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INVESTIGATIONS IN THE TRITERPENOID FIELD

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A THESIS

submitted to

THE UNIVERSITY OF GLASGOW

in fulfilment of the

requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

By

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The Royal College of Science and Technology

Glasgow.

November, 1961

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CONTENTS

INTRODUCTION

The two sections of this thesis deal with (a) the complete structural elucidation of soyasapogenols B and D and (b) the chemistry of some compounds derived from lupeol, betulin, and betulinic acid by dehydrogenation with mercuric acetate. All of these compounds belong to the pentacyclic class of triterpenoids and more specifically to the oleanane and lupane sub-groups respectively.

The triterpenoids are a class of natural products which have been isolated from a wide range of vegetable material, sheep wool wax, fungi, and fish oil. This class of compounds was originally defined as the triterpenes, consisting of hydrocarbons and oxygenated hydrocarbons whose molecular skeleton was made up of thirty carbon atoms derivable from six isoprene units. In order to accommodate several recently discovered compounds showing definite triterpene characteristics but containing thirty one carbon atoms[1], this group of natural products is now classified under the more general term of the triterpenoids.

With the exception of the hydrocarbon squalene (I) all triterpenoids are polycyclic compounds and the majority are pentacyclic or tetracyclic. They may be classified in three main groups according to the nature of their carbon skeletons.

(a) The Squalene Group.

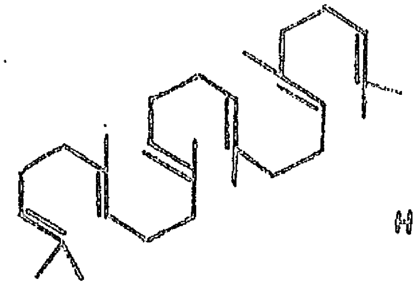
Besides the aliphatic hydrocarbon, squalene, this group contains the tricyclic alcohol, ambrein,[2] and the tetracyclic diol, onocerin[3] (XXI) which is unique in the triterpenoid field because it has a completely symmetrical molecule. Another noteworthy feature is that onocerin has recently been synthesised.[4]

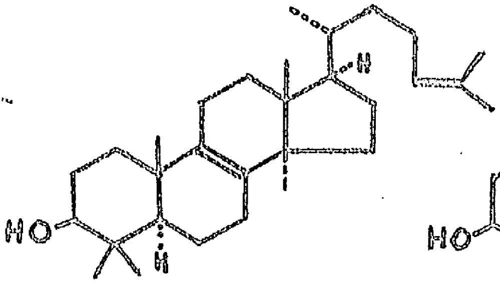
(b) The Tetracyclic Group.

The members of this group show a close structural relationship to the steroids. Subdivision of the tetracyclic group is based on two structural types which differ in the configuration at positions 13, 14 and 17, and are exemplified by lanosterol (II) and euphol (III). A ready differentiation can be made between derivatives of the two series from the characteristic molecular rotation changes[5] which accompany the formation of the corresponding 7,9(11)-dienyl derivatives and also from the significant differences in the positions of the ultraviolet maxima of these dienes.[5]

Some naturally occurring members of the lanosterol series are listed as follows:-

lanosterol, agnosterol, parkeol, eburicoic acid, the polyporenic acids A and C, pinicolic acid, cycloartenol, cyclolaudenol, cyclo-orysterol, cycloeucalenol,[6] 21-hydroxylanosta-7,9(11),24-trien-3-one,[7] 3β,21-dihydroxylanosta-7,9(11),24-triene[7] and elaterecin A and B.[8] Of these, the fungal acids are mostly C_{31} compounds and cycloeucalenol has recently been shown to be 4β-demethyl-24-methylenecycloartanol.[8] Cycloartenol, cyclolaudenol, cyclo-orysterol and cycloeucalenol each contain a cyclopropane ring and are therefore pentacyclic. However, because of their ready conversion to lanosterol derivatives they are included in the tetracyclic group.


(I)



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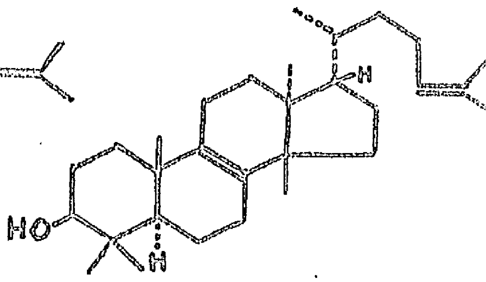


(III)

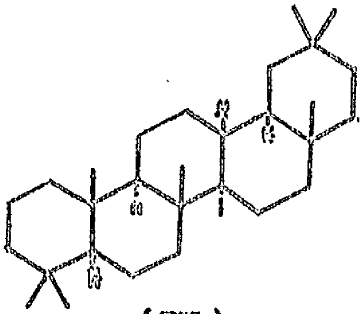
The euphol group contains euphol, euphorbol, elemadienolic acid, masticadienonic acid, butyrospermol and tirucallol. Although tirucallol is classified under the euphol sub-group it differs from euphol in the stereochemistry of the centre at $C_{(20)}$.[9] Dipterocarpol and the dammar resin triterpenoids are also regarded as members of the euphol sub-group.

(c) The Pentacyclic Group.

Sub-division of this group is based on five different types of carbon skeleton which occur. The largest of these sub-groups is that based on the hydrocarbon, oleanane (IV), and contains such compounds as β-amyrin, oleanolic acid, hederagenin, soyasapogenols A, B and C, and bassic acid.[10] Also included in this group are taraxerol, friedelin, cerin, and glutinone, all of which are readily converted into derivatives of the oleanane type. Certain aspects of the chemistry of the oleanane group will be dealt with in the following section when some reactions of the soyasapogenols are considered. The total synthesis of oleana-11,13(18)-diene has recently been reported.[11]

Closely related to the oleanane group is the group based on the hydrocarbon ursane (V). Included in the ursane sub-group are:-

α-amyrin, β-boswellic acid, uvaol, brein, quinovic acid, and bauerenol.[12] Phyllanthol is also included in this section although it contains a cyclopropane ring and is therefore hexacyclic. Its classification in the ursane group is on account of its ready conversion to an α-amyrin derivative.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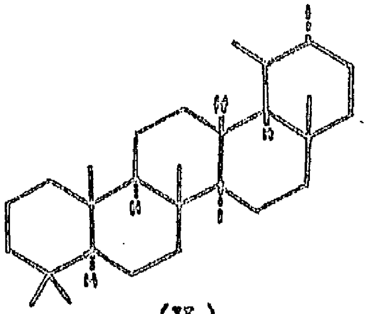
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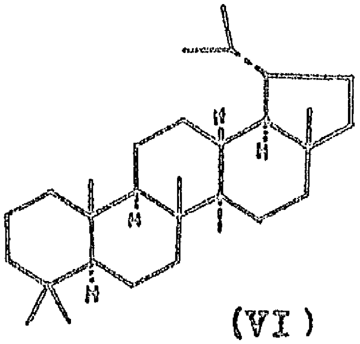
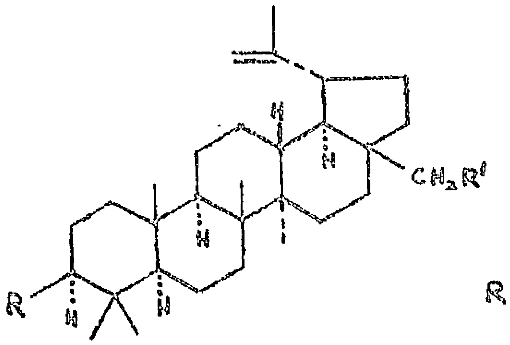
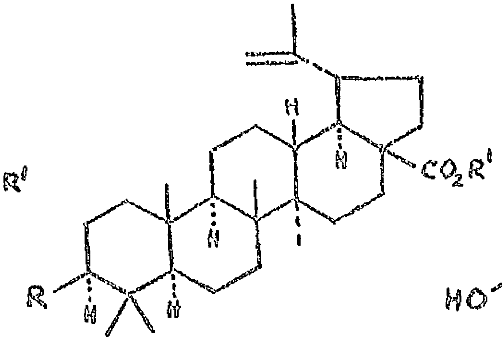
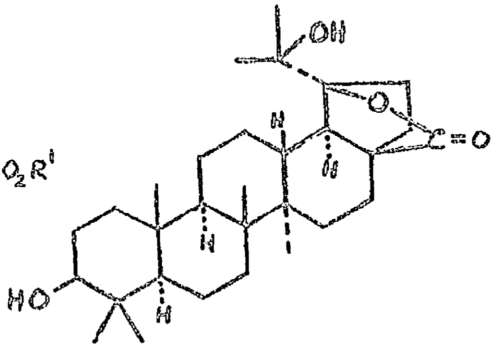

(VI)

The third group, based on the hydrocarbon, lupane (VI), is of special note because the second section of this thesis is concerned with compounds derived from lupeol (VIIa), betulin (VIIb), and betulinic acid (VIIIa), three members of the lupane group. Stellatogenin (IX) and thurberogenin (X) are also derivatives of lupane and are noteworthy as they are naturally occurring lactones.[13] Only two other triterpenoid lactones have been isolated from natural products, namely, dumortierigenin[14] and lucernic acid.[15] The structure of the latter compound has not yet been established but the presence of a γ-lactone has been deduced from the band at 1770 cm.$^{-1}$ in the infrared spectrum. Thurberogenin was of particular interest to the author as its properties showed a marked similarity to those of the lactone (XIa) derived from betulinic acid by the action of mercuric acetate. The two other members of the lupane group are 23-hydroxybetulin[16] and 3β,20-dihydroxylupane.[17] Extracted from rowan bark (*Sorbus aucaparia*) and from the fruits of the Osage orange (*Maclura pomifera*) respectively, these recently discovered triterpenes have been allocated the structures (XII) and (XIII).



