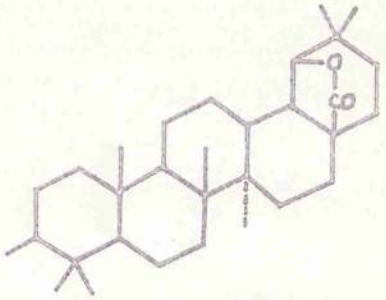
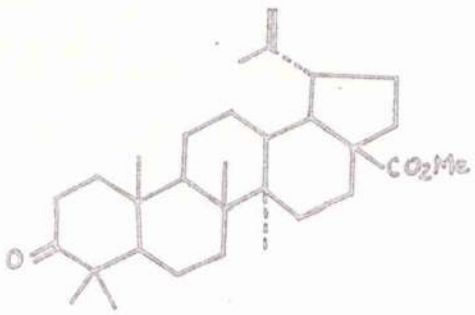


(XVI)



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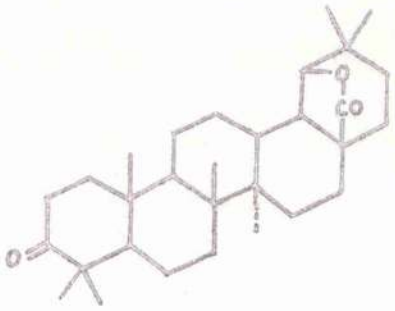

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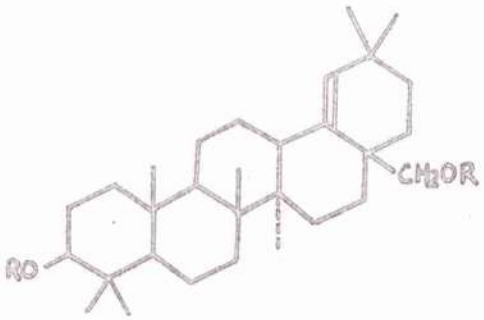

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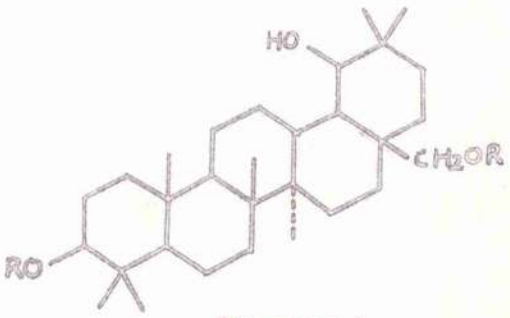

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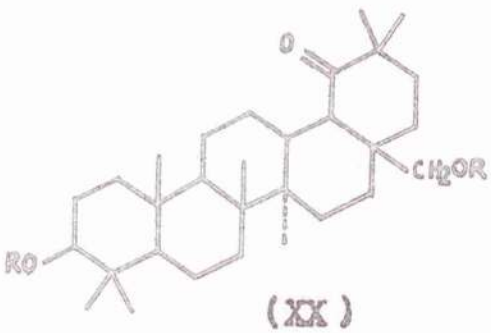
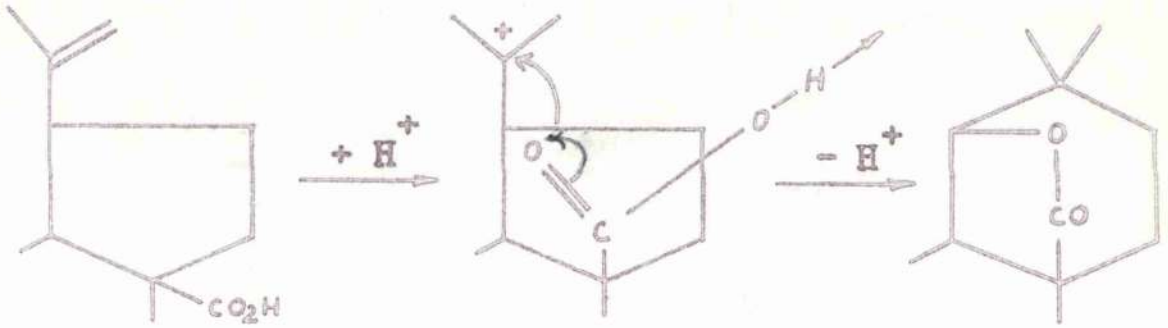


(XX)

was confirmed by the oxidation of this compound to a ketodiacetate (XX, R = Ac) which could be reconverted to the parent triol with lithium aluminium hydride.

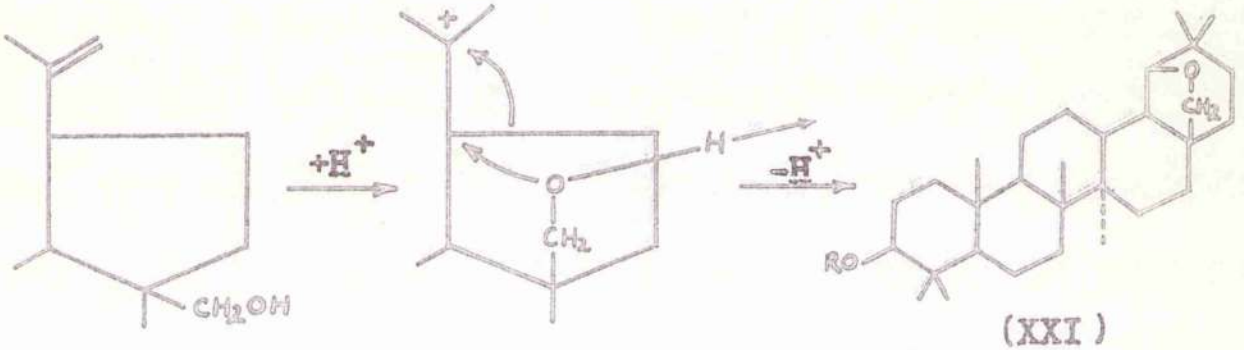
The mechanism for lactone formation under the influence of an acid reagent was represented by Davy, Halsall and Jones[18] thus:



Allobetulin.

In 1922, Schulze and Pieroh[8] attempted to prepare betulin diformate by heating betulin with 90 per cent formic acid. The product obtained contained only one formate group and on hydrolysis gave a saturated monohydric alcohol isomeric with betulin, which was named allobetulin. The isomer was also obtained by the action of hydrobromic acid in boiling chloroform,[17] and its acetate was formed when betulin was heated with amalgamated zinc in hydrochloric acid - acetic acid solution.[8] The structure assigned to allobetulin is (XXI, R = H) and the mechanism suggested for its formation

is similar to that proposed for the acid-induced lactonisation of betulinic acid.



(XXI)

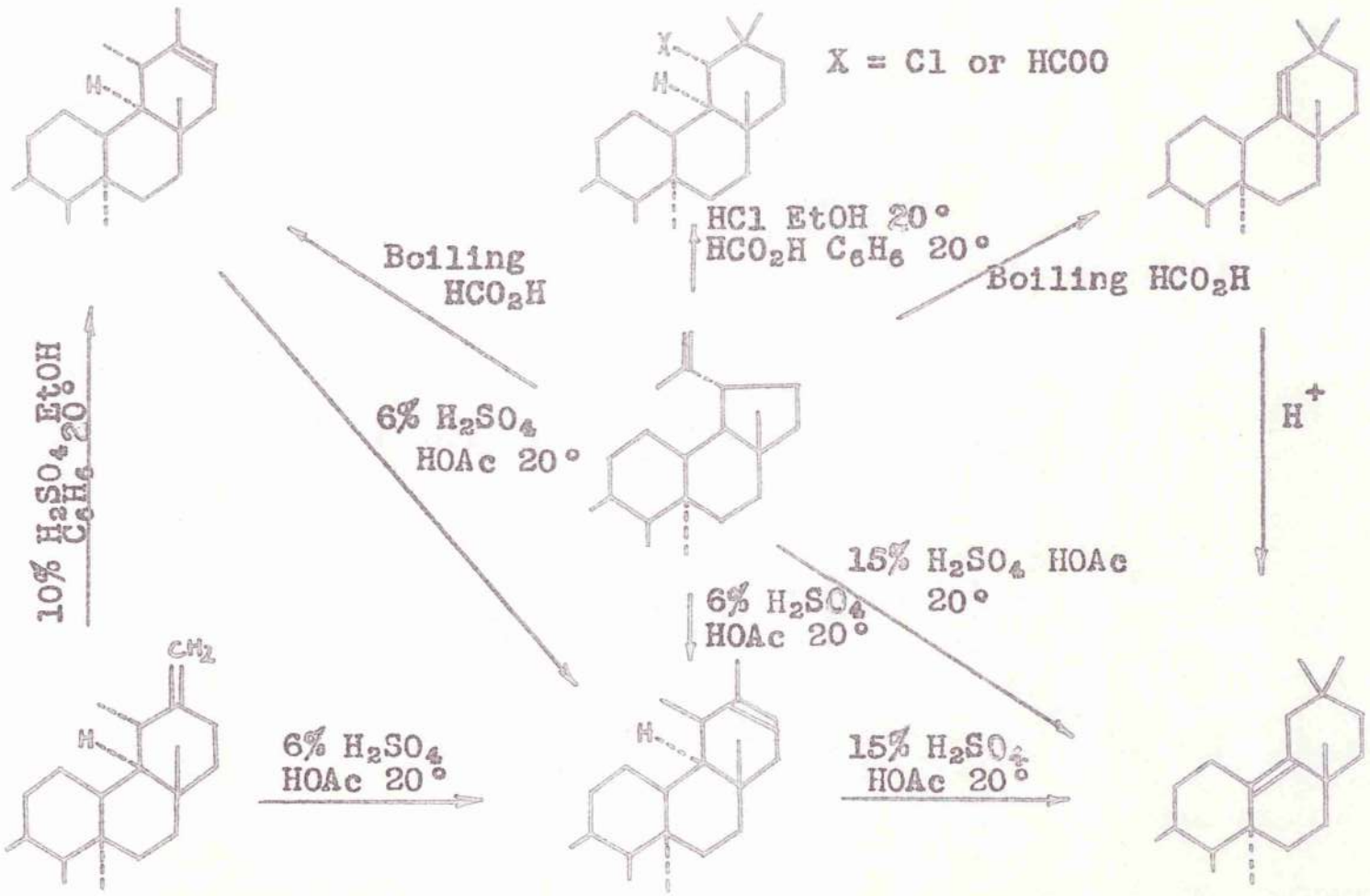
This structure was arrived at by oxidation of allobetulin acetate (XXI, R = Ac) with chromic acid, when it was found that the product (the so-called "oxyallobetulin" acetate) was the lactone to which the structure (XVI, R = Ac) had already been assigned.

Some aspects of the chemistry of lupeol.

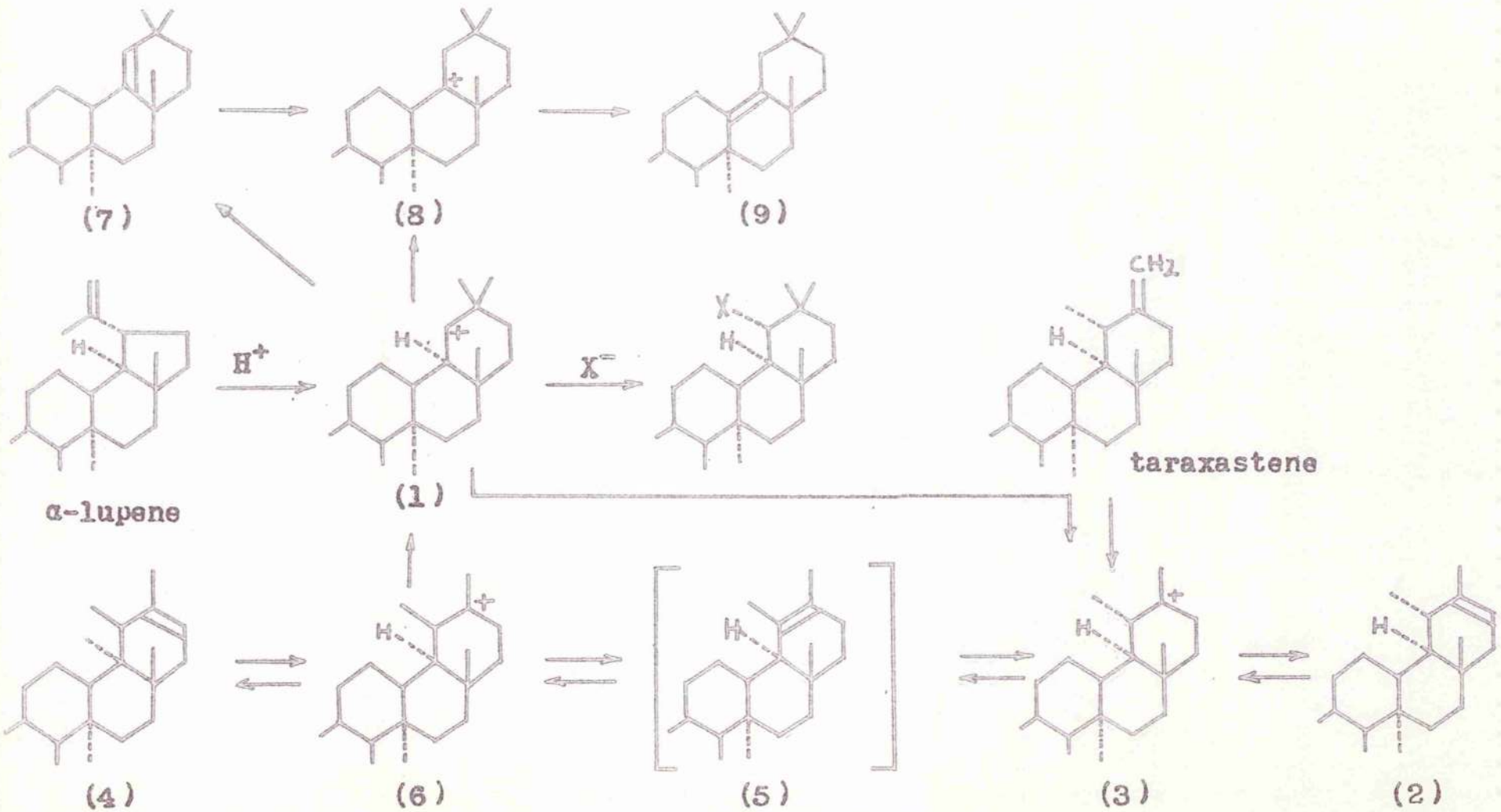
Lupeol (V) was first isolated in 1889 by Schulze and Steiger [18] from the seeds of the lupine plant (Lupinus albus). It appears to be the most widely distributed of all the triterpenes, being encountered in more plant species than the α- and β-amyrins with which it is frequently associated.

Its structure has been elucidated by a series of reactions similar to that employed for betulin, but with this principal difference; since lupeol has no hydroxyl function at C_{28}, the acid-induced rearrangements at ring E in lupeol are

S C H E M E I.