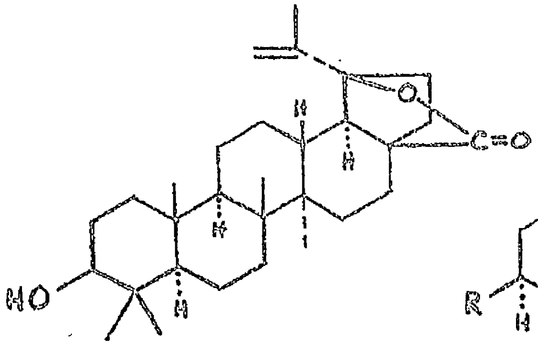


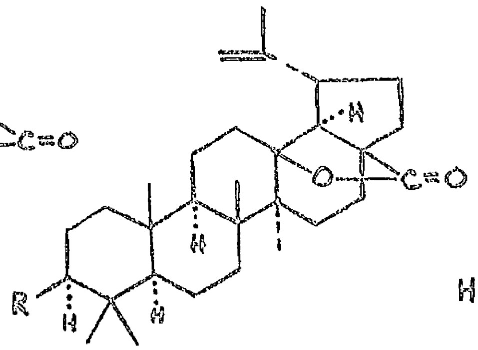


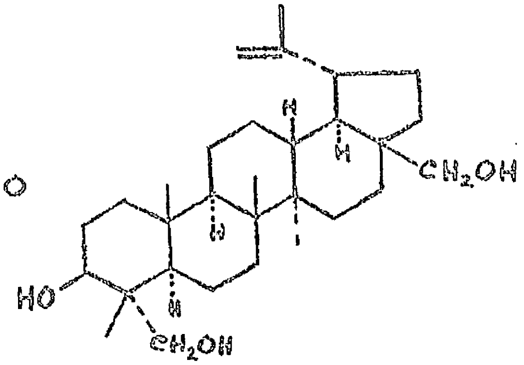
(VII)(a) R = OH; R' = H
(b) R = OH; R' = OH

(VIII)(a) R = OH; R' = H
(b) R = OAc; R' = H
(c) R = OAc; R' = Me

(IX)



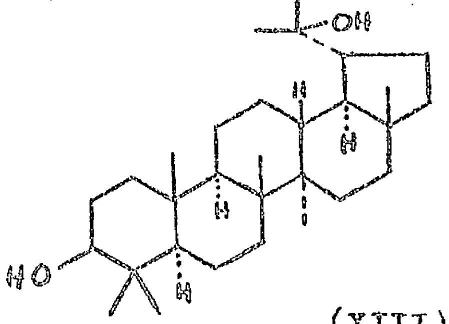




(X)

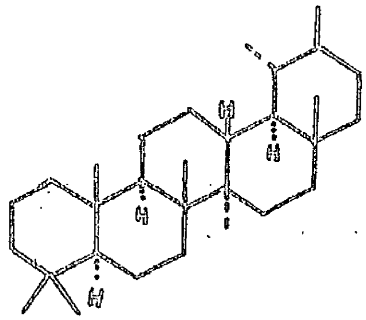
(XI)(a) R = OH; (b) R = OAc

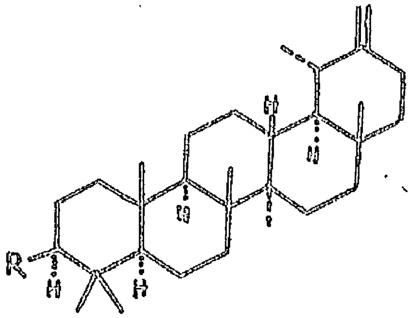
(XII)



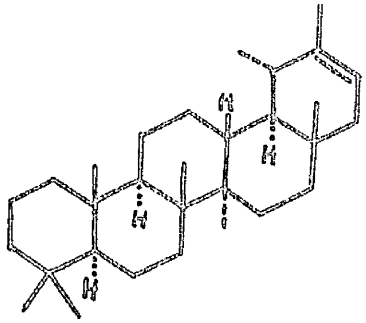
(XIII)

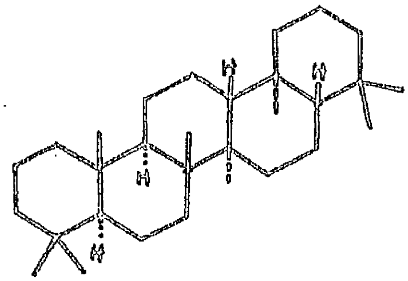
The fourth group is based on the hydrocarbon taraxastane (XIV) which differs from ursane (V) only in the stereochemistry[18,19] at positions 18, 19, and 20. Included in this group are taraxasterol (XVb), arnidiol,[20] and taraxastene (XVa), the last named compound being the only polycyclic triterpenoid hydrocarbon which has been reported as occurring naturally.[21] Faradiol[20] has been related to Ψ-taraxastene (XVI) which may also be regarded as a member of this group since it is derived from taraxastene by reaction with mineral acid.[20] The acid isomerisation of lupenone (XVII) to δ-amyrenone (XVIII) is very similar and will be dealt with more fully in the theoretical section (Table V).

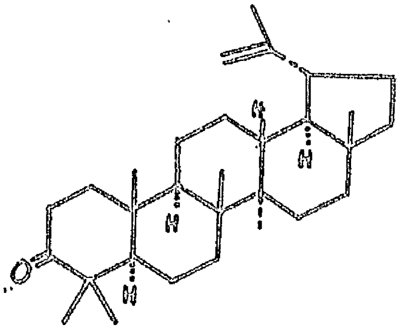

(XIV)



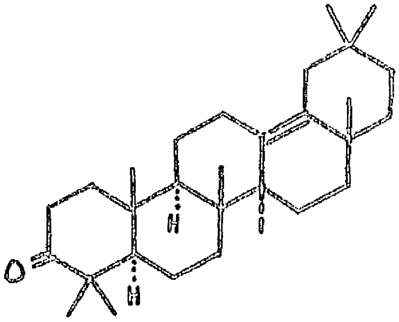
(XV) (a) R = H (b) R = OH


(XVI)


(XIX)



(XVII)



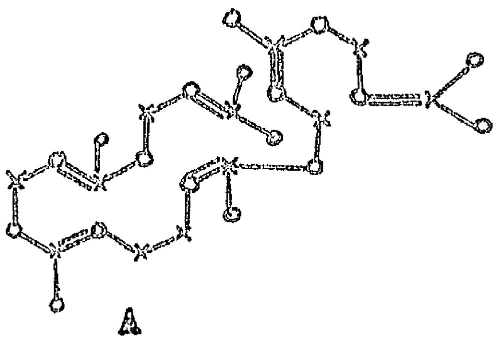
(XVIII)

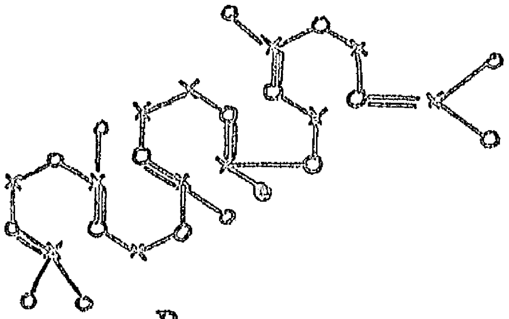
In recent years the study of the triterpenes zeorin[23] and hydroxyhopanone[24] has led to the recognition of a new sub-group of pentacyclic triterpenoids based on the hydrocarbon, gammacerane (XIX). The triterpenes of this group have not been related to any of the other groups but it is possible to derive them from squalene which can be regarded as the precursor of all pentacyclic and tetracyclic triterpenes.[25-27] Hydroxyhopanone and zeorin are the only pentacyclic triterpenes which retain the intact carbon skeleton of squalene i.e. no migration of axial methyl groups or hydrogen atoms is necessary during the postulated cyclisation from a squalenoid type precursor. In the ensuing section on the biogenesis of the triterpenoids the formation of these unusual compounds is discussed.