X = Cl or HCOO
HCl EtOH 20°
HCO2H C6H6 20°
Boiling HCO2H
Boiling HCO2H
6% H2SO4 HOAc 20°
H+
10% H2SO4 EtOH C6H6 20°
15% H2SO4 HOAc 20°
6% H2SO4 HOAc 20°
6% H2SO4 HOAc 20°
15% H2SO4 HOAc 20°
CH2

SCHEME II



different to those which occur with betulin. Ames, Beton, Bowers, Halsall and Jones[19] have discussed fully the mechanism of the isomerisation of lupeol and its derivatives. The compounds formed, together with the conditions required for their formation, are shown in scheme I, the suggested mechanisms being outlined in scheme II. It was suggested that the first step was the formation of the carbonium ion (1). Weakly acidic conditions, coupled with the presence of an excess of reactive anion, resulted in the formation of 18α,19α-oleanane derivatives, whilst, under the influence of stronger acid, the axial C_{20}-methyl group migrated to C_{19} giving initially a C_{19}α-(equatorial) methyl derivative. This required the formation of the ion (3) which lost a proton giving (2). Ψ-Taraxastene (2) isomerised to lupene-I (4) probably by way of an ion (3), the unstable ditertiary olefine (5), and ion (6). Lupene-I (4) derivatives had the more stable configuration and were produced unless the intermediate Ψ-taraxastane derivatives were insoluble and thus were removed from the system. Loss of a proton from ion (1) gave germanicol (7). If rearrangement of the ion (1) to the ion (8) preceded the elimination of the proton, δ-amyrin (9) derivatives were formed. The low yield of germanicol derivatives in these isomerisation reactions could be attributed to the rapid conversion of germanicol (7) into the carbonium ion (8), or

to the formation of the ion (8) directly from ion (1).

Lupeol and its derivatives may be systematically named from the parent hydrocarbon (III) (see p.2).

The cactus triterpenes thurberogenin and stellatogenin.

Djerassi and coworkers[20] have postulated structures for thurberogenin and stellatogenin, triterpenes which were isolated in 1953, from the cacti Lemaireocereus thurberi[21] and Lemaireocereus stellatus,[22] and their findings are summarised below. Dehydration of stellatogenin was shown[22] to give thurberogenin, and from infrared evidence, both were shown to contain a five-membered lactone ring.

Reactions of the double bond.

Although thurberogenin gave no colour with tetranitromethane, nor did it show typical ultraviolet absorption normally associated with a double bond, the presence of a reactive centre of unsaturation was demonstrated by several reactions.

(a) Catalytic hydrogenation of thurberogenin gave a dihydro-derivative.

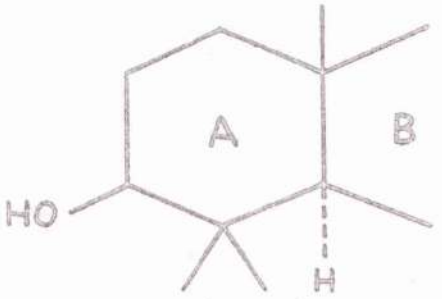
(b) Treatment with perbenzoic acid readily yielded an epoxide.

(c) Selenium dioxide converted thurberogenin acetate into an α,β-unsaturated aldehyde which, in turn, could be hydrogenated to a dihydroaldehyde.

These reactions suggest that thurberogenin belongs to the lupeol class of triterpenes, an assumption which was supported by the ozonolysis of thurberogenin to a nor-ketone with formaldehyde as a by-product.

Structure of ring A.

The structure of ring A was demonstrated to be typical of that found in other pentacyclic triterpenes by a series of standard reactions which it is not necessary to describe at this time. That is to say, ring A contains a 3β-hydroxyl group and is trans-fused to ring B (XXII).

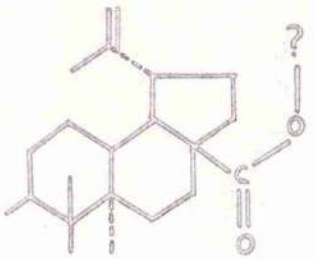


(XXII)

The five-membered lactone ring.

The presence of a γ-lactone ring was shown by the infrared absorption at 1770 $cm.^{-1}$. The ring could be opened by warming with alkali, but re-lactonisation occurred immediately on acidification or when methylation was attempted. Since stellatogenin occurs along with betulinic acid and oleanolic acid, while thurberogenin occurs with oleanolic acid, it was tentatively assumed that the carbonyl group of the

lactone originates at C_{17}, thus the two possible partial structures (XXIII) and (XXIV) may be postulated.



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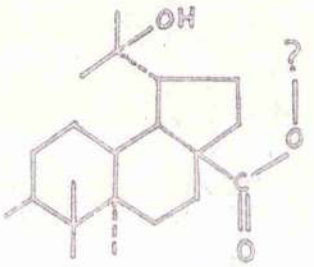
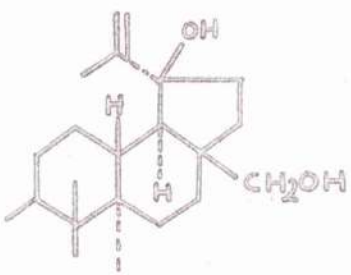


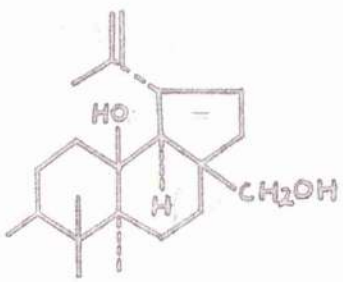
(XXIV)

On the basis of the partial structure (XXIII) for thurberogenin, only four possibilities (C_{12}, C_{15}, C_{19} and C_{21}) remained for terminal points of the lactone ring.

Reduction of thurberogenin with lithium aluminium hydride afforded the expected triol, which formed a diacetate, the remaining hydroxyl group proving stable to oxidation and thus demonstrating that it must be tertiary. The triol from thurberogenin could thus be (XXV) or (XXVa) since C_{15} and C_{21} had then been eliminated.



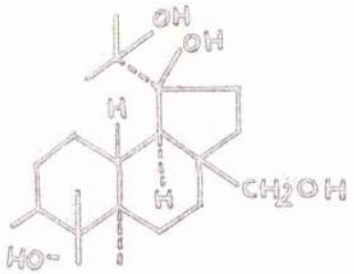
(XXV)



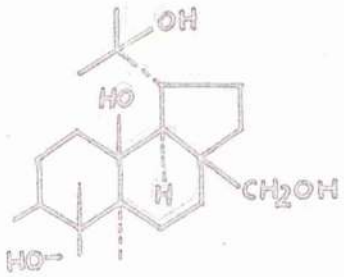
(XXVa)

The structures of thurberogenin and stellatogenin.

Reduction of stellatogenin with lithium aluminium hydride gave a tetrol (XXVI) or (XXVII), which did not react with lead tetracetate or sodium bismuthate indicating the absence of a vicinal glycol system and that structures (XXVII)



(XXVI)



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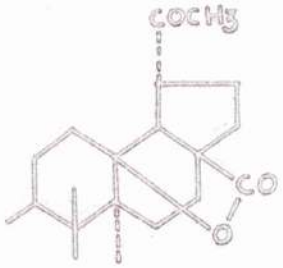

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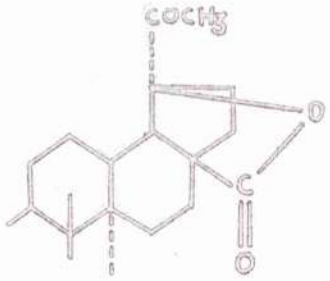

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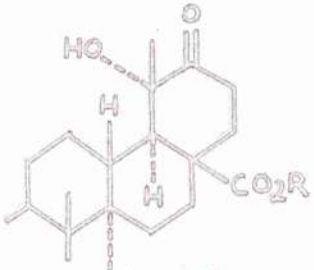
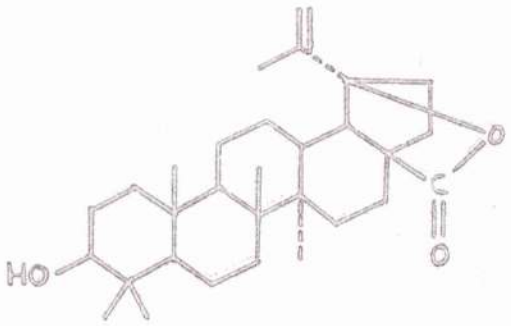


(XXX)

and (XXVa) were correct. However attention was then turned to the nor-ketone (XXVIII) or (XXIX). It was found that the nor-ketone underwent a base-catalysed rearrangement analogous to that of a 17-hydroxy-ketosteroid,[28] a reaction which would be impossible were the correct structure of the nor-ketone (XXVIII).

Djerassi and his co-workers therefore decided that the most probable structures for thurberogenin and stellatogenin were (VIII) and (IX) respectively, although no satisfactory explanation is given for the failure of the tetrol (XXVI) to react with lead tetracetate.



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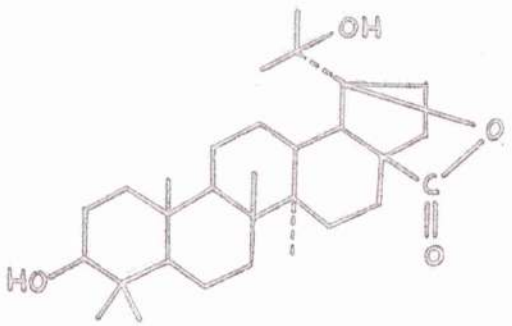


(IX)

Further evidence favouring (VIII) and (IX) as the correct structures of thurberogenin and stellatogenin will be given in section II of this thesis.

This description of some of the principle reactions involved in the structural elucidation of betulin, the ring E

enlargements of lupeol and the structural elucidation of thurberogenin and stellotogenin, will serve as a background for the chemistry involved in the researches for this thesis. For a complete account of the chemistry of lupeol and betulin, the reader's attention is directed to "The Terpenes", vol. IV, by Simonsen and Ross. A comprehensive discussion of the triterpenes as a whole, and description of the general methods employed in structural elucidation, is contained in this volume, in the reviews of Haworth,[24] Spring,[25] Noller,[26] Jeger,[27] Birch,[28] and Barton,[29] and in Elsevier's "Encyclopaedia of Organic Chemistry".

S E C T I O N I

Triterpenoids in the Bark of Mountain Ash

(Sorbus aucuparia L.)

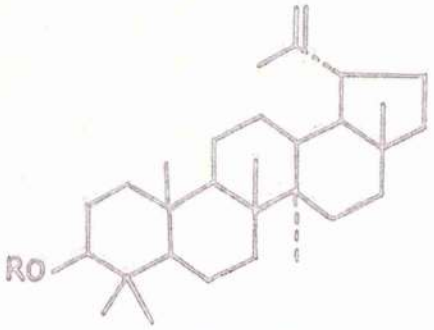
A petroleum extract of the bark of mountain ash (Sorbus aucuparia L.) has yielded lupeol and betulin. From an ether extract of the defatted bark, 23-hydroxybetulin has been isolated and its structure determined.

An examination of the bark of the mountain ash or rowan tree (Sorbus aucuparia L.) was conducted by Danoff and Zellner[30] in 1932, who reported the isolation, from the petroleum extract of the bark of a low-melting alcohol and a compound (m.p. 193°) which had triterpenoid characteristics and to which they assigned the molecular formula, $C_{38}H_{60}O$. No value was quoted for the specific rotation of this substance, which they designated sorbikortol I. Subsequent extraction of the bark with ether, followed by saponification of the extract, produced a second substance (m.p. 263°, $[\alpha]_D$ - 28.9°), again showing triterpenoid characteristics, which was named sorbikortol II. This substance was recognised to be an alcohol but crystalline derivatives could not be prepared. It was decided that further examination of these compounds would be useful.

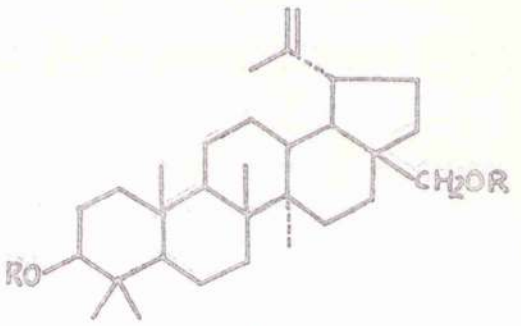
Examination of the petroleum extract.

After extracting the bark with petroleum and ether successively, a careful examination of the petroleum extract was conducted. Saponification of the fat gave a non-saponifiable mixture of alcohols which could be crystallised from ethyl acetate to give an impure alcohol (m.p. 185-194°) as orange needles. This product was further purified by crystallisation from benzene, the resultant solid being

acetylated and crystallised from chloroform-methanol to give the acetate of the commonly occurring β-sitosterol. Chromatography of this mother liquor gave lupenyl acetate (XXXI, R = Ac) and betulin diacetate (X, R = Ac), both of which were identified by the usual comparisons with authentic specimens.



(XXXI)



(X)

Chromatography of the gum which had proved soluble in ethyl acetate, gave a large quantity of a low-melting aliphatic alcohol which, although considered to be in all probability ceryl alcohol, was not carefully examined to confirm this suspicion. A further quantity of β-sitosterol was also obtained, and from the mother liquors of selected fractions, additional lupenyl acetate was isolated by acetylation and chromatography.

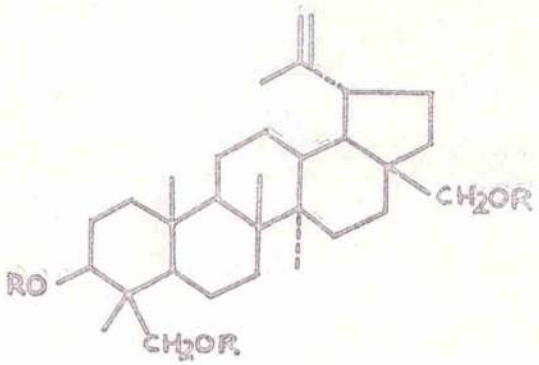
Similarly, by acetylation and chromatography of the benzene-soluble material (see above), varying quantities

of the four compounds already described, were obtained.

Since these attempts to isolate a pure compound corresponding to sorbikortol-I have proved abortive, it is now believed that the substance isolated by Danoff and Zellner[30] and so designated, is in fact a mixture consisting largely of lupeol (XXXI, R = H) and betulin (X, R = H).

The isolation and structural elucidation of 23-hydroxybetulin.

The ethereal extract of the defatted bark was then examined. The neutral, non-saponifiable material obtained from it, having proved to be only sparingly soluble in non-polar solvents, was purified by dissolving in ether containing 1 per cent of methanol and filtering through alumina, the product being acetylated, chromatographed on alumina and selected fractions hydrolysed. Repeated crystallisation of the resultant material afforded a compound, m.p. 259-260°, $[\alpha]_D$ + 25° (cf. Danoff and Zellner,[30] sorbikortol-II, m.p. 263°, $[\alpha]_D$ - 28.9°). This compound has been examined in some detail and its structure (XXXII, R = H) has been elucidated by a series of reactions which will now be described. It should be noted that, at this stage of the discussion, formulae will be given without detailed stereochemistry.

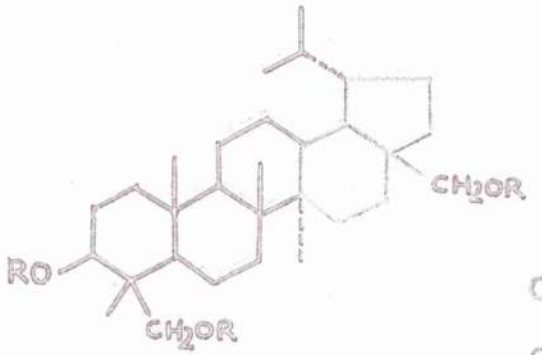


(XXXII)

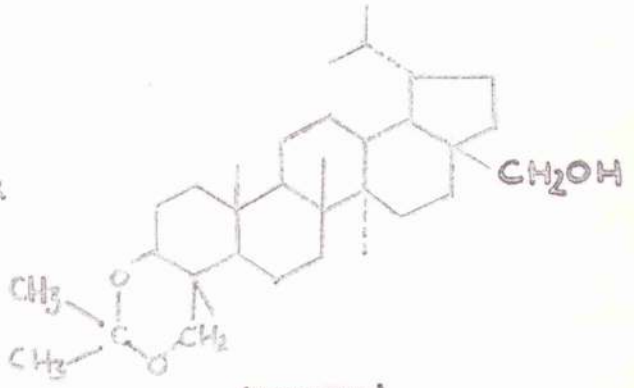
The compound gives a reaction typical of a pentacyclic triterpene on application of the Liebermann-Burchard test, and its infrared spectrum showed strong hydroxyl absorption at 3278 cm.$^{-1}$ with bands of medium intensity at 1640 cm.$^{-1}$ and 880 cm.$^{-1}$, indicating a vinylidene group. The constants of the compound resembled those of betulin[31] (X, R = H) m.p. 261°, $[\alpha]_D$ + 21°, but on admixture with betulin a substantial depression of melting point was observed. Acetylation of the compound failed to give a crystalline derivative but the resultant gum showed strong acetate absorption in the infrared at 1740 and 1248 cm.$^{-1}$, and no hydroxyl absorption. Hydrolysis of the resinous acetate regenerated the original crystalline alcohol. Attempts to form crystalline derivatives of the alcohol with benzoyl chloride and with 3,5-dinitrobenzoyl chloride were

equally unsuccessful. Analysis of the alcohol suggested the molecular formula $C_{30}H_{50}O_3$. The presence of a double bond was confirmed by the low intensity absorption at 2040 Å. in the ultraviolet region of the spectrum and also by a yellow colouration produced with tetranitromethane.

Hydrogenation of the resinous acetate gave a fully saturated dihydro-derivative which could not be crystallised but which, on hydrolysis, gave the saturated alcohol $C_{30}H_{52}O_3$ (XXXIII, R = H).



(XXXIII)

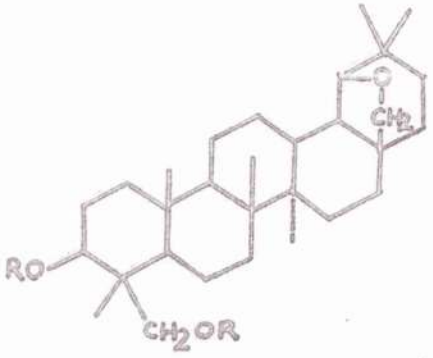


(XXXIV)

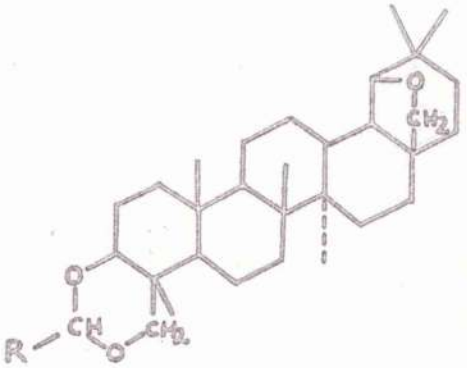
Treatment of the dihydroalcohol with acetone in the presence of mineral acid yielded a crystalline isopropylidene derivative (XXXIV) showing absorption in the infrared at 1171, 1112, 1068 and 864 $cm.^{-1}$ indicative of an isopropylidene-dioxy group,[32] and also hydroxyl absorption at 3390 $cm.^{-1}$. The dihydro-compound and its unsaturated parent must therefore contain three hydroxyl groups, two of which are in close

proximity.

Hydrochloric acid in ethanol caused isomerisation of the unsaturated triol (XXXII, R = H) to a saturated diol (XXXV, R = H) which was transparent to ultraviolet light and gave no colour with tetranitromethane. Moreover this diol formed a crystalline diacetate (XXXV, R = Ac) which did not show hydroxyl absorption in the infrared region of the spectrum. Consequently it might be concluded that protonation of the triol has resulted in a rearrangement involving the double bond and one hydroxyl group. This suggested a rearrangement of the betulin-allobetulin type (see introduction, p.10) a suspicion which was supported by the close correspondence of the infrared spectrum of the diol (XXXV, R = H) with that of allobetulin (XXI, R = H).



(XXXV)



(XXXVI)

At this stage, it was suspected that since the unsaturated triol (XXXII, R = H) had been isolated from a

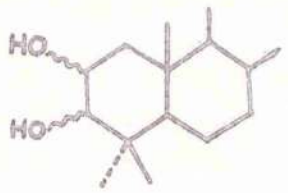
source which was already known to contain lupeol and betulin, its carbon skeleton might well be of the same type, and indeed that the unknown substance might be a hydroxy-derivative of betulin.

With a view to supporting this postulate, betulin was subjected to treatment with hydrochloric acid in ethanol according to the method which caused the isomerisation of the triol (XXXII, R = H) to the diol (XXXV, R = H), but was recovered from the reaction mixture unchanged.

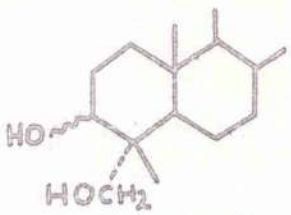
However, the similarity between the triol and betulin was further demonstrated by treatment of the triol with formic acid when a saturated diformate (XXXV, R = Fo) was obtained, which gave the diol (XXXV, R = H) on hydrolysis. Under similar conditions betulin (X, R = H) may be converted to allobetulin formate (XXI, R = Fo) and allobetulin (XXI, R = H)[8].

The saturated diol (XXXV, R = H) readily formed crystalline benzylidene (XXXVI, R = Ph) and ethylidene (XXXVI, R = Me) derivatives, but the corresponding isopropylidene derivative was less stable and tended to decompose on crystallisation. The formation of these derivatives indicates that the vicinal hydroxyl groups concerned are not involved in the allomerisation process, and if it is assumed that one of these hydroxyl groups is at C_3 as in most naturally

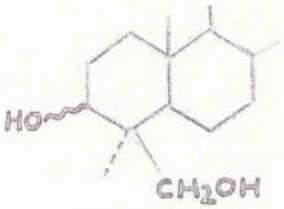
occurring triterpenes, then the other is likely to be at C_2 (XXXVII), C_{23} (XXXVIII) or C_{24} (XXXIX).



(XXXVII)



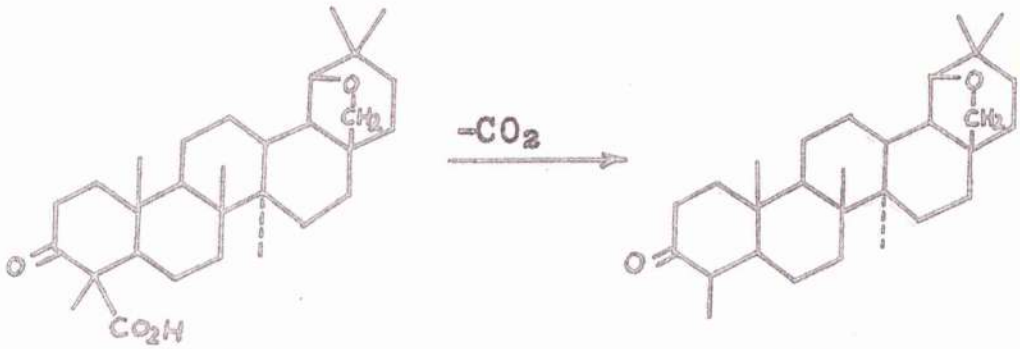
(XXXVIII)



(XXXIX)

Evidence for rejecting structure (XXXVII) was obtained when it was found that the saturated diol (XXXV, R = H) did not react with periodic acid, precluding the presence of a 1,2-glycol system.

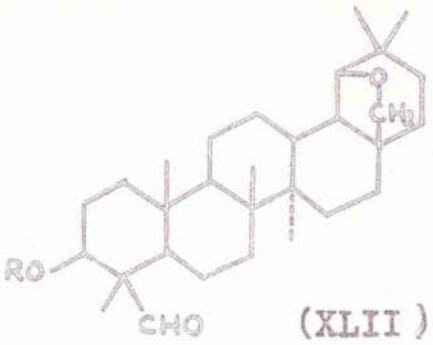
Oxidation of the diol (XXXV, R = H) with chromium trioxide in pyridine gave two crystalline products which were separated on alumina. Elution with benzene yielded a norketone (XLI) showing a strong band in the infrared at 1715 $cm.^{-1}$ which is indicative of a six-membered ring ketone.



(XL) (XLI)

Analysis indicated the molecular formula $C_{29}H_{46}O_2$, presumably the ketone having been formed *via* the unstable β-ketoacid (XL) which was not isolated.

The other oxidation product (XLII, R = H) which was eluted with ether, showed absorption bands in the infrared at



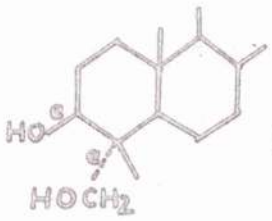
(XLII)

3340 $cm.^{-1}$ (hydroxyl), and at 1733 and 2660 $cm.^{-1}$ (aldehyde) Acetylation of the hydroxyaldehyde (XLII, R = H) furnished the corresponding acetate (XLII, R = Ac) which on reduction under Huang-Minlon[33] conditions gave allobetulin (XXI, R = H) identified by the usual comparisons with an authentic specimen and by conversion into the acetate (XXI, R = Ac) which was in turn compared with an authentic sample of allobetulin acetate.

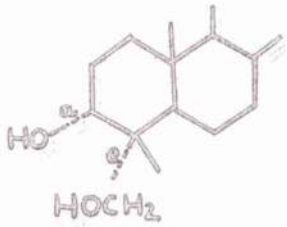
The conversion of the triol (XXXII, R = H) into allobetulin (XXI, R = H) fixes the position of two of the three hydroxyl groups, namely those at C_3 and C_{28}. No decision regarding the configuration of the C_3-hydroxyl group could be taken at this stage, since the strongly alkaline

Huang-Minlon conditions utilised to convert the aldehyde (XLII, R = Ac) into allobetulin would inevitably lead to the alcohol adopting the more stable β-configuration.[34] The third hydroxyl group is not present at C_2 for reasons already stated, but since the diol (XXXV, R = H) forms condensation products with benzaldehyde and acetaldehyde, the remaining hydroxyl group must be present at C_{23} or C_{24}. Further evidence in support of this is the formation of the aldehyde (XLII, R = H) indicating that the hydroxyl group is primary, and the ready decarboxylation of the intermediate ketoacid (XL) to the norketone (XLI).

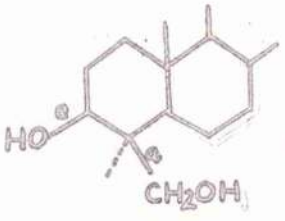
The triol may now be represented by one of the four partial structures (XLIII)-(XLVI), of which (XLVI) can be immediately rejected as in it the 3-hydroxyl group and the 4-hydroxy-methyl group are <u>trans</u> and diaxial and would not be



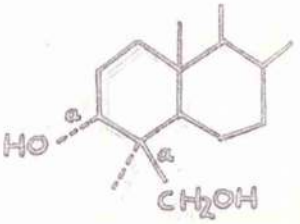
(XLIII)



(XLIV)

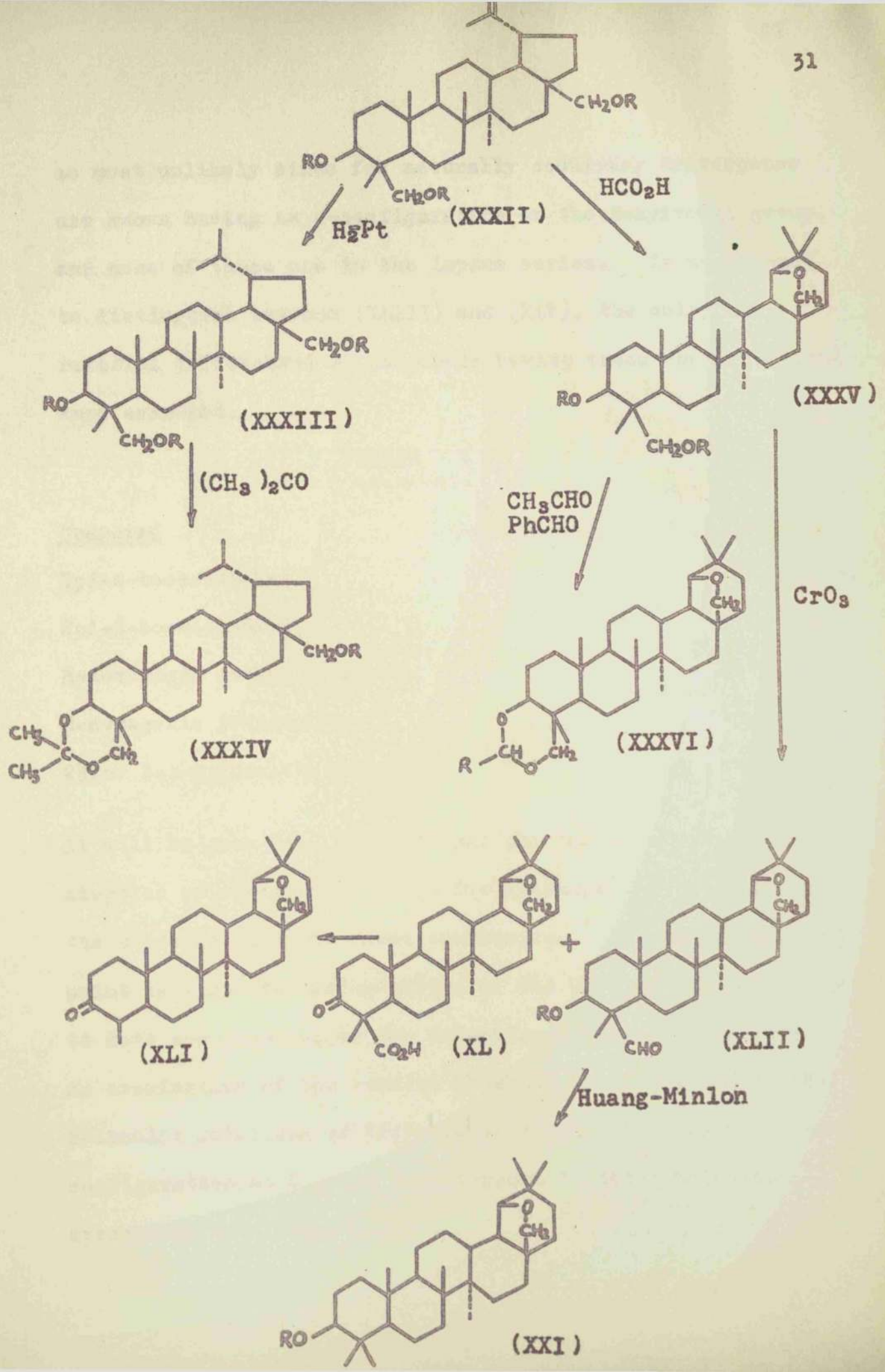


(XLV)



(XLVI)

expected to form condensation products with acetone, benzaldehyde, or acetaldehyde.[35] Although structure (XLIV) cannot as yet be categorically excluded, it may be regarded


RO
CH2OR
CH2OR
(XXXII)
H2Pt
HCO2H
(XXXIII)
(CH3)2CO
(XXXIV
(XXXV)
CH3CHO
PhCHO
CrO3
(XXXVI)
(XLI)
(XL)
CO2H
(XLII)
CHO
Huang-Minlon
(XXI)

as most unlikely since few naturally occurring triterpenes are known having an α-configuration of the 3-hydroxyl group, and none of these are in the lupane series. In an attempt to distinguish between (XLIII) and (XLV), the molecular rotation differences of compounds having these configurations were examined.