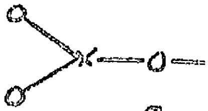
The Biogenesis of the Triterpenoids.

It was first noted by Channon[28] that when rats were fed with squalene the amount of cholesterol in their liver was markedly increased. Guided by this observation, Robinson postulated that squalene might be the biogenetic precursor of cholesterol on the grounds that squalene comprises the complete molecular skeleton of cholesterol plus a further three carbon atoms.[29] Robinson also suggested that the cyclisation took place from squalene folded as in Fig. A followed by loss of three methyl groups from positions 3, 13 and 14.

Robinson's hypothesis was apparently substantiated by the experimental evidence of Langdon and Bloch[30,31,32] who showed that squalene is an intermediate in the biological synthesis of cholesterol (XX) and also that acetic acid, which is the principal carbon source in cholesterol synthesis, is a precursor of squalene. Such a cyclisation scheme could be completely reconciled with the hitherto known distribution of acetate carbon in cholesterol[33,34] on the assumption that each isoprene unit of squalene contained three methyl and two carboxyl carbon atoms of acetate arranged as in Fig. C.


A


B

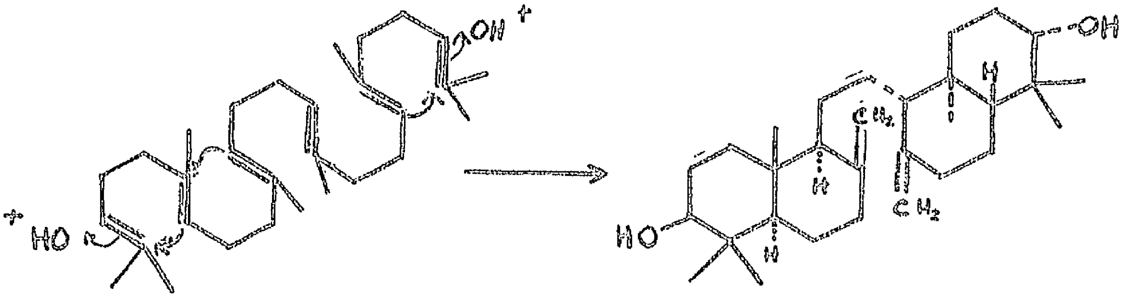

C

However, using epiandrosterone obtained from isotopically labelled dihydrocholesterol, Woodward and Bloch[35] have shown that the carbon atom at position 13 is derived from the methyl carbon of acetate. This conclusion was later confirmed independently by Cornforth *et al.*[36] The scheme suggested by Robinson was therefore invalid so Woodward and Bloch[35] proposed an alternative mode of cyclisation from squalene, folded as in Fig B, and involving the migration of methyl groups. Substantial evidence for this scheme has been supplied by several workers[36-39] whose combined results have established the origin of all the carbon atoms in cholesterol from either the methyl or the carboxyl carbon of acetate. These results agree completely with Woodward's proposed scheme which also explains the part played by lanosterol in the biogenesis of steroids. Moreover the fact that methyl group migrations take place during the cyclisation offers a rational explanation for the non-conformity of lanosterol with the classical isoprene rule.

In order to accommodate lanosterol, euphol, friedelin, α-amyrin, taraxasterol and many other compounds which do not follow the classical isoprene rule Ruzicka and his fellow workers formulated the "biogenetic isoprene rule"[40] which so far has no exceptions in the triterpenoid field. Using the biogenetic isoprene rule it is possible to derive those compounds which are exceptions to the classical isoprene rule from conforming intermediates. The above postulates are of great significance because it is now possible to derive similar modes of cyclisation for all groups of triterpenes starting from a squalene type precursor and using accepted mechanisms based on Wagner-Meerwein type rearrangements (Table I).

It has been established[41] that natural squalene occurs as the all-*trans* isomer and also that synthetic squalene, which differs from natural squalene in the positions of the double bonds, cannot be converted biologically into cholesterol.[31] It is now recognised that the all-trans configuration and the natural arrangement of ethylenic linkages in squalene are essential for the postulated cyclisation. Tchen and Bloch[42] have also established that squalene is not transformed into lanosterol in the absence of oxygen and have therefore suggested that the reaction sequence is initiated by attack of activated molecular oxygen at a terminal double bond.

Only the cyclic members of the squalene group of triterpenes, ambrein and onocerin (XXI), are formed by simultaneous cyclisation from both ends of the squalene (I) chain.



(I) (XXI)

In all other cases the initial attack at one end of the molecule is followed by cyclisation in one of three different ways. The first route leads to lanosterol (II) and thence to cholesterol (XX), both of which may be derived from the cation (XXII) by the mechanism shown in Table I. It has been postulated that (XXII) is formed from squalene (I) by concerted cyclisation of rings A and B, immediately followed by closure of rings C and D thus giving rise to the known stereochemistry of the steroids and the lanosterol group.

The carbonium ion (XXIII) may be regarded as the precursor of the majority of pentacyclic triterpenes and of the euphol group of tetracyclic triterpenes. It can be derived from squalene by synchronous formation of rings A, B, C, and D. The route to euphol (III) from (XXIII) involves methyl group migration and finally loss of a proton from $C_{(9)}$ whereas a Wagner-Meerwein type ring enlargement of (XXIII) to give carbonium ion (XXIV) and subsequent closure of ring E produces the lupanyl cation (XXV). The pentacyclic triterpenes lupeol (VIIa), α-amyrin (XXXIIIa), β-amyrin (XXXIIa), and taraxasterol (XVb) can all be derived from (XXV) by well established mechanisms (Table II).

Hydroxyhopanone (XXVII) exemplifies the third type of cyclisation and on the basis of its recently established stereochemistry[43] it is possible to postulate its formation by a completely concerted cyclisation of squalene folded in the all chair conformation. Carbonium ion (XXVI) is thus formed and may be stabilised by addition of an anion such as OH^- (Table I).

TABLE I.

The Cyclisation of Squalene.