TABLE I

Compound	Configuration	M_D Diol	M_D Diacetate	
Epi-α-boswelladiol	XLV	398	367	-31
Epi-β-boswelladiol	XLV	451	386	-65
Hederagenin methyl ester[37]	XLIII	380	428	+48
Hederagenin 18α-lactone	XLIII	78	189	+111
23(or 24)-hydroxyallobetulin	?	211	371	+160

It will be seen from Table I that the results favour the adoption of structure (XLIII) for hydroxyallobetulin although the evidence is by no means conclusive. Another significant point is that the configuration of the C_{18}-hydrogen atom appears to have some bearing on the molecular rotation difference. An examination of the results obtained by comparison of the molecular rotations of triterpenes which differ only in their configuration at C_{18} has lent support to the conclusions arrived at thus far.

TABLE II

Compound	M_D Alcohol	Acetate	
β-amyranol	78	99	+ 21
18α-β-amyranol	154	207	+ 53
β-amyrin	379	399	+ 20
18α-β-amyrin	193	248	+ 55

From Table II it can be observed that the apparent effect of the 18α-hydrogen atom is to accentuate the change in molecular rotation due to acetylation. However, insufficient data is available to allow adequate testing of these conclusions, and no compound is known which has the configuration (XLIV) in ring A and an 18α-hydrogen atom, for comparison purposes.

Comparison of infrared data from diols of known configuration in ring A has lent support to the belief that structure (XLIII) is correct. Cole and Müller[36] have studied the infrared absorption characteristics of a number of these 3-hydroxy-4-hydroxymethyl triterpenoids in the lower (2.5-3 u) region of the spectrum and they have demonstrated that diols having diaxial groups [compounds of type (XLVI)] do not show hydrogen bonding, whereas compounds in which the groups are both equatorial, (XLII), or in which one group is axial and the other equatorial, (XLIV and XLV),

show evidence of intramolecular hydrogen bonding, the bonds being of varying strength and location according to the stereochemistry of the groups involved.

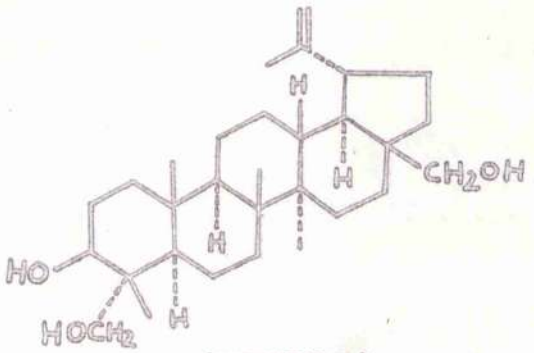
The measurements carried out in our laboratories were on samples dissolved in carbon tetrachloride, using a calcium fluoride prism.

TABLE III

Compound	Configuration	Free OH	Bonded OH
Urs-12-en-3α,24 diol	XLVI	3738 $cm.^{-1}$	-
Olean-12-en-3α,24 diol	XLVI	3738	-
Olean-12-en-3β,24 diol	XLV	3738	3575 (weak)
Methyl hederagenin	XLIII	3738	3603
Hydroxyallobetulin	?	3738	3603

The results shown in Table III confirm that the 3α,24-diols (diaxial, XLVI) show no absorption band due to hydrogen bonding; a 3β,24-diol (equatorial: axial, XLV) shows a very weak band at 3575 $cm.^{-1}$; whereas a 3β,23-diol (diequatorial, XLIII) shows a band due to hydrogen bonding at 3603 $cm.^{-1}$ which has an intensity similar to that of the free hydroxyl band. Once again, a sample of a 3α,23-diol (XLIV) was not available, but as this is an axial:equatorial diol, its hydrogen bonding characteristics would be expected to resemble those of a diol

of type (XLV, equatorial:axial). The striking similarity of the absorption characteristics in this region of the spectrum of hydroxyallobetulin and methyl hederagenin (XLIII), together with the molecular rotation evidence, leads to the conclusion that the former is also a 3β,23-diol, the parent triol is therefore 23-hydroxybetulin (XLVII).



(XLVII)

While the melting points of sorbikortol-II and 23-hydroxybetulin are in agreement, and the compounds resemble each other in that neither forms a crystalline acetate or benzoate, the rotation quoted by Danoff and Zellner, ^{30} $[\alpha]_D$ -28.9° is of the same magnitude as that given by 23-hydroxybetulin, $[\alpha]_D$ + 24.8°, but of opposite sign. It may therefore be that sorbikortol-II is identical to 23-hydroxybetulin and that the earlier workers misquoted the sign of the rotation.

Further examination of the ether extract from the mountain ash bark yielded no further triterpenoid material.

EXPERIMENTAL

Rotations were determined in chloroform at room temperature unless otherwise stated. Ultraviolet spectra were determined in ethanol. Infrared spectra were determined with a Grubb-Parsons double beam spectrophotometer using nujol mull unless otherwise stated. Light petroleum refers to the fraction of b.p. 60-80°.

Light Petroleum Extraction of Bark. - Dry crushed bark (10 lb.) of mountain ash was extracted continuously with light petroleum for 15 hr. and the extract (100 g.) was saponified by refluxing with benzene (250 ml.) and potassium hydroxide (60 g.) in methanol (350 ml.). Working up in the usual way through ether gave the non-saponifiable matter (80 g.), a portion of which (10 g.) was chromatographed on alumina (400 g.) from light petroleum--benzene (1:1). Development of the column was conducted through benzene, benzene-ether, ether, ether-methanol, 80 fractions (each 150 ml.) being collected but no appreciable elution occurred until ether was used as eluting solvent.

Lupenyl Acetate. - Fraction 25-27, eluted with ether, were acetylated in pyridine-acetic anhydride on the

steam bath (1 hr.) and worked up through ether. Three crystallisations of the product from chloroform-methanol gave lupenyl acetate (1 g.), needles, m.p. 216°, $[\alpha]_D$ + 43.2° identified by mixed m.p. and infrared comparison with an authentic specimen. The mother liquors yielded a further quantity (0.7 g.) of lupenyl acetate.

Ceryl Alcohol. - Fractions 28-30, eluted with ether gave an aliphatic alcohol, m.p. 72-73°, probably ceryl alcohol.

β-Sitosteryl Acetate. - Fractions 31-33, eluted with ether, were evaporated and the residue crystallised from ethanol. The first crop (0.09 g.) consisted of ceryl alcohol and the mother liquor on standing deposited impure β-sitosterol (0.8 g.), m.p. 120-130°. Acetylation in pyridine-acetic anhydride and working up as usual gave β-sitosteryl acetate (0.8 g.) as blades from chloroform-methanol, m.p. and mixed m.p. 127-128°, $[\alpha]_D$ - 36°. Infrared comparison with an authentic sample confirmed identity.

Betulin Diacetate. - Fractions 34-50, eluted with ether containing 0.5% methanol, gave first crops of material melting in the range 130-138°. These were bulked and recrystallised when an additional crop of β-sitosterol (0.9 g.) was obtained. The mother liquor was taken to dryness (vacuum) and the residue (1.1 g.) acetylated with pyridine-acetic anhydride. Working up through ether gave crude acetates

(1.2 g.) which were chromatographed on alumina (30 g.) from benzene-light petroleum (1:2). Elution with benzene-petroleum (2:1) gave betulin diacetate (0.4 g.) as needles from chloroform-methanol, m.p. 223-224°, $[\alpha]_D$ + 22°.

Examination of Non-Saponifiable Matter using Zellner's Procedure[30]. - The non-saponifiable fraction (70 g.) was dissolved in ethyl acetate (200 ml.) and the solution was left aside for four days. The crystalline deposit (15.1 g.) which separated had m.p. 185-194° (Zellner[30] records m.p. 194° for Sorbikortol I). Crystallisation of the solid (15.1 g.) from benzene (100 ml.) gave material (7 g.) m.p. 100-130° which was acetylated in the usual manner and chromatographed on alumina to give β-sitosteryl acetate (1.4 g.), m.p. and mixed m.p. 127-128°, $[\alpha]_D$ - 35°. The benzene mother liquors were evaporated under vacuum to give an oil (5.5 g.) which was chromatographed on alumina (150 g.). Elution with benzene-light petroleum (1:3) gave lupenyl acetate (0.4 g.), m.p. 216°, $[\alpha]_D$ + 44°, identified as above. Continued elution with the same solvent mixture gave betulin diacetate (0.93 g.), m.p. 223-224°, $[\alpha]_D$ + 23° identified as above.

The non-saponifiable fraction which was soluble in ethyl acetate was recovered by evaporation and a portion (30 g.)

in benzene-light petroleum (2:1) was chromatographed on alumina (1 kg.). Elution with benzene (2 l.), benzene-ether mixtures (8 l.) and ether (9 l.) produced only intractable material (5.2 g.). Elution with ether and methanol (1%; 1.2 l.) afforded a fraction (12.3 g.) which crystallised from chloroform-methanol to give ceryl alcohol (7.5 g.), m.p. 74-75°. Further elution with ether-methanol (1%; 800 ml.) and crystallisation of the eluted material (3.8 g.) from chloroform-methanol gave β-sitosterol, as plates, m.p. and mixed m.p. 134-135°, $[\alpha]_D$ - 34°. The mother liquors from which β-sitosterol had separated were combined with those from the crystallisation of ceryl alcohol (above) and the solution was taken to dryness. The residue (6.2 g.) was treated with acetic anhydride in pyridine, and a solution of the dry acetylated product in benzene-light petroleum (1:4), was chromatographed on alumina (180 g.). Elution with the same solvent gave lupenyl acetate (1.4 g.) as needles (from chloroform-methanol) m.p. and mixed m.p. 215-216°, $[\alpha]_D$ + 41°.

Ether Extraction of Bark. - Mountain ash bark (4 lb.) which had been defatted with light petroleum was extracted continuously with ether for 24 hr. Removal of the solvent left an extract (80 g.) which was hydrolysed by refluxing with methanolic potassium hydroxide (6%; 1.5 l.). Working

up through ether gave the non-saponifiable material (14 g.).

23-Hydroxybetulin. - The non-saponifiable matter (7.3 g.) was dissolved in ether-methanol (1%) and chromatographed on alumina (200 g.). Elution with ether-methanol (1.2 l.) yielded fractions (total wt. 2.4 g.) which were bulked and crystallised from ethyl acetate-methanol to give needles, m.p. 246-258°, $[\alpha]_D$ + 22.1°, showing strong hydroxyl absorption in the infrared (3278 $cm.^{-1}$). Acetylation of the alcohol in pyridine-acetic anhydride on the steam bath (1 hr.) and working up through ether gave a resinous acetate in which the hydroxyl absorption band had been replaced by acetate bands at 1740 and 1248 $cm.^{-1}$. The resinous acetate (0.7 g.) in light petroleum was filtered through a short column of alumina, taken to dryness and hydrolysed with methanolic potassium hydroxide (100 ml.) for 2.5 hr. Working up through ether and crystallisation from chloroform-light petroleum gave 23-hydroxybetulin as needles, m.p. 259-260°, $[\alpha]_D$ + 24.6°, showing infrared absorption bands at 3278 $cm.^{-1}$ (hydroxyl) and 1642 and 880 $cm.^{-1}$ (vinylidene). (Found: C,78.7; H,11.1. $C_{30}H_{50}O_3$ requires C,78.6; H,11.0%).

20,30-Dihydro-23-hydroxybetulin. - The resinous acetate (0.54 g.), obtained from 23-hydroxybetulin, was hydrogenated in ether (150 ml.) containing acetic acid (25 ml.), in the presence of platinum oxide (0.4 g.). The resulting

dihydro-acetate failed to crystallise. The resinous product was boiled under reflux for 1 hr. with methanolic potassium hydroxide (5%) and the dihydro-alcohol was isolated by means of ether. Filtration of the dried ethereal solution through a short column of alumina and crystallisation of the product from methanol-light petroleum afforded 20,30-dihydro-23-hydroxybetulin as needles, m.p. 257-260°, $[\alpha]_D$ - 18.4° (ethanol-chloroform) (Found: C, 78.7; H, 11.5. $C_{30}H_{52}O_3$ requires C, 78.2; H, 11.4%). There was no absorption in the ultraviolet.

Isopropylidene derivative, from acetone and concentrated hydrochloric acid (4 drops). Crystallised from aqueous acetone, decompeses at 147°, $[\alpha]_D$ - 19.6° $\nu_{max.}$ 1171, 1112, 1068 and 864 $cm.^{-1}$ (isopropylidenedioxy) (Found: C, 75.4; H, 10.95. $C_{33}H_{56}O_3 . 2(CH_3)_2CO$ requires C, 75.9; H, 11.1%).

23-Hydroxyallobetulin. - (a) 23-Hydroxybetulin (0.33 g.) was refluxed in ethanol (85 ml.) and concentrated hydrochloric acid (15 ml.) for 5 hr. Isolation of the product through ether gave 23-hydroxyallobetulin which was crystallised from methylene chloride-acetone as plates, m.p. 252-253°, $[\alpha]_D$ + 44.3° (Found: C, 76.4; H, 10.7. $C_{30}H_{50}O_3 . (CH_3)_2CO$ requires C, 76.7; H, 10.9%).

The benzylidene derivative was prepared by treatment of the diol (0.1 g.) with benzaldehyde (15 ml.) and concentrated

sulphuric acid (10 drops) at room temperature overnight. Treatment with sodium carbonate, followed by extraction with ether and evaporation under vacuum left an oil which was dissolved in light petroleum-benzene and chromatographed on alumina (20 g.). Elution with the same solvent gave the benzylidene derivative as needles (from aqueous acetone) m.p. 230° (decomp.), $[\alpha]_D$ + 24° (Found: C,81.3; H,10.05. $C_{37}H_{54}O_3$ requires C,81.35; H,9.95%).

The ethylidene derivative prepared as above using acetaldehyde, crystallised from aqueous acetone as plates, m.p. 217-222°, $[\alpha]_D$ + 48° (Found: C,79.2; H,11.1. $C_{32}H_{52}O_3$ requires C,79.3; H,10.8%).

23-Hydroxyallobetulin (0.16 g.) in ethanol (25 ml.) was recovered quantitatively after treatment for 24 hr. at room temperature with periodic acid (15%; 1 ml.). Acetylation of the diol with acetic anhydride in pyridine at 100° for 1 hr. followed by purification on alumina gave the diacetate as needles, (from chloroform-methanol), m.p. 211-212°, $[\alpha]_D$ + 68.5°. $\nu_{max.}$ 1740 and 1248 cm.$^{-1}$ (acetate) (Found: C,75.1; H,10.0. $C_{34}H_{54}O_3$ requires C,75.2; H,10.0%).

(b) 23-Hydroxybetulin (0.19 g.) was boiled under reflux (1 hr.) with formic acid (98%; 20 ml.). Isolation through ether and crystallisation from aqueous acetone gave

23-formyloxy-allobetulin formate as needles, m.p. 217-218°, $[\alpha]_D$ + 62° (Found: C,75.1; H,10.0. $C_{32}H_{50}O_5$ requires C,74.7; H,9.8%). Hydrolysis of the diformate (0.3 g.) with methanolic potassium hydroxide (5%; 100 ml.) at 100° for 1 hr. gave 23-hydroxy-allobetulin as plates from methylene chloride-acetone, m.p. and mixed m.p. with specimen prepared as in (a) above, 252-253°, $[\alpha]_D$ + 44°.

Oxidation of 23-Hydroxyallobetulin. - A solution of 23-hydroxyallobetulin (0.8 g.) in pyridine (50 ml.) was added to a solution of chromium trioxide (1.06 g.) in pyridine (60 ml.) and left overnight. The mixture was poured into potassium hydroxide (2N., 200 ml.), extracted with ether and worked up in the usual way to give a gum (0.7 g.) which was dissolved in benzene and chromatographed on alumina (15 g.). Benzene eluted crystalline material (0.2 g.) which after two recrystallisations from methylene chloride-light petroleum afforded 23-norallobetulone, m.p. 214-215°, $[\alpha]_D$ + 84°. $\nu_{max.}$ 1715 $cm.^{-1}$ (carbonyl) (Found: C,81.2; H,11.2. $C_{29}H_{46}O_2$ requires C,81.6; H,10.9%). Subsequent elution of the column with ether and crystallisation of the resinous eluate (0.4 g.) from chloroform-light petroleum gave 23-oxoallobetulin, (rosettes), m.p. 243-244°, $[\alpha]_D$ + 56.5°. $\nu_{max.}$ 3340 $cm.^{-1}$ (hydroxyl) and 1738 and 2660 $cm.^{-1}$ (aldehyde) (Found: C,78.8; H,10.7. $C_{30}H_{48}O_3$ requires C,78.9; H,10.6%). Acetylation of 23-oxo-

allobetulin (0.17 g.) in pyridine (10 ml.) with acetic anhydride (2 ml.) at 100° for 1 hr. and working up through ether gave 23-oxoallobetulin acetate, from chloroform-methanol, plates, m.p. 252-253°, $[\alpha]_D$ + 62.7°). ν_{max} 1734, 1245 cm.$^{-1}$ (acetate) and 2800, 1718 (aldehyde) (Found: C,76.8; H,9.8. $C_{32}H_{50}O_4$ requires C,77.1; H,10.1%).

Conversion of 23-Oxoallobetulin Acetate to Allobetulin.- 23-Oxoallobetulin acetate (0.65 g.) in diethylene glycol (80ml.) and hydrazine hydrate (100%, 6 ml.) was heated under reflux for 1 hr. Potassium hydroxide (13 g.) in water (20 ml.) was added to the cooled solution which was again refluxed for 0.5 hr. and then distilled until the vapour temperature reached 220°. The solution was again boiled under reflux (2.5 hr.), cooled, poured into water and acidified with 5N.HCl. Isolation of the product through ether afforded a dark red oil (0.6 g.) which was chromatographed on alumina (15 g.). Elution with ether and crystallisation of the product from chloroform-petrol gave allobetulin as needles, m.p. and mixed m.p. 267-268°, $[\alpha]_D$ + 49°. Acetylation in pyridine-acetic anhydride gave allobetulin acetate, crystallised from chloroform-ethanol as plates m.p. and mixed m.p. 288-290°, $[\alpha]_D$ + 58°. Infrared comparisons of the synthetic allobetulin and its acetate with authentic specimens of these showed complete identity.

S E C T I O N II.

The Dehydrogenation of Betulin and its Diacetate with Mercuric Acetate.

The dehydrogenation of betulin and its diacetate with mercuric acetate has yielded compounds which have been shown to be 3β-hydroxy-13β,28-epoxylup-20(30)-ene and lupa-12,20(30)-dien-3β,28-diol diacetate respectively.

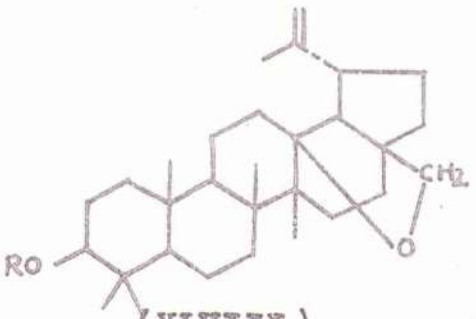
In 1942, Biedebach[38] investigated the dehydrogenation effect of mercuric acetate on a number of triterpenes. He observed that whereas lupeol, lupenyl acetate, lupenyl benzoate, betulin and betulin diacetate all reacted with mercuric acetate, α-amyrin, β-amyrin, lupanyl acetate, allobetulin, etc., did not react. Of the reactions performed he only characterised the product from lupeol, which he referred to as dehydrolupeol.

While the author has been investigating the dehydrogenation of betulin and betulin diacetate, another worker in these laboratories, J. M. Allison, has been concerned with the analogous reactions of lupeol, lupenyl acetate, betulinic acid and its methyl ester. Frequent reference will be made to this work in the course of this discussion, and for greater detail of his researches, the reader is referred to the thesis at present in preparation by Allison.

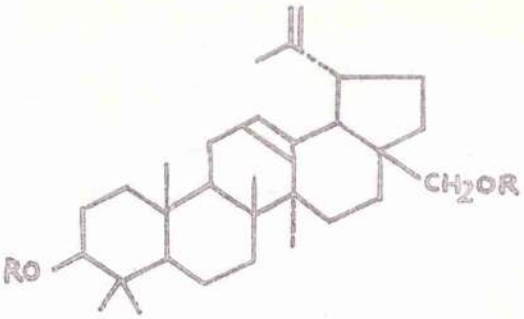
Betulin (X, R = H) and betulin diacetate (X, R = Ac) were treated with a large excess of mercuric acetate in chloroform-acetic acid solution and the resultant mercury complexes were destroyed, initially with hydrogen sulphide, and in the later preparations with hydrazine hydrate in alkaline ethanol, the method employed by Henbest and Nicholls.[39] Acetylation of the product obtained from betulin gave an

acetate which proved to be different from the acetate resulting from dehydrogenation of betulin diacetate. These compounds will be shown to have the structures (XLVIII, R = H) and (XLIX, R = Ac) respectively.

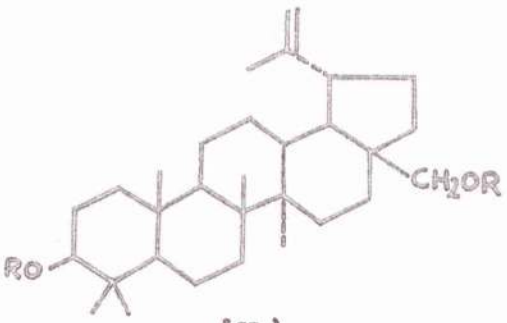
Acetylation of the product from betulin (XLVIII, R = H) gave the acetate (XLVIII, R = Ac) which showed no hydroxyl absorption in the infrared spectrum, and the compound



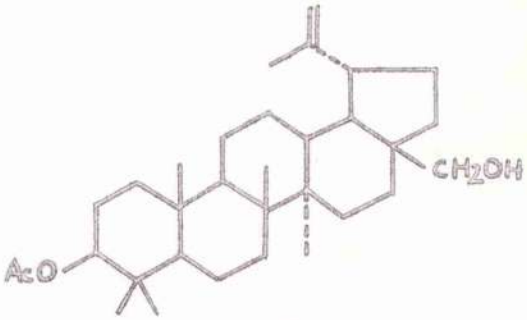
(XLVIII)



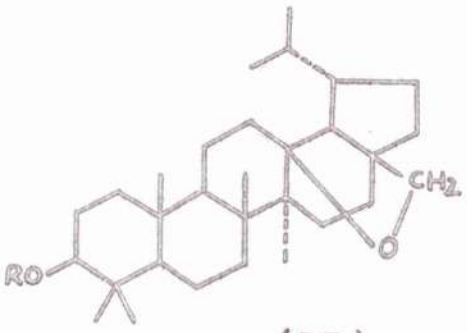
(XLIX)



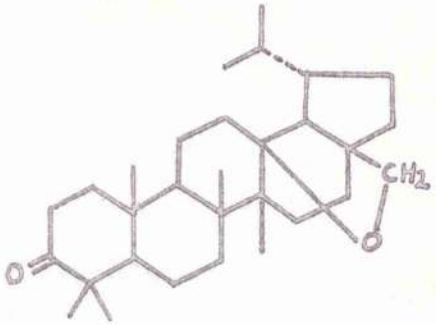
(X)



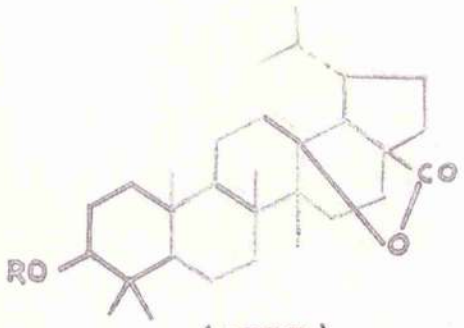
(L)



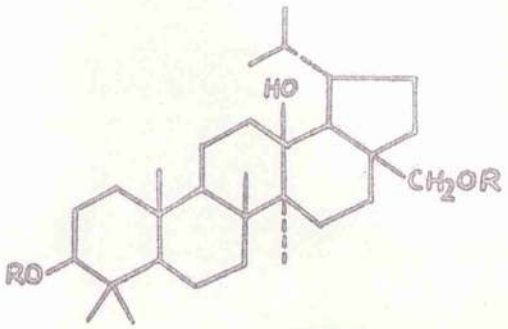
(LI)



(LII)



(LIII)



(LIV)

analysed as a monoacetate, $C_{32}H_{50}O_3$. The ultraviolet spectrum showed absorption at 2060 Å. (ε, 6000) suggesting the presence of one double bond. Infrared absorption at 1630 and 896 $cm.^{-1}$ indicated that this double bond was present as a vinylidene group. Catalytic hydrogenation of the acetate (XLVIII, R = Ac) gave a fully saturated dihydromonoacetate, $C_{32}H_{52}O_3$ (LI, R = Ac), readily hydrolysed to the alcohol (LI, R = H). These compounds showed no ultraviolet absorption, gave no colouration with tetranitromethane, and the characteristic vinylidene absorption in the infrared spectrum had disappeared on hydrogenation.

The presence of only one hydroxyl function in the alcohol (LI, R = H) was confirmed by preparation of a fully esterified monoformate, $C_{31}H_{50}O_3$ (LI, R = Fo). Hence, the dihydroalcohol (LI, R = H) and its unsaturated parent contain only one hydroxyl group, which was shown to be secondary by

oxidation of the dihydroalcohol (LI, R = H) with chromium trioxide, to a ketone, $C_{30}H_{48}O_2$ (LII) showing carbonyl absorption at 1700 cm.$^{-1}$ in the infrared region of the spectrum. Thus the hydroxyl function still present after dehydrogenation of betulin must be at C_3.

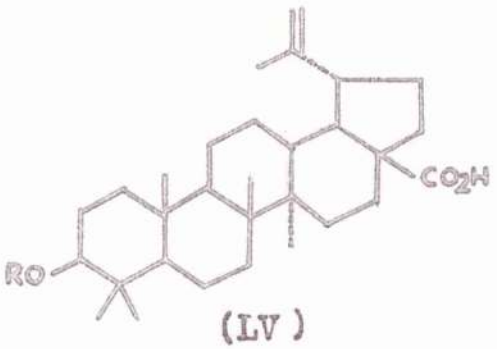
It is evident from these reactions that the primary hydroxyl group at C_{28} in betulin has been involved in some rearrangement, perhaps similar to that occurring in the formation of allobetulin from betulin (see p.10), resulting in the formation of a cyclic ether, a supposition which was confirmed by subjecting betulin monoacetate (L) to similar mercuric acetate dehydrogenation; the reaction yielding the dehydroether acetate (XLVIII, R = Ac) as expected.

The saturated ether acetate (LI, R = Ac) was subjected to a series of reactions analogous to that used for the conversion of allobetulin to moradiol.[15,16]

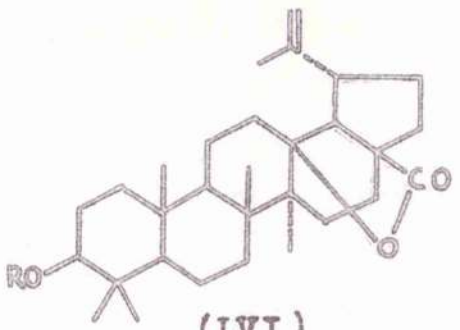
The compound (LI, R = Ac) was refluxed with chromium trioxide in glacial acetic acid to give a crystalline product, $C_{32}H_{50}O_4$, (LIII, R = Ac), which showed infrared absorption at 1770 $cm.^{-1}$ characteristic of a γ-lactone.[40]

The compound was later obtained by Allison *via* betulinic acid by the following methods. Acetyl betulinic acid (LV, R = Ac) was dehydrogenated with mercuric acetate to give a compound (LVI, R = Ac) showing absorption at 1790 $cm.^{-1}$ in its infrared spectrum. Although this γ-lactone was unsaturated it showed little or no colour with tetranitromethane and negligible absorption of ultraviolet light, and in this respect shows great similarity to thurberogenin (VIII).[20] However, on hydrogenation, the lactone (LVI, R = Ac) absorbed two atoms of hydrogen to give a product identical to the saturated lactone (LIII, R = Ac).