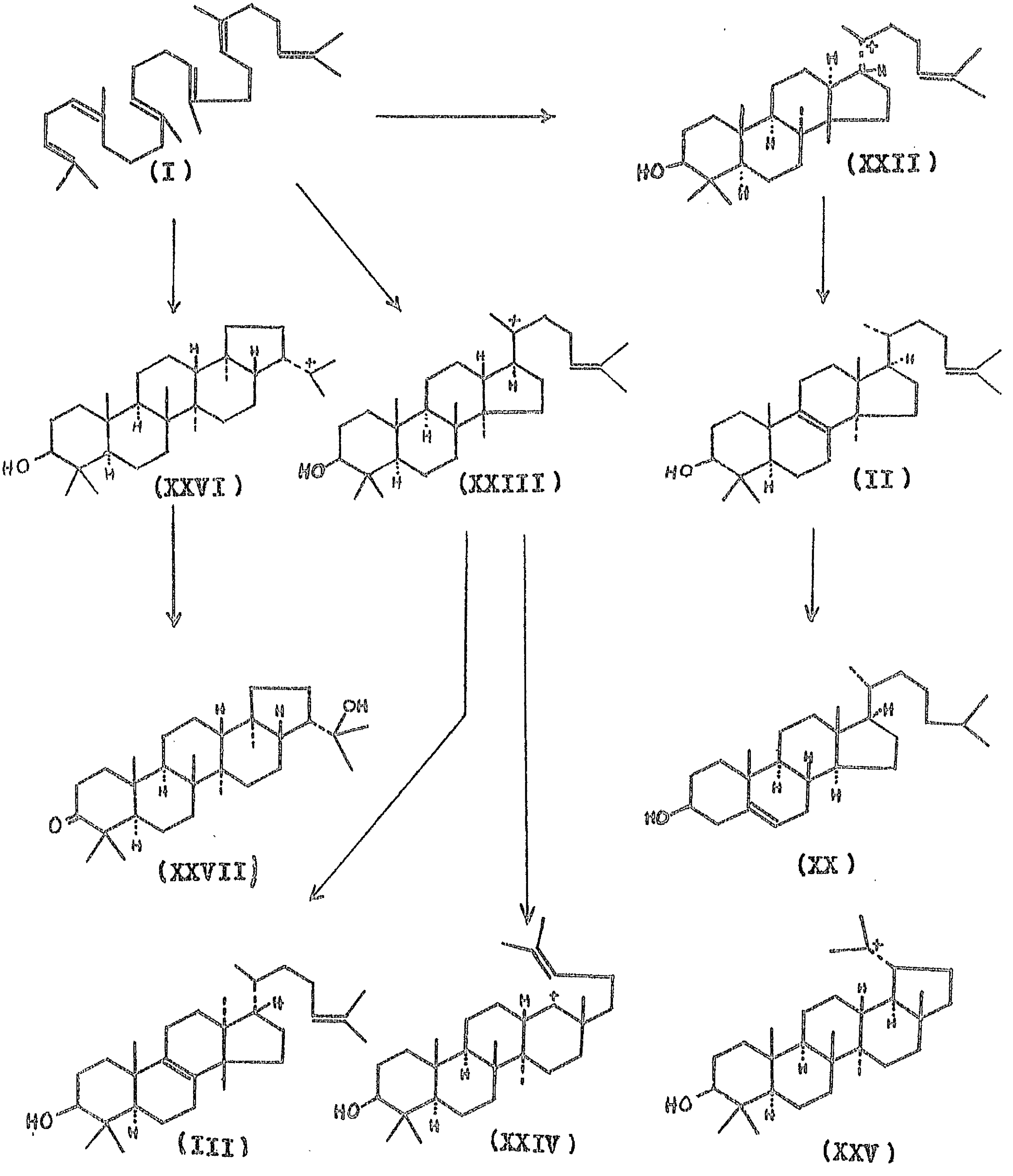
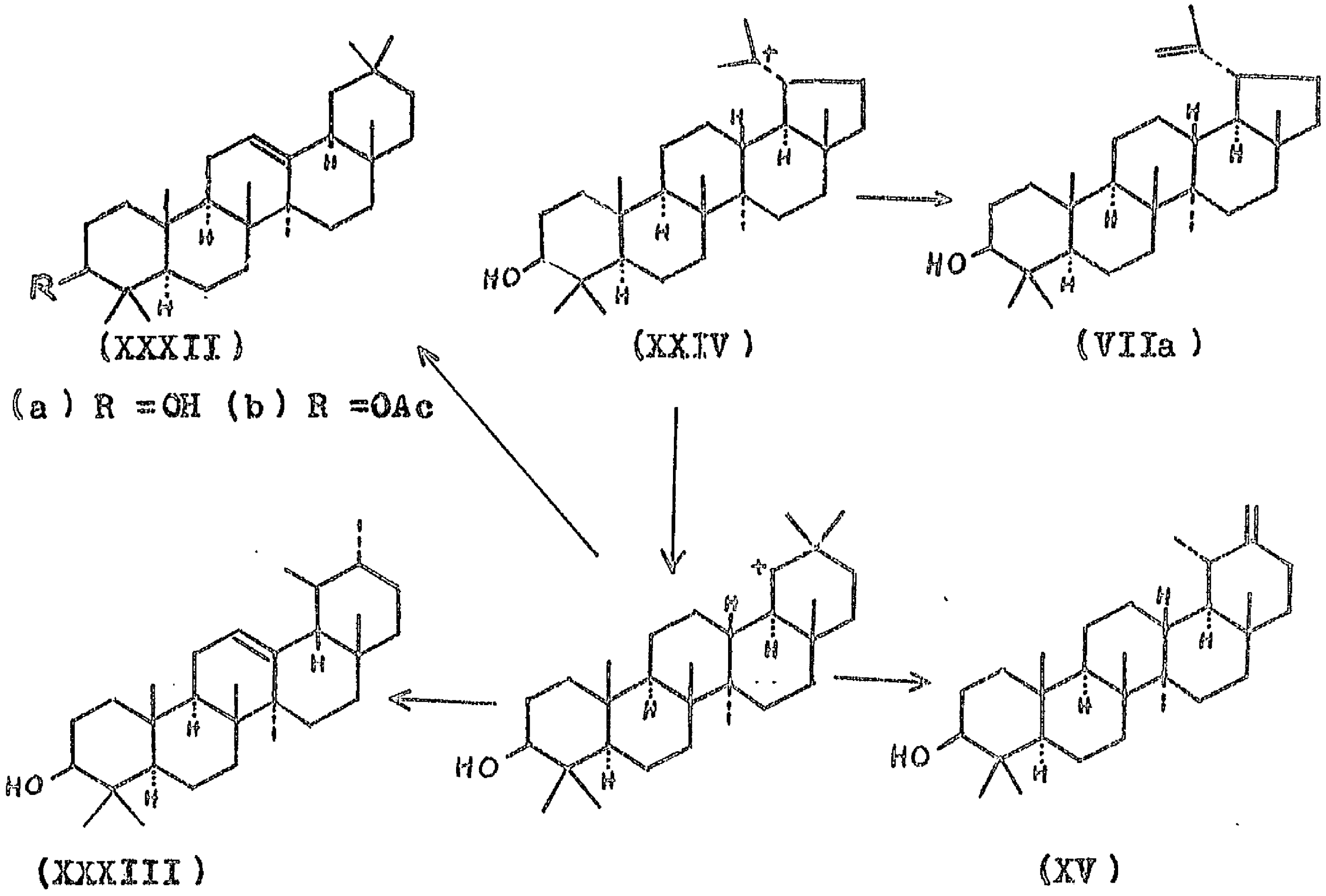
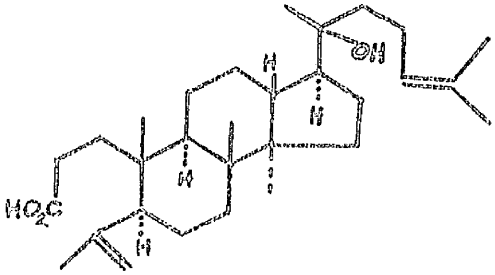


TABLE II.

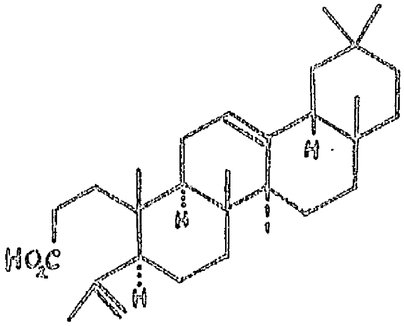


The mechanisms postulated above are to some extent borne out by the observation that the configuration of the D-E ring junction is governed by the position of the double bond. If it remains in, or exocyclic to ring E as in ψ-taraxasterol, taraxasterol, or lupeol the ring junction is <u>trans</u>. However, in the case of the amyrins, taraxerol, glutinone, friedelin etc., where migration of the double bond to rings D, C, B, and A has occurred the ring junction is <u>cis</u>.

The basic principles of biogenesis enumerated in the foregoing discussion have been extremely helpful in establishing the structure of several triterpenes. Recently the structures of dammarenolic acid[44] (XXVIII) and nyctanthic[45] acid (XXIX) have been elucidated.



(XXVIII)

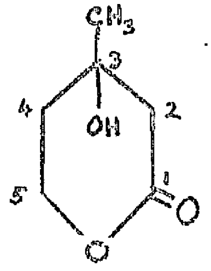


(XXIX)

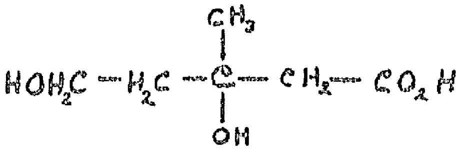
Unusual as they are, these structures are not exceptions to the biogenetic rule and can be rationally derived by acceptable mechanisms e.g. the carboxyl groups in (XXVIII) and (XXIX) may arise from biogenetic fission of the corresponding 3-ketones.

The Use of Mevalonic Acid Derivatives

Since the discovery in distillers soluble residues[46,47] of mevalonolactone (XXX), both it, and the parent mevalonic acid (XXXI) have played an increasingly important part in the biosynthetic confirmation of the hypothetical mechanisms postulated for the formation of the triterpenoids from squalene.