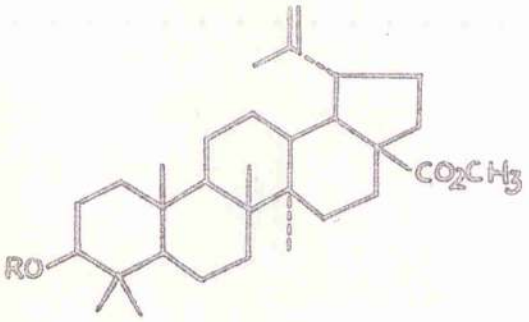
Allison then dehydrogenated acetyl methyl betulinate (LVII, R = Ac) to obtain a diene (LVIII, R = Ac) which could be hydrogenated catalytically to give the dihydro compound



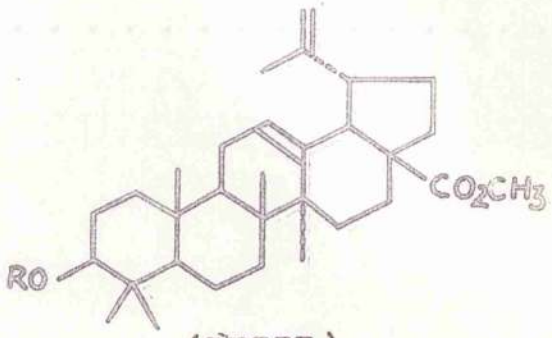
(LV)



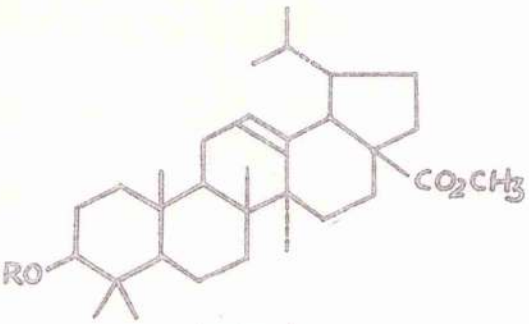
(LVI)



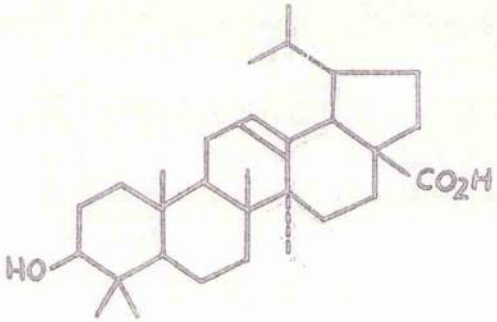
(LVII)



(LVIII)

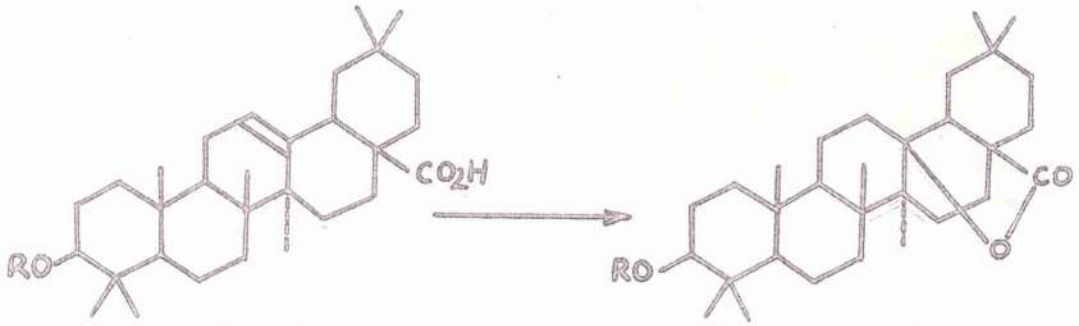


(LIX)



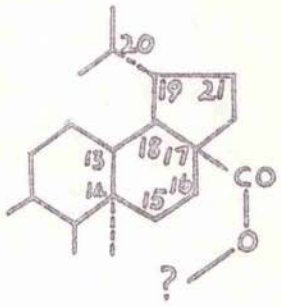
(LX)

(LIX, R = Ac). Vigorous hydrolysis of the ester (LIX, R = Ac) with sodium ethoxide gave the hydroxy acid (LX) which lactonised in the presence of mineral acid to the same fully saturated γ-lactone (LIII, R = H). This reaction is similar to that required for the lactonisation of oleanolic acid[41]



(LXI) (LXII)

(LXI→LXII). The presence of a γ-lactone system indicates that the terminal carbon atom of the lactone (and therefore of the ether) ring could be at C_{15}, C_{18}, C_{19} or C_{21}. Rupture of

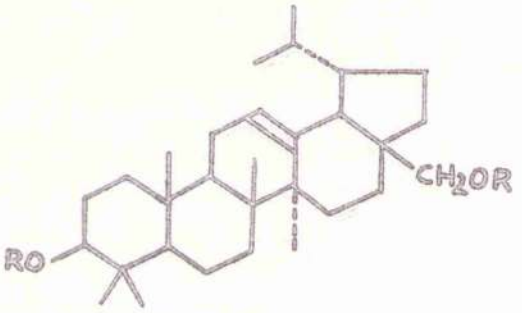


the lactone ring with lithium aluminium hydride gave a triol, $C_{30}H_{52}O_3$ (LIV, R = H) which on acetylation gave a diacetate, $C_{34}H_{56}O_5$ (LIV, R = Ac), showing that the newly formed hydroxyl group is tertiary or very strongly hindered. Thus, C_{18} and C_{21} are eliminated as possible locations of this hydroxyl function.

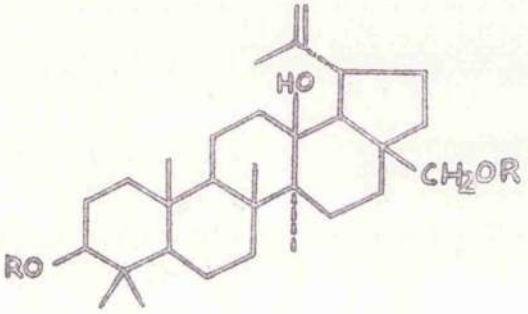
However, since the lactone (LVI) and the dihydrolactone (LIII) are not identical to thurberogenin and dihydrothurberogenin respectively, compounds of known structure, we may conclude that C_{15}, and not C_{19}, is the terminal carbon atom of the lactone ring. This was confirmed by the following series of reactions.

It was found that dehydration of the triol diacetate (LIV, R = Ac) could readily be effected by treatment with

phosphorus oxychloride to give a compound, $C_{34}H_{54}O_4$, (LXIII, R = Ac) showing absorption at 2060 Å. (ε, 7900) in the ultraviolet region of the spectrum, indicative of a trisubstituted double bond.



(LXIII)

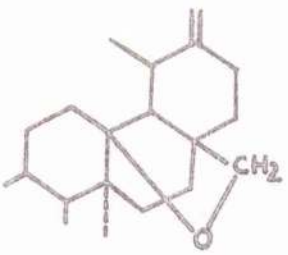


(LXIV)

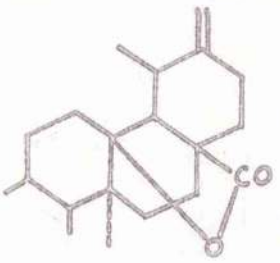
Allison therefore treated the unsaturated lactone acetate (LVI, R = Ac) with lithium aluminium hydride to obtain the expected unsaturated triol (LXIV, R = H) which gave the diacetate (LXIV, R = Ac) on acetylation. Dehydration of this compound with phosphorus oxychloride gave the known diene diacetate (XLIX, R = Ac) showing ultraviolet absorption at 2040 Å. (ε, 8000), results which show that this diene is not conjugated. Now, if the unsaturated lactone (LVI) had the structure of thurberogenin, it would have given rise to a conjugated diene, or a mixture of conjugated dienes, which would have shown a characteristic absorption of ultraviolet light. Thus, not only has the structure of the series of

ethers and lactones obtained by mercuric acetate dehydrogenation of betulin and its derivatives been proved, but the structures of thurberogenin, and hence of stellatogenin, have been confirmed.

One possible source of error existed in this line of reasoning. Since the dehydrogenation reactions were performed in acid solution, it appeared possible that rearrangement of the carbon skeleton of ring E might have occurred, resulting in compounds having partial structures (LXV) and (LXVI).



(LXV)

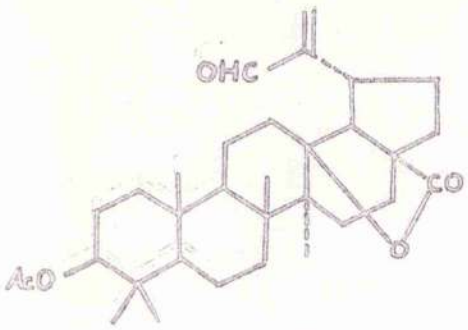


(LXVI)

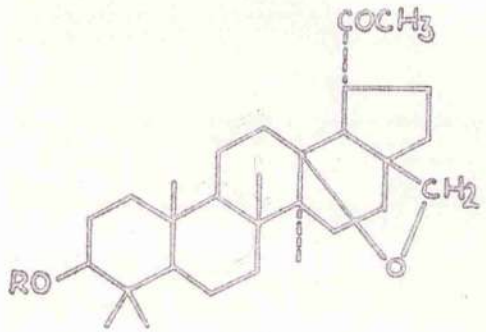
To distinguish between these possible structures and the unchanged five-membered ring E structures, a number of experiments were carried out.

Allison oxidised the unsaturated lactone acetate (LVI, R = Ac) with selenium dioxide and obtained a product (LXVII) showing ultraviolet absorption at 2220 Å. (ε, 6500)

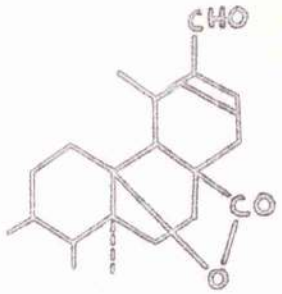
indicative of the expected α,β-unsaturated aldehyde. Infrared data confirmed the presence of this grouping.



(LXVII)



(LXVIII)



(LXIX)

Had the six-membered ring E structure (LXVI) been present, this would have given rise to an α,β-unsaturated aldehyde (LXIX) which is more highly substituted than the αβ-unsaturated aldehyde (LXVII) and would be expected to absorb at a higher wavelength, namely 2340 Å.

Oxidation of the dehydroether acetate (XLVIII, R = Ac), either with ozonised oxygen or chromium trioxide was

found to give a norketone acetate (LXVIII, R = Ac) which on hydrolysis gave the free alcohol which was not readily crystalline. Benzoylation of the alcohol readily yielded the benzoate (LXVIII, R = Bz).

Examination of the nuclear magnetic resonance spectra of the unsaturated ether acetate (XLVIII, R = Ac) and of the ketobenzoate (LXVIII, R = Bz) was then undertaken with the following results.

The spectrum of the unsaturated ether acetate (XLVIII, R = Ac) contains the following features:

(i) bands at + 13c/s. and + 26 c/s due to $-CH_2$ protons.

(ii) weak bands between + 63 c/s and + 89 c/s due to $C - CH_2 - O$ protons.

(iii) peaks at + 140 c/s and + 150 c/s due to $Me - \overset{\|}{C}$ - protons.

(iv) a band at + 160 c/s due to cyclic $- CH_2 -$ protons.

(v) peaks between + 176 c/s and + 186 c/s due to $Me - C\equiv$ protons.

There must also be bands due to $\equiv CH$ protons, but these cannot be identified with certainty. They probably will be within the range + 10 c/s to + 100 c/s, and will, in many cases, be too weak to be detected above the noise owing to their resonances being split into multiplets by adjacent $- CH_2 -$ protons. Of the two peaks attributed to $Me-\overset{\|}{C}-$

protons, that at + 150 c/s is absent in the spectrum of the ketobenzoate (LXVIII): this must therefore be due to the ester methyl group MeCOO. The presence of the other peak at + 140 c/s supports structure (XLVIII, R = Ac).

There is no evidence of a 7 c/s double peak in the Me - C $\lesssim$ region which would be expected from the Me - CH methyl group in the alternative structure (LXV).

The spectrum of the ketobenzoate (LXVIII) has the following features.

(i) bands at - 102 c/s and - 85 c/s due to aromatic ring protons.

(ii) a peak at + 131 c/s, presumably due to the Me - $\overset{|}{C}$ = O protons in structure (LXVIII).

(iii) a band at + 159 c/s, due to cyclic - CH_2 - protons

(iv) peaks at + 175 c/s and + 180 c/s due to Me - C $\lesssim$ protons.

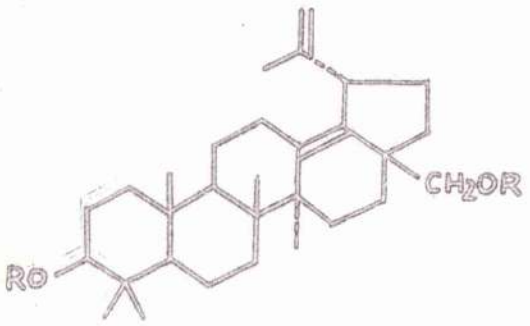
The = CH_2 bands are, as expected, not present in this spectrum. The apparent absence of C - CH_2 - O bands is surprising, but these bands might be too weak in this case to be evident above the noise level. The signal-to-noise ratio is limited by the solubility of the sample in carbon tetrachloride.

To summarise, the nuclear magnetic resonance spectra

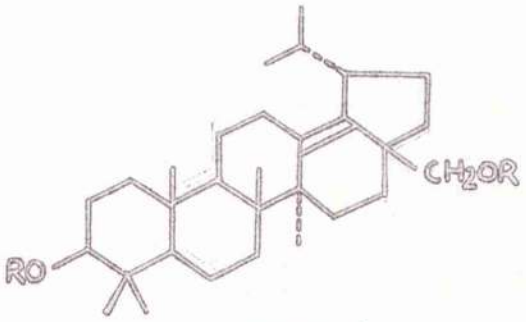
favour the adoption of structures (XLVIII) and (LXVIII) with a five-membered ring, because (a) there is no Me - CH methyl doublet in either spectrum, (b) there is a Me - C = peak in both spectra.

Thus (LXV) and (LXVI) have been eliminated, proving that the correct structure of the unsaturated ether acetate is (XLVIII).

There remains only two possible structures for the diene obtained by dehydrogenation of betulin diacetate, (XLIX, R = Ac) or (LXX, R = Ac) having a fully substituted double bond.



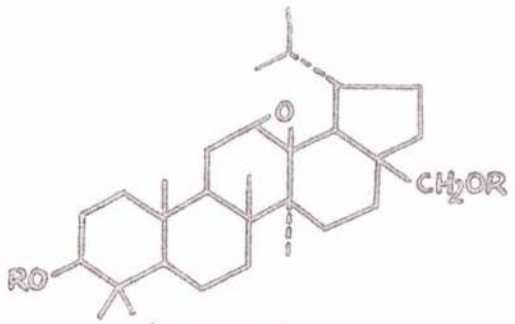
(LXX)



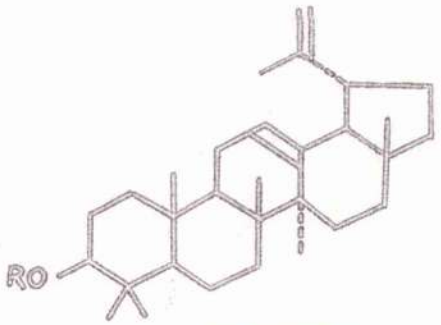
(LXXI)

The presence of the diene system was confirmed by hydrogenation of the diacetate in the presence of platinium oxide to give a product identical to that obtained by dehydration of the triol diacetate (LIV, R = Ac), which will

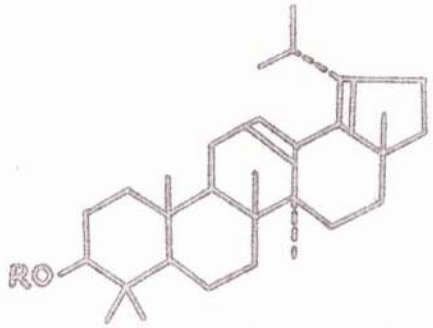
have the structure (LXIII, R = Ac) or (LXXI, R = Ac). As has already been mentioned, ultraviolet evidence favours the trisubstituted double bond. Treatment of the mono-unsaturated derivative with performic acid, results in the uptake of one atom of oxygen, to give a fully substituted oxide (LXXII, R = Ac) which is transparent to ultraviolet light and shows no colouration with tetranitromethane.



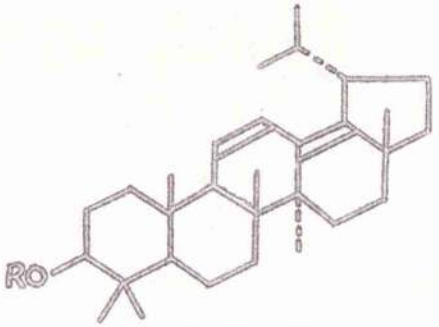
(LXXII)



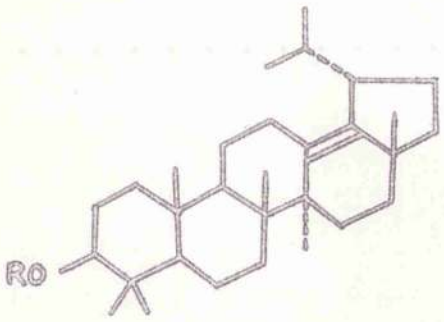
(LXXIII)



(LXXIV)



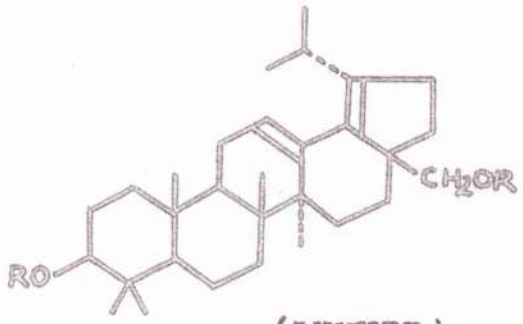
(LXXV)



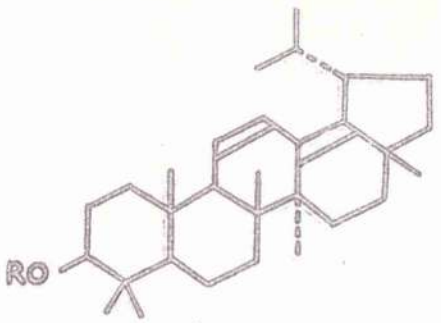
(LXXVI)

Once again reference must be made to the work of Allison. He found that mineral acid caused rearrangement of dehydrolupenyl acetate (LXXIII, R = Ac) to give a mixture of conjugated dienes (LXXIV and LXXV, R = Ac) which were readily separable by chromatography. Catalytic hydrogenation of these two compounds individually gave a mono-unsaturated acetate which has been formulated as (LXXVI, R = Ac). This compound shows ultraviolet absorption at 2060 Å. (ε, 13700) indicative of a tetrasubstituted double bond.

The analogous reaction was therefore carried out on the diene diacetate (XLIX, R = Ac) to obtain a mixture of dienes, formulated as (LXXVII and LXXVIII, R = Ac)



(LXXVII)



(LXXVIII)

These, however, were not separated, but on catalytic hydrogenation of the mixture, a product isomeric with the dihydroacetate (LXIII, R = Ac) was obtained. This compound showed absorption in the ultraviolet region of the spectrum at 2090 Å. (ε, 13400) indicative of a tetrasubstituted double bond. It has already been stated that the ultraviolet absorption shown by the dihydroacetate (LXIII, R = Ac) occurs at 2060 Å. (ε, 7900) and by comparison of these two intensities, it is evident that the latter compound contains a less highly substituted double bond. Since dehydration of the triol diacetate (LIV, R = Ac) produces the compound showing the lower intensity of absorption, this product must therefore have the structure (LXIII, R = Ac) and the presence of the more highly substituted ethylenic linkage in the isomer shows that this isomer has the structure (LXXI, R = Ac). Hence, of the two possible structures for the diene obtained by dehydrogenation of betulin diacetate with mercuric acetate (XLIX, R = Ac) and (LXX, R = Ac), we have shown that the former is in fact the correct structure.

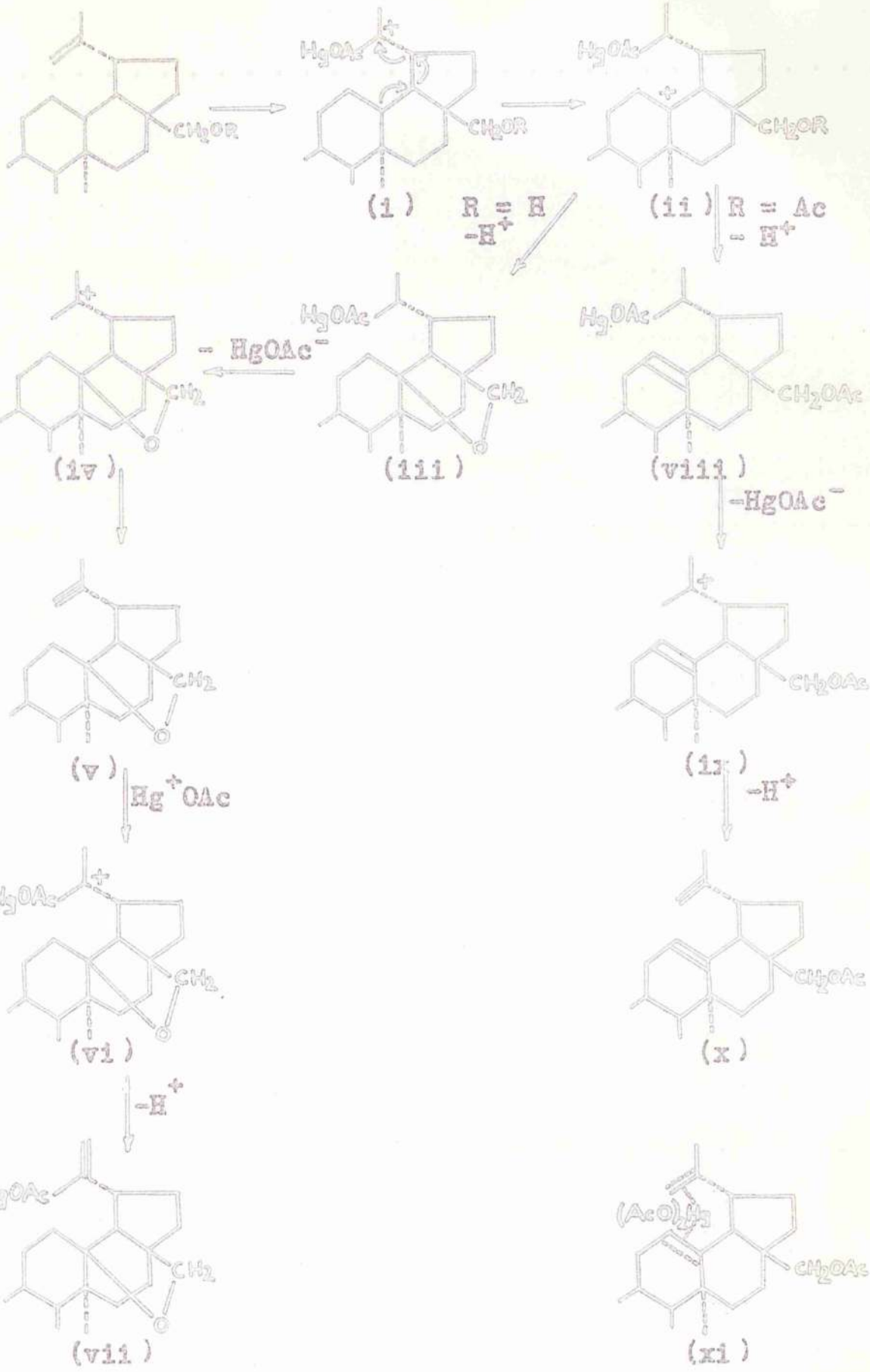
To determine whether or not lupa-12,20(30)-dien-3β,28-diol is an intermediate in the formation of 3β-hydroxy-13β,28-epoxylup-20(30)-ene, the diol was treated with mercuric acetate

in the usual way. However, the only solid product obtained from the reaction was unchanged starting material. The following mechanism is therefore suggested as most probable:

Mechanism of the reactions leading to the formation of 3β-hydroxy-13β,28-epoxylup-20(30)-ene and lupa-12,20(30)-dien--3β,28-diol diacetate.

The following mechanism is proposed for the action of mercuric acetate on betulin and betulin diacetate. Mercuric acetate is regarded as $[Hg^{+}OAc][OAc^{-}]$.

Electrophilic attack of $Hg^{+}OAc$ on the double bond gives (i) in which there is a positive charge at C_{20}, followed by hydride ion transfer from C_{13} to C_{20}, by a concerted mechanism to form the carbonium ion (ii). In the case of betulin the ion (ii, R = H) loses a proton from the hydroxymethyl function resulting in the formation of the ether (iii) which then loses $HgOAc^{-}$ by combination with $Hg^{+}OAc$ to give mercurous acetate, the resulting ion (iv) then losing a proton to give the ether (v). A second attack at the regenerated double bond by $Hg^{+}OAc$ gives the ion (vi) which can lose a proton to form the unsaturated mercury complex (vii). The mercury complex will be readily reduced by hydrogen sulphide or hydrazine hydrate to the ether (v).


HgOAc
CH2OR
(i)
R = H
-H+
(ii)
R = Ac
- H+
- HgOAc−
CH2
(iv)
(iii)
CH2OAc
(viii)
-HgOAc−
(v)
Hg+OAc
(ix)
-H+
(vi)
(x)
-H+
(vii)
(AcO)Hg
(xi)

In the case of betulin diacetate, we may regard the ion (ii, R = Ac) as losing a proton to form the mercury complex (viii) which loses $HgOAc^-$ and a proton yielding the dienyl diacetate (x). Probably the dienyl diacetate (x) is formed in preference to isomeric dienes, as its geometry is such that it could form a complex of type (xi) with excess of mercuric acetate.

EXPERIMENTAL

Rotations were determined in chloroform at room temperature unless otherwise stated. Ultraviolet spectra were determined in ethanol. Infrared spectra were determined with a Grubb-Parsons double beam spectrophotometer using Nujol Mull, unless otherwise stated. Light petroleum refers to the fraction of b.p. 60-80°.

Lupa-12,20(30)-dien-3β,28-diol Diacetate. - Betulin diacetate (1 g.) in chloroform (20 ml.) was treated with mercuric acetate (17 g.) in glacial acetic acid (370 ml.) to give a yellow solution which was heated on the steam bath for 3 hr. The reaction mixture was allowed to cool, the needles of mercurous acetate which had formed were filtered off, and the solution was poured into water, and extracted twice with chloroform. The chloroform extract was then washed repeatedly with water, dried (sodium sulphate) and taken to dryness under vacuum. The resultant mercury complex was dissolved in ethyl acetate (150 ml.) and hydrogen sulphide bubbled through the solution for 3 hr., the solution then being taken to dryness and the residue triturated with ether. The ether solution was washed with water, evaporated to dryness, and the product

dissolved in an excess of benzene and filtered through alumina (50 g.). Crystallisation of the resultant gum from aqueous ethanol gave lupa-12,20(30)-dien-3β,28-diol diacetate as needles (0.06 g.), m.p. 176.5-177.5°, $[\alpha]_D$ + 46.4° (c,1.3) showing infrared absorption bands at 1730, 1256 and 1240 $cm.^{-1}$ (acetate), and 1625 and 896 $cm.^{-1}$ (vinylidene). Absorption in the ultraviolet region of the spectrum occurred at 2040 Å. (ε,8000). (Found: C,77.9; H,9.9. $C_{34}H_{52}O_4$ requires C,77.82; H,9.99%). A further quantity (0.09 g.) was obtained from the mother liquors.

Lupa-12,20(30)-dien-3β,28-diol. - The diacetate (0.06 g.) was hydrolysed by refluxing with 5% methanolic potassium hydroxide (60 ml.) for 15 hr. Working up through ether (without washing with acid) gave a gum which crystallised from chloroform-light petroleum to yield lupa-12,20(30)-dien-3β,28-diol as needles (0.055 g.) melting at 196-198°, resolidifying and remelting at 211-214°, $[\alpha]_D$ + 8.3° (c,1.2), showing infrared absorption bands at 3220 $cm.^{-1}$ (hydroxyl) and 1620 and 885 $cm.^{-1}$ (vinylidene). Infrared comparison with a sample prepared by Allison by an alternative route showed identity.

3β-Hydroxy-13β,28-epoxylup-20(30)-ene. - Betulin (1 g.) dissolved in a minimum of warm chloroform (ca. 30 ml.) was heated with mercuric acetate (17 g.) in glacial acetic acid (370 ml.) on the steam bath for 3 hr. After cooling and filtering, the solution was poured into water, extracted twice with chloroform, the extract being washed with water, dried (sodium sulphate) and evaporated to dryness. The mercury complex thus obtained was dissolved in ethyl acetate (150 ml.) and destroyed by reduction with hydrogen sulphide over a period of 3 hr. The ethyl acetate was removed under vacuum, the residue dissolved in excess methanolic ether (5%) and filtered successively through Kieselguhr and alumina to give a yellow gum (0.86 g.) which was dissolved in ether (30 ml.) and chromatographed on alumina (15 g.). Elution with ether gave fractions (0.535 g.) which crystallised from methylene chloride-light petroleum as needles of 3β-hydroxy--13β,28-epoxylup-20(30)-ene, m.p. 266°, $[\alpha]_D$ + 60.2° (c,1.4). $\nu_{max.}$ 3460 cm.$^{-1}$ (hydroxyl), and 1620 and 893 cm.$^{-1}$ (vinylidene) (Found: C,81.5; H,11.1. $C_{30}H_{48}O_2$ requires C,81.76; H,10.98%).