(XXX)



$$HOH_2C-H_2C-\overset{CH_3}{\underset{OH}{C}}-CH_2-CO_2H$$

(XXXI)

It has been demonstrated by Tavormina, Gibbs and Huff[48] that as a precursor of cholesterol mevalonic acid is superior to all other compounds previously tried, and they suggested that the atoms in an isoprenoid unit may be derived directly from the five carbon atoms of the lactone. The mechanism by which mevalonic acid is incorporated into compounds of higher molecular weight has been investigated and it has been shown[49] that during this process the acid loses its carboxyl group. Furthermore the $C_{(5)}$ to $C_{(2)}$ (head to tail) condensation of mevalonic acid derivatives has been amply demonstrated by the localisation of C^{14} introduced into several natural products from 2-C^{14} mevalonate.[50-54]

The elegant research carried out by Arigoni[54] on the soyasapogenols and sterols isolated from soya beans grown on a radioactive substrate represents the first biosynthetic route to isotopically labelled pentacyclic triterpenes from a plant source. A second noteworthy feature of these experiments has been the observation that sterols and triterpenes isolated from soya beans have shown very comparable radioactivity thus supporting the hypothesis that squalene is a single common precursor of steroids and triterpenes. Such an assumption is not unreasonable in view of the fact that squalene has been isolated from oils of plant origin[55,56] and occurs in a wide variety of other natural products.[57-60]

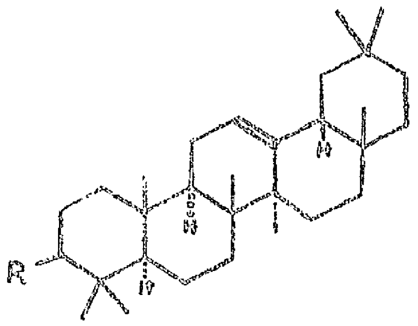
All the results so far obtained are in agreement with the general scheme for the cyclisation of squalene proposed by Woodward and Bloch.[35] The fact that so many variations of structure can be explained on the basis of one hypothesis is in itself good evidence that it is correct. The squalene hypothesis therefore remains of primary importance in the field of triterpenoid chemistry.

THEORETICAL

SECTION I.

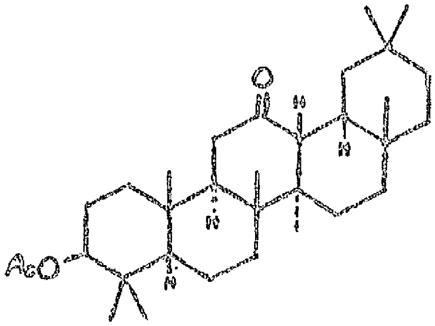
Some Reactions of β-Amyrin

Some reactions of β-amyrin (XXXIIa), 3β-hydroxy-olean-12-ene, are outlined below. These reactions are of particular interest since they have been applied to the soyasapogenols which are hydroxylated olean-12-ene derivatives.

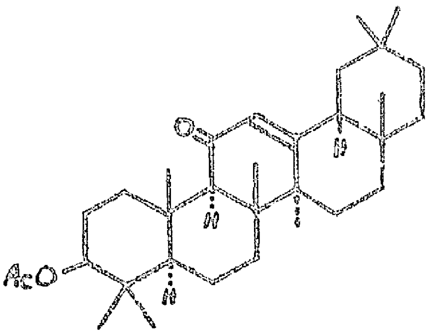


(XXXII)

(a) R =OH (b) R =OAc

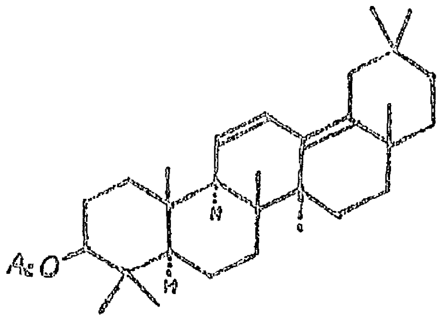


(XXXIV)

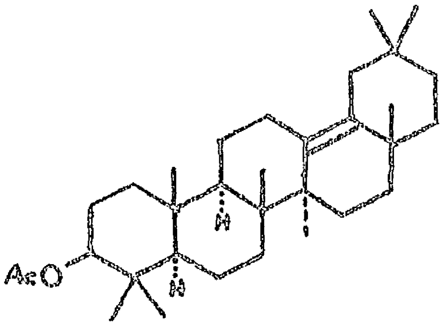


(XXXV)

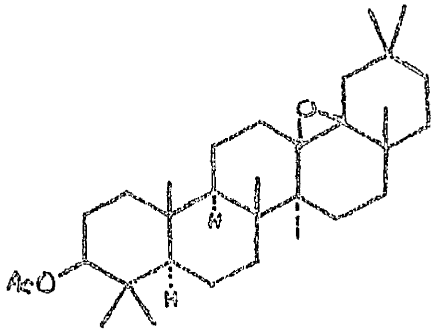
The double bond in β-amyrin derivatives is relatively unreactive and, for instance, cannot be hydrogenated. However, it is readily attacked by oxidising agents such as performic acid, chromium trioxide and selenium dioxide. Treatment of β-amyrin acetate (XXXIIb) with performic acid yields the saturated ketone[61] (XXXIV) whereas oxidation with chromium trioxide leads to the αβ-unsaturated ketone[62] (XXXV). The products obtained by oxidation of β-amyrin acetate with selenium dioxide vary with the conditions used. Oxidation of the acetate (XXXIIb) with selenium dioxide in boiling acetic acid gives the characteristic 11,13(18)-dienyl acetate[63] (XXXVI) which can be hydrogenated to δ-amyrin acetate[64] (XXXVII). On treatment with performic acid δ-amyrin acetate gives the 13(18)-epoxide (XXXVIII) which is converted, under acid conditions, into the 11,13(18)-dienyl acetate[65] (XXXVI).



(XXXVI)

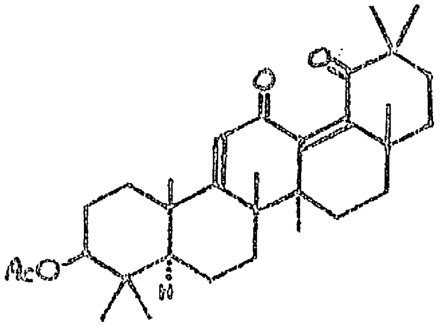


(XXXVII)

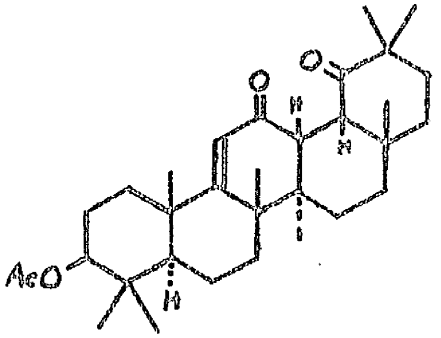


(XXXVIII)

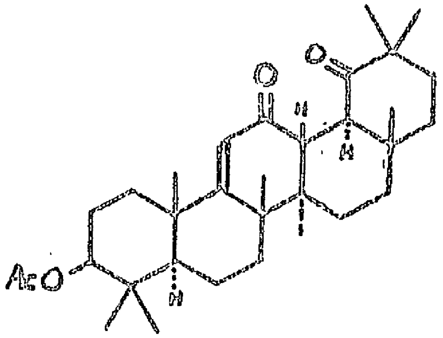
More vigorous oxidation of β-amyrin acetate with selenium dioxide e.g. in a sealed tube at 200°, yields the dioxodienyl derivative[65,66] (XXXIX). In this compound the 13(18)-double bond is readily reduced either by catalytic hydrogenation[64] or by means of zinc in ethanol.[67] The product is 3β-acetoxy-12,19-dioxo-olean-9(11)-ene (XL) and on treatment with boiling methanolic potassium hydroxide followed by acetylation it yields the more stable 18α-epimer[67] (XLI).



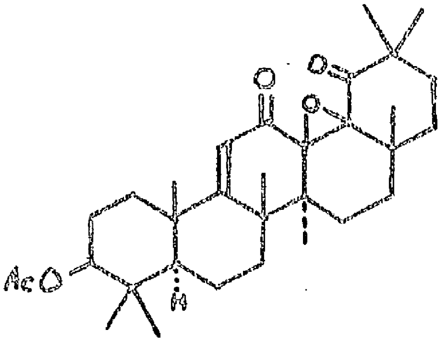
(XXXIX)



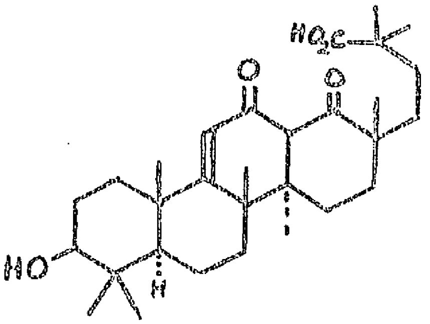
(XL)



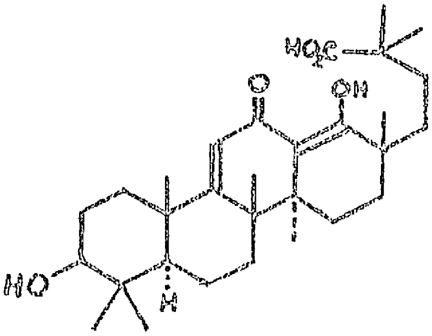
(XLI)



(XLII)



(XLIII)



(XLIV)

Further oxidation of the dioxodienyl acetate (XXXIX) can be effected by treatment with chromium trioxide in acetic acid at 90° when the 13(18)-epoxide (XLII) is produced.[68] Under vigorous alkaline conditions this compound yields a hydroxy-keto-acid (XLIII) which has been characterised as the acetate, methyl ester, and methyl ester acetate. The keto acid (XLIII), which can also exist in the enolic form (XLIV) is produced by fission of the bond between $C_{(18)}$ and $C_{(19)}$.

The Structures of Soyasapogenols B and D.

The soyasapogenols A,B,C and D comprise a group of triterpenoids which were originally isolated by hydrolysis of the saponin from soya beans.[69] Of these four compounds the first three have since been obtained from other sources.[70,71] The present author has shown that under the conditions used for the acid hydrolysis of the saponin, soyasapogenol B is readily converted into soyasapogenol D. It therefore seems likely that soyasapogenol D does not occur naturally but is an artefact. That soyasapogenol D does not occur in the other sources mentioned[70,71] is therefore readily explained since different methods of isolating the sapogenins were used in these cases.

In 1923 Murumatsu[72] reported the isolation of hispidic acid from the ethanolic extract of defatted soya beans. Later workers showed that hispidic acid was in fact a saponin which on hydrolysis gave a mixture of sapogenins.[73,74] Three of these sapogenins, corresponding to soyasapogenols A, B, and C, were first isolated in a pure state by Miyasaka.[75] Shortly afterwards Nozoye and Katsura[76] reported the isolation, from the same source, of four compounds, three of which had constants corresponding to those of the compounds obtained by Miyasaka. All four sapogenols were isolated from the calcium salt of the soya bean saponin by Ochiai, Tsuda, and Kitagawa.[77] The constants of these compounds agreed closely with those obtained previously by Nozoye[76] and further evidence of their purity was later provided by Meyer, Jeger and Ruzicka,[78,79] and finally by Smith, Smith and Spring,[80] all of whom obtained the sapogenins by methods other than that used by Ochiai et al.[77]

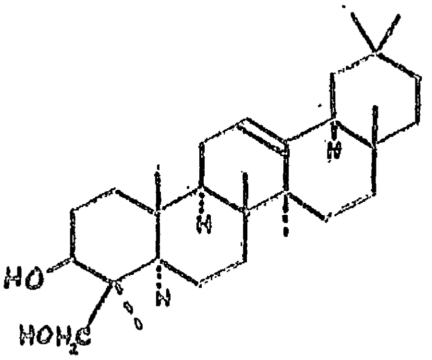
The triterpenoid nature of the soyasapogenols was demonstrated by Ochiai, Tsuda, and Kitagawa who dehydrogenated sapogenol B with selenium and obtained products characteristic of triterpenes,[81] namely, sapotalene and 1,2,5,6-tetramethyl naphthalene.[82] Sapogenols A, B, C, and D have been formulated as $C_{30}H_{46}(OH)_4$, $C_{30}H_{47}(OH)_3$, $C_{30}H_{46}(OH)_2$, and $C_{31}H_{30}O(OH)_2$ respectively and each contains a comparatively inert ethylenic linkage although soyasapogenol C has, in addition, a reactive double bond which can be hydrogenated.[79] Each of these compounds is pentacyclic and only sapogenol D contains a functional group, viz. an ether linkage,[83] in addition to the hydroxyl groups shown in the formulae above.

In the case of soyasapogenol B the reactivity of the unsaturated centre was comparable to that in oleanolic acid[83] and the double bond was therefore deduced to be at position 12(13). Experiments by Meyer et al.[78] have confirmed this postulation. From oxidation experiments similar to those carried out on hederagenin,[84] Tsuda and Kitagawa[85] have shown the existence of a 1:3-glycol group in ring A of soyasapogenol B.

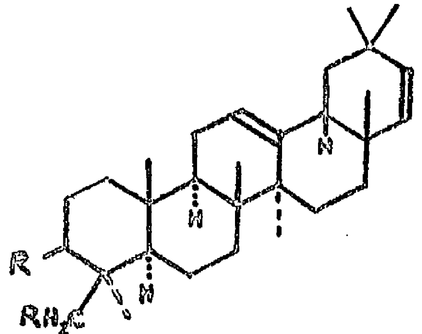
By methods which will receive more detailed treatment below, sapogenol B has been related directly to sapogenol C and thence to sapogenol A. Thus it has been conclusively demonstrated that the 1:3 glycol system in ring A and the 12(13)-double bond is common to all three compounds. Furthermore, interrelation of derivatives of sapogenols B and D has indicated the presence of the same 1:3 glycol system and of a 13(18)-double bond in the latter.

Irrevocable proof of the nature of the soyasapogenols was obtained by Meyer, Jeger, and Ruzicka[79] who identified dihydro soyasapogenol C as 3β,24-dihydroxyolean-12-ene (XLV) and thus established the structure and stereochemistry of soyasapogenol C except for the position of the reactive double bond. Therefore all of the soyasapogenols contain the 3β,24 diol system in ring A and three of them, A, B, and C have a 12(13)-double bond. Thus the only functions which still had to be placed were:- the *cis* glycol group[79] in sapogenol A, the remaining hydroxyl group in sapogenol B, the reactive double bond in sapogenol C, and the other linkage in sapogenol D.

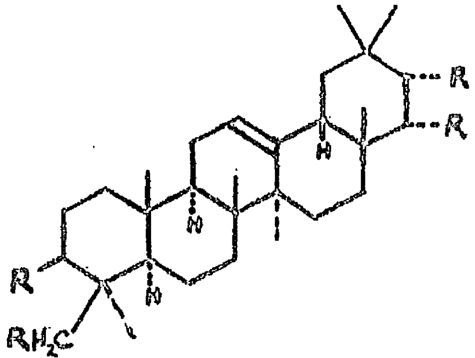
The structure of soyasapogenol C has recently been established as (XLVIa)[80] and its interrelationship with the other



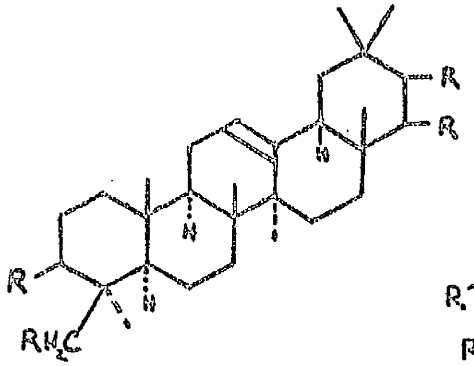
(XLV)