3β-Acetoxy-13β,28-epoxylup-20(30)-ene. - (a) 3β--Hydroxy-13β,28-epoxylup-20(30)-ene (0.1 g.) was acetylated using pyridine (25 ml.) and acetic anhydride (10 ml.) at 100° for 1 hr. Isolation of the product through ether in the usual

way gave 3β-acetoxy-13β,28-epoxylup-20(30)-ene crystallising from chloroform-methanol as needles, m.p. 242-244°, $[\alpha]_D$ + 64.4° (c,1.2), showing infrared absorption bands at 1730 and 1255 $cm.^{-1}$ (acetate) and 1630 and 896 $cm.^{-1}$ (vinylidene). The absorption in the ultraviolet showed a maximum at 2060 Å. (ε, 6000) (Found: C,79.1; H,10.6. $C_{32}H_{50}O_3$ requires C,79.6; H,10.44%).

(b) Betulin monoacetate (0.7 g.) in chloroform (50 ml.) was treated in the usual way with mercuric acetate (25 g.) in glacial acetic acid (400 ml.) for 5 hr. The reaction mixture was worked up in the normal manner via chloroform and the resulting mercury complex destroyed with hydrogen sulphide as before. The product was chromatographed on alumina (15 g.), elution with benzene giving a product (0.21 g.), which on crystallisation from chloroform-methanol, gave needles, m.p. 242-244°, $[\alpha]_D$ + 67°, (c,1.1) showing no depression of melting point on admixture with a sample of 3β-acetoxy-13β,28-epoxylup-20(30)-ene obtained by method (a). The infrared spectra of the two samples were identical.

3β-Acetoxy-13β,28-epoxylupane. - 3β-Acetoxy-13β,28--epoxylup-20(30)-ene (0.21 g.) was hydrogenated overnight in dry ether (150 ml.) containing glacial acetic acid (10 ml.) in the presence of platinium oxide (0.15 g.). The resulting

3β-acetoxy-13β,28-epoxylupane crystallised from chloroform--methanol as needles, m.p. 281-282° (in vacuo), $[\alpha]_D$ + 48.3° (c,1.5). The compound shows absorption maxima in the infrared region of the spectrum at 1734 and 1248 cm.$^{-1}$ (acetate); it is transparent to ultraviolet light and shows no colouration with tetranitromethane. (Found: C,79.5 (79.4); H,11.1 (11.1) $C_{32}H_{52}O_3$ requires C,79.28; H,10.81%).

Hydrolysis of the acetate (0.7 g.) with 5% methanolic potassium hydroxide (200 ml.) under reflux for 1.5 hr., and isolation of the product by means of ether gave 3β-hydroxy--13β,28-epoxylupane (0.61 g.) crystallising from methylene chloride-acetone as needles, m.p. 213°, $[\alpha]_D$ + 39.6° (c,1.4), $\nu_{max.}$ 3380 cm.$^{-1}$ (hydroxyl) (Found: C,81.4; H,11.3. $C_{30}H_{50}O_2$ requires C,81.39; H,11.38%).

3β-Formyloxy-13β,28-epoxylupane. - The alcohol (0.32 g.) in ethyl acetate (13 ml.) was treated at 45° with a solution of hydrogen peroxide (100 vol., 2.5 ml.) in formic acid (99-100%, 11ml.), added dropwise over a period of 3 hr. The reaction mixture, was poured into water, worked up in the usual way through ether, and the ethereal extract taken to small bulk in the presence of methanol. The product which separated was filtered off and crystallised from chloroform--methanol as small plates of 3β-formyloxy-13β,28-epoxylupane,

m.p. 254-255°, $[\alpha]_D$ + 49.5° (c,1.1). $\nu_{max.}$ 1700 and 1175 $cm.^{-1}$ (formate) (Found: C,78.8; H,11.0. $C_{31}H_{50}O_3$ requires C,79.1; H,10.71%).

Hydrolysis of this product with 5% methanolic potassium hydroxide under reflux for 1 hr. gave back unchanged 3β-hydroxy-13β,28-epoxylupane, identified by melting point, mixed melting point and infrared comparison with an authentic specimen.

13β,28-Epoxylupan-3-one. - Oxidation of 3β-hydroxy--13β,28-epoxylupane (0.38 g.) in pyridine (30 ml.) with chromium trioxide (0.3 g.) in pyridine (40 ml.) over a period of 20 hr. at room temperature, the reaction mixture being poured into 2N potassium hydroxide and worked up through ether in the usual way, gave needles (0.35 g.) which were dissolved in light petroleum and chromatographed on alumina. Elution with benzene-light petroleum mixtures gave 3β,28-epoxylupan-3-one (0.27 g.) crystallising from chloroform-methanol as needles, m.p. 252-253° (in vacuo), $[\alpha]_D$ + 67.9° (c,1.1). $\nu_{max.}$ 1700 $cm.^{-1}$ (carbonyl) (Found: C,80.4; H,10.9. $C_{30}H_{48}O_2 . \frac{1}{2}CH_3OH$ requires C,80.3; H,10.97%).

Elution of the column with methanol in ether gave a small quantity (0.08 g.) of unchanged starting material.

3β-Acetoxylupan-28,13β-olide. - To a boiling solution of 3β-acetoxy-13β,28-epoxylupane (0.5 g.) in glacial acetic acid (50 ml.), chromium trioxide (1 g.) in glacial acetic acid (150 ml.) was added slowly over a period of 20 min. The reaction mixture was refluxed for a further 1.5 hr. and then worked up in the usual manner through ether, after adding methanol and allowing to stand. Crystallisation of the product from chloroform-methanol gave 3β-acetoxylupan-28,13β--olide as plates (0.47 g.), m.p. 299-300° (decomp.), $[\alpha]_D$ + 50.6° (c,1.1). Absorption in the infrared region of the spectrum occurred at 1734 and 1250 $cm.^{-1}$ (acetate) and 1770 $cm.^{-1}$ (γ-lactone) (Found: C,76.0; H,9.9. $C_{32}H_{50}O_4.\frac{1}{2}CH_3OH$ requires C,75.88; H,10.12%).

3β,13β,28-Trihydroxylupane. - The lactone acetate (0.25 g.) in dry ether (200 ml.) was refluxed with lithium aluminium hydride (1 g.) for 1.5 hr. and the product isolated via ether. Crystallisation from chloroform-benzene gave 3β,13β,28-trihydroxylupane (0.2 g.) as beautiful needles, m.p. 240-242° (decomp.), $[\alpha]_D$ + 21.7°, (c,1.0 in minimum ethanol and chloroform). The infrared spectrum of the compound shows strong hydroxyl absorption at 3220 $cm.^{-1}$, with no carbonyl absorption present. (Found: C,78.0; H,11.2. $C_{30}H_{52}O_3$ requires C,78.2; H,11.38%).

3β,28-Diacetoxy-13-hydroxylupane. - Acetylation of the triol (0.19 g.) was effected by heating with pyridine (30 ml.) and acetic anhydride (10 ml.) for 2 hr. at 100°. Isolation of the product through ether in the usual way gave a compound (0.2 g.) crystallising from chloroform-methanol as needles of 3β,28--diacetoxy-13-hydroxylupane, m.p. 272-273°, $[\alpha]_D$ + 18.6° (c, 1.0) $\nu_{max.}$ 3470 cm.$^{-1}$ (hydroxyl); 1745, 1712 and 1242 cm.$^{-1}$ (acetate) (Found: C, 75.3; H, 10.0. $C_{34}H_{58}O_5$ requires C, 74.95; H, 10.36%).

Lup-12-en-3β,28-diol Diacetate. - (a) Lup-12,20(30)--dien-3β,28-diol diacetate (0.92 g.) in ether (200 ml.) and glacial acetic acid (20 ml.) was hydrogenated overnight in the presence of platinium oxide (0.5 g.) to give lup-12-en-3β,28-diol diacetate, crystallising from chloroform-methanol as needles, m.p. 209-210°, $[\alpha]_D$ + 15.4° (c, 1.2) and showing absorption in the infrared region of the spectrum at 1730 and 1240 cm.$^{-1}$ (acetate). $\lambda_{max.}$ 2060 Å (ε, 7900) (Found: C, 77.5; H, 10.5. $C_{34}H_{54}O_4$ requires C, 77.52; H, 10.33%).

(b) 3β,28-Diacetoxy-13-hydroxylupane (0.08 g.) in dry pyridine (15 ml.) and in the presence of phosphorus oxychloride (4 ml.), was heated on the steam bath for 1.5 hr. The reaction mixture was allowed to cool, poured cautiously into water, cooled once again and extracted with ether. The ether extract was worked up in the usual way to give a gum (0.05 g.), which crystallised

from chloroform-methanol to give needles, m.p. 210°, $[\alpha]_D$ + 18° (c,1.3), which showed no depression of melting point on admixture with lup-12-en-3β,28-diol diacetate obtained by method (a). Infrared comparison of the two samples confirmed the identity (Found: C,77.3; H,9.8. $C_{34}H_{54}O_4$ requires C,77.52; H,10.3%).

Lup-12-en-3β,28-diol. - Hydrolysis of lup-12-en-3β,28-diol diacetate (0.4 g.) by refluxing with 5% methanolic potassium hydroxide (75 ml.) for 1 hr., followed by the usual work up through ether gave lup-12-en-3β,28-diol crystallising as needles from chloroform-methanol. The compound melts over the range 209-218° and repeated crystallisation will not improve this result, $[\alpha]_D$ - 14° (c,1.1) (Found: C,81.3; H,11.6. $C_{30}H_{50}O_2$ requires C,81.39; H,11.38).

Lup-12,13-epoxy-3β,28-diol Diacetate. - A solution of lup-12-en-3β,28-diol diacetate (0.19 g.) in ethyl acetate (15 ml.) was heated at 45°, while hydrogen peroxide (100 vol., 3 ml.) in formic acid (98-100%; 15 ml.) was added dropwise over a period of 30 min., the temperature being maintained at 40-50° for a further 1 hr. The reaction mixture was then poured into water, extracted with ether, and the ether extract worked up in the usual way. Concentration of the ether extract in the presence of methanol gave needles, which were filtered off and recrystallised from chloroform-methanol to give lup-12,13-epoxy-3β,28-diol

diacetate as needles, m.p. 207-208°, $[\alpha]_D$ + 21.7° (c,1.1). This compound was transparent to ultraviolet light and showed no colouration with tetranitromethane. Absorption occurred in the infrared region of the spectrum at 1738, 1725 and 1245 $cm.^{-1}$ (acetate) (Found: C,75.3; H,10.2. $C_{34}H_{54}O_5$ requires C,75.23; H,10.03%).

Oxidation of 3β-Acetoxy-13β,28-epoxylup-20(30)-ene. - (a) 3β-Acetoxy-13β,28-epoxylup-20(30)-ene (0.41 g.) in methylene chloride (60 ml.) was treated with ozonised oxygen at -45° for 35 mins. The reaction mixture was allowed to come to room temperature, glacial acetic acid (15 ml.) and zinc dust (1 g.) were added to it over a period of 20 mins., with continuous stirring. The whole was stirred for a further 40 mins., filtered, washed with water, dried (sodium sulphate), and evaporated to dryness. Crystallisation of the product from chloroform-methanol gave 3β-acetoxy-13β,28-epoxy-20-oxo--30-norlupane as plates, m.p. 317-319°, $[\alpha]_D$ + 3.1° (c,1.3), showing infrared absorption at 1736 and 1245 $cm.^{-1}$ (acetate) and 1706 $cm.^{-1}$ (carbonyl) (Found: C,77.1; H,10.1. $C_{31}H_{48}O_4$ requires C,76.81; H,9.98%)

(b) To 3β-acetoxy-13β,28-epoxylup-20(30)-ene (2.31 g.) in

boiling glacial acetic acid (ca. 200 ml.), chromium trioxide (7 g.) in glacial acetic acid (ca. 250 ml.) was added slowly and the reaction mixture refluxed for 1.5 hr., poured into aqueous methanol and allowed to stand. The whole was then extracted with ether and the ether extract worked up in the usual way. The resultant gum was dissolved in benzene-light petroleum (1:1) and chromatographed on alumina (40 g.). Elution with benzene-light petroleum mixtures gave a resinous product (0.31 g.), which, after crystallisation from chloroform-methanol had m.p. 317-319°, $[\alpha]_D$ + 3.7°, (c,1.3). Comparison with 3β-acetoxy-13β,28-epoxy-20-oxo-30-norlupane showed identity.

The bulk of the material (1.98 g.) required to be stripped off the column with glacial acetic acid, indicating that it was acidic in nature. However, this acid fraction was not further investigated.

Hydrolysis of the norketone acetate gave the norketone alcohol which was not readily crystalline.

3β-Benzoyloxy-13β,28-epoxy-20-oxo-30-norlupane was prepared by heating a solution of the alcohol (0.56 g.) in pyridine (30 ml.) with benzoyl chloride (2ml.), on the steam bath for 1.5 hr. Water was added cautiously to the cooled solution and the whole allowed to stand for 3 hr., when it was

extracted with ether and worked up in the usual way, the product being chromatographed on alumina. Elution with benzene-light petroleum (1:1) gave a crystalline residue (0.51 g.) which on recrystallisation from chloroform-methanol yielded tiny needles of 3β-benzoyloxy-13β,28-epoxy-20-oxo-30-norlupane, m.p. 313°, $[\alpha]_D$ + 26.5° (c, 1.5), showing absorption in the infrared region of the spectrum at 1725, 1280 and 1120 cm.$^{-1}$ (benzoate). The ketonic absorption is masked by the absorption due to the ester group. Absorption in the ultraviolet region of the spectrum occurs at 2280 Å. (ε, 13900). (Found: C, 76.7; H, 9.2. $C_{36}H_{50}O_4$. CH_3OH requires C, 76.8; H, 9.3%).

Lup-13(18)-en-3β,28-diol Diacetate. - Lupa-12,20(30)-dien-3β,28-diol diacetate (1.5 g.) dissolved in glacial acetic acid (100 ml.) at 100°, was treated with concentrated hydrochloric acid (5 ml.). After 15 min., the whole was taken to dryness under vacuum. The residual gum was dissolved in petrol and chromatographed in an attempt to separate the two isomeric conjugated dienes. This proved unsuccessful and the fractions were therefore bulked, dissolved in ether (150 ml.) and glacial acetic acid (20 ml.), and hydrogenated overnight in the presence of platinum oxide. The product was chromatographed on alumina (15 g.). Elution with

benzene-light petroleum (1:1) gave lupa-13(18)-en-3β,28-diol diacetate, m.p. 205-206° (in vacuo) showing absorption in the ultraviolet region of the spectrum at 2090 Å. (ε, 13400).

Treatment of lupa-12,20(30)-dien-3β,28-diol with Mercuric Acetate. - Lupa-12,20(30)-dien-3β,28-diol (6 g.) in chloroform (150 ml.) was heated with mercuric acetate (100 g.) in glacial acetic acid (1500 ml.) at 100° for 3 hr. Working up in the usual way through chloroform gave a brown gum which was treated with methanolic potassium hydroxide (7.5%, 750 ml.) to which hydrazine hydrate (90%, 10 ml.) had been added. The mixture was refluxed for 3 hr., poured into water and extracted with ether. The ether extract was washed in the usual way, taken to dryness and acetylated with pyridine-acetic anhydride on the steam bath. Working up in the usual way through ether gave a brown gum which was chromatographed on alumina to give lupa-12,20(30)-dien--3β,28-diol diacetate as the only solid product.

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