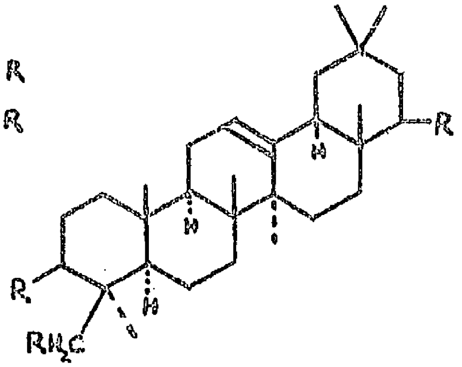
(XLVI)(a) R = OH
(b) R = OAc



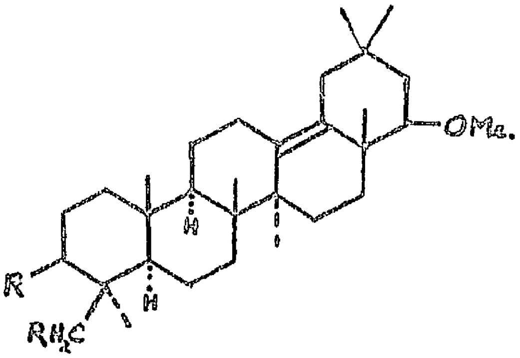
(XLVII)(a) R = OH
(b) R = OAc



(XLVIII)
(a) R = OH
(b) R = OAc



(XLIX)
(a) R = OH
(b) R = OAc

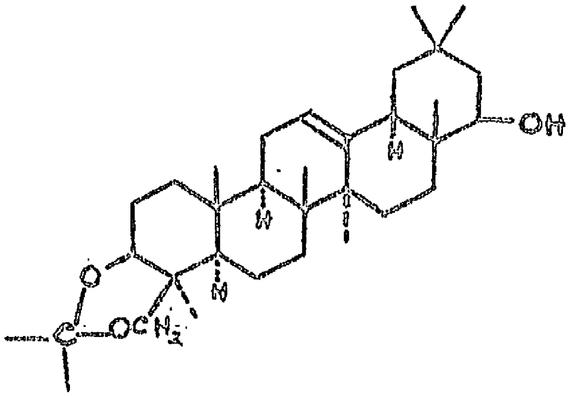


(L)
(a) R = OH
(b) R = OAc

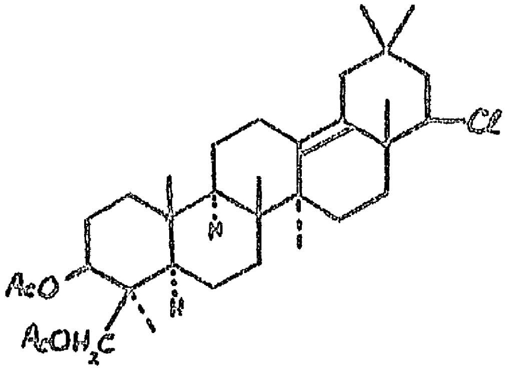
members of the group can thus be represented as follows. Treatment of soyasapogenol C (XLVIa) with osmium tetroxide[78,80] gave a mixture of isomeric tetrols which was resolved by chromatography of the corresponding acetates. Of the two components, (XLVIIb) and (XLVIIIb), separated in this way, that formed in lower yield was identified as soyasapogenol A tetra-acetate. Since it was formed in lower yield, and from a study of molecular models, Smith et al.[20] suggested that sapogenol A was produced by attack from the more hindered α-face of ring E and could therefore be represented by (XLVIIa) while the more abundant epimer (XLVIIIa) was produced by attack from the less hindered β-face. However, this evidence is not completely conclusive with regard to stereochemistry.

On the basis of evidence presented below sapogenols B and D may be represented by (XLIXa) and (La) respectively. The positions of the third hydroxyl group in sapogenol B and of one end of the ether linkage in sapogenol D were fixed relative to the reactive double bond in sapogenol C by the following reactions.

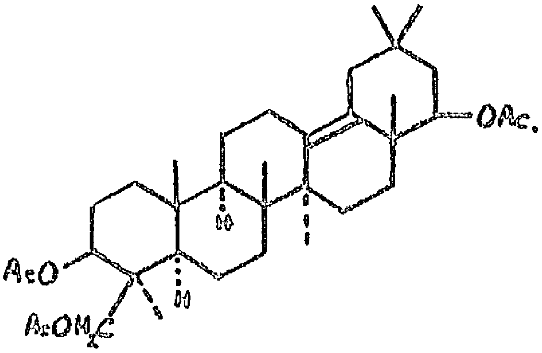
Dehydration of the isopropylidene derivative (LI) of soyasapogenol B followed by acid hydrolysis, to remove the protecting group in ring A, and acetylation gave soyasapogenol C diacetate (XLVIb) in good yield.[20] Since the active double bond in sapogenol C is in the 21(22) position the third hydroxyl group in sapogenol B must therefore be located at either $C_{(21)}$ or $C_{(22)}$. The oxide bridge in sapogenol D is readily attacked by ether-splitting reagents. Thus treatment of sapogenol D diacetate with hydrogen chloride gave the chloro-compound (LII) which was converted directly into the 13(18)-double bond isomer (LIII) of sapogenol B triacetate by treatment with sodium acetate and acetic anhydride.[88] In this way it was established that the oxygen functions in ring E of soyasapogenols B and D were attached to the same carbon atom.



(LI)



(LII)

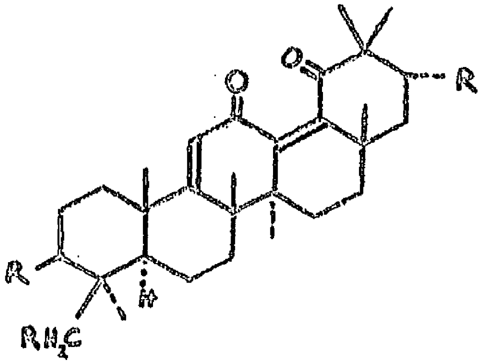


(LIII)

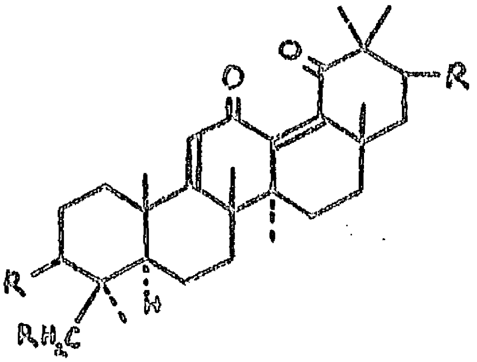
The Structure of Soyasapogenol B.

Several methods have been used in attempts to differentiate between positions 21 and 22 for the third hydroxyl group in sapogenol B. Smith *et al.*[80] were able to show that the hydroxyl group in ring E of soyasapogenol B was axially orientated and it must therefore be either 21α or 22β. This conclusion was drawn from experiments which showed that the free alcohol group in the isopropylidene derivative (LI) of sapogenol B was more hindered than that of the corresponding epimeric compound which differed from (LI) only in the configuration of the ring E substituent.

In the dioxodienyl derivative (LIVa) of sapogenol B the ring E hydroxyl group becomes equatorial due to the introduction of the 13(18)-double bond while the epimeric compound (LVa) has an axial substituent in ring E. Treatment of the epimeric dioxo-dienyl acetate (LVb) with alkali followed by acetylation gave the corresponding derivative (LIVb) of sapogenol B in almost quantitative yield. The inversion of configuration was attributed to the presence of a 21α-hydroxyl group which caused retroaldolisation, resulting in the opening of the β-hydroxy ketone system in ring E followed by its closure in the alternate sense.[80]



(LIV)(a) R = OH
(b) R = OAc

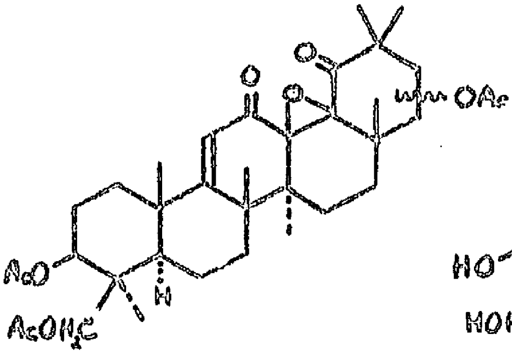


(LV) (a) R = OH
(b) R = OAc

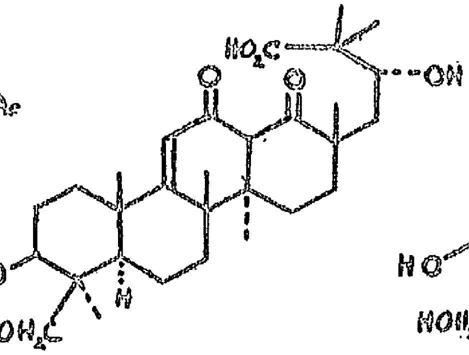
However, the above interpretation of the epimerisation is not completely unambiguous and Arigoni *et al*[88] have suggested that if the third hydroxyl group occupied the 22β- position the reversal of configuration could be caused by the activating influence of the 13(18)-double bond on that position.[88] The reactions described below were therefore undertaken in an attempt to differentiate conclusively between positions 21 and 22.

The first of these experiments was based on a series of reactions which have been well characterised in the β-amyrin series. Oxidation of the dioxodienyl derivative of sapogenol B triacetate with chromic acid at 90° gave a neutral compound, $C_{36}H_{50}O_{9}$, which showed very similar light absorption properties, $\lambda_{max.}$ 2560 Å. (ε, 12,000), to those of the 13(18)-epoxide (XLII) obtained in the β-amyrin series.[88] The sapogenol B derivative is therefore represented by structure (LVI). By further analogy with the β-amyrin series it was expected that treatment of the epoxide (LVI) with alkali at high temperature would give an acid, either (LVII) or (LVIII) depending on the position of the hydroxyl group.

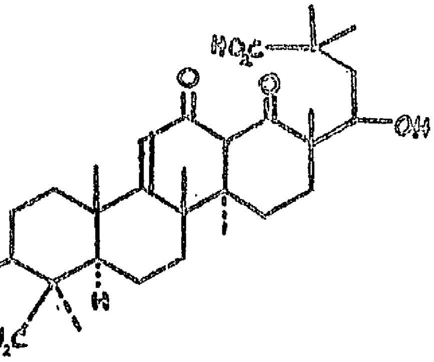
Differentiation between these two compounds could readily be made by simple degradative experiments. However, neither the acidic nor the neutral fraction from the alkaline fission reactions gave any tractable material and this line of approach was consequently abandoned.



(LVI)

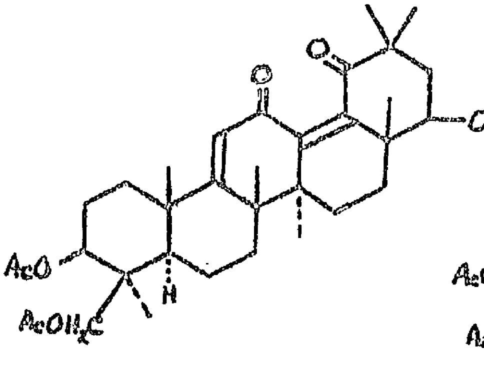


(LVII)

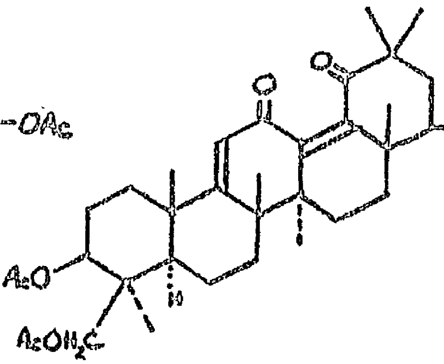


(LVIII)

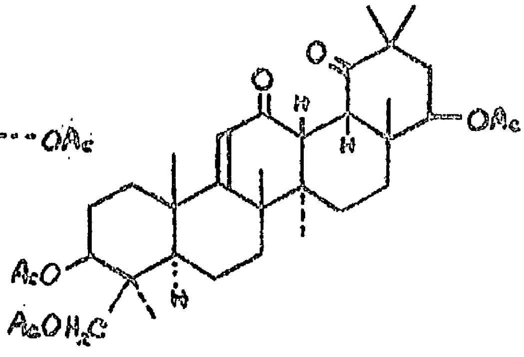
As stated above, Arigoni et al.[88] have suggested that the ring E substituents of soyasapogenols B and D are attached to $C_{(22)}$ and are therefore influenced by the 13(18)-double bond. It was decided that the simplest method of testing this hypothesis was by saturation of the 13(18)-double bond in the epimeric dioxodienyl derivatives (LIX) and (LX) to give compounds (LXI) and (LXII) which would not be expected to epimerise at position 22 under alkaline conditions since the source of activation had been removed. If on the other hand the hydroxyl group was attached to $C_{(21)}$, epimerisation could still take place by the retro-aldol type reaction as in the case of the dihydrodioxodienyl derivative of soyasapogenol A[60] which definitely contains a 21-hydroxyl group but not a 13(18)-double bond.



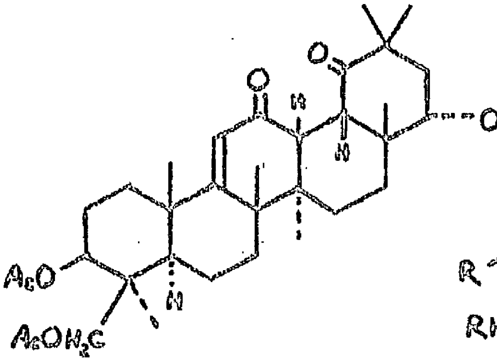
(LIX)



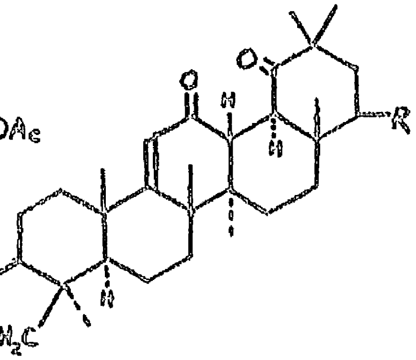
(LX)



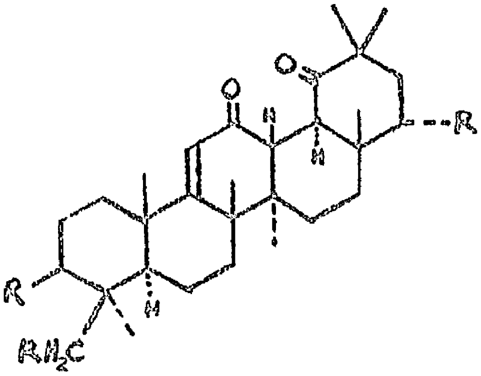
(LXI)



(LXII)



(LXIII) (a) R = OH
(b) R = OAc



(LXIV) (a) R = OH
(b) R = OAc

Treatment of the dioxodienyl triacetates (LIX) and (LX) with zinc and ethanol gave the corresponding ene-diones (LXI) and (LXII) respectively.[cf. 67] These dihydro compounds were then boiled under reflux with methanolic alkali in a nitrogen atmosphere to yield the trihydroxy derivatives (LXIIIa) and (LXIVa) respectively. The triols were not identical and on acetylation they yielded the corresponding triacetates (LXIIIb) and (LXIVb) which again differed from each other. Furthermore, these triacetates were different from both of the original compounds (LXI)and (LXII) and it was evident that epimerisation had occurred at $C_{(18)}$ although there had been no epimerisation of the ring E hydroxyl group. Only in this way can the formation of the two different triacetates (LXIIIb) and (LXIVb) be explained. It was therefore concluded that in the case of the epimeric sapogenol B dioxodienyl derivatives[60] (LIX) and (LX), the inversion of configuration took place at the 22 position and was due to the proximity of the 13(18)-double bond. Although the above evidence is of a negative nature the conclusion has been substantiated to some extent by the reactions of soyasapogenol D which supply considerable evidence for the activating influence of the 13(18)-double bond on the 22-position. It is therefore the author's opinion that soyasapogenol B is best represented by structure (XLIXa).

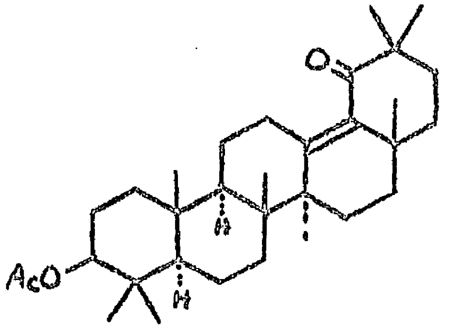
The Structure of Soyasapogenol D.

In the earlier investigation of the soyasapogenols, Tsuda and Kitagawa[83] demonstrated that sapogenol D contained two hydroxyl groups, an inert oxygen function and a double bond. That the double bond was tetrasubstituted was shown by Meyer et al.[79] who converted soyasapogenol D into the 13(18)-isomer of soyasapogenol B triacetate. This finding has been completely confirmed by all the subsequent reactions of soyasapogenol D which have given products expected of a fully substituted double bond.

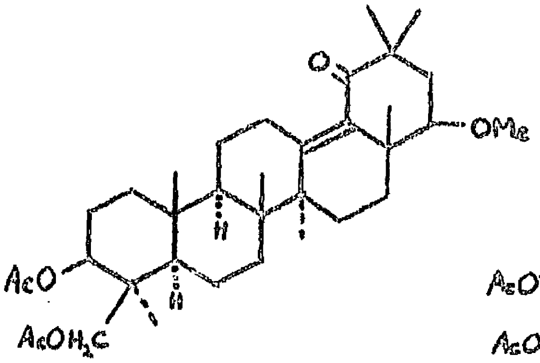
Oxidation of sapogenol D diacetate with chromium trioxide in acetic acid at room temperature gave a mixture which was resolved by chromatography on alumina into four components each one of which still showed the band, at 1100 cm.$^{-1}$ in the infrared spectrum, characteristic of an ether linkage. The first of these compounds was a cisoid αβ-unsaturated ketone which showed maximal absorption in the ultraviolet at 2540 Å. (ε, 7,000)cf.[87] and a band in the infrared spectrum at 1667 cm.$^{-1}$ Similar oxidation of δ-amyrin acetate yielded a compound which had an ultraviolet absorption maximum at 2560 Å. (ε, 6,800) and showed a band in the infrared spectrum at 1670 cm.$^{-1}$. Since this compound was most likely an impure sample of 19-oxo-olean-13(18)-en-3β-yl acetate[84] (LXV) and the molecular rotation differences of the δ-amyrin and sapogenol D derivatives were very similar the latter has been formulated as (LXVI).

Molecular Rotation Differences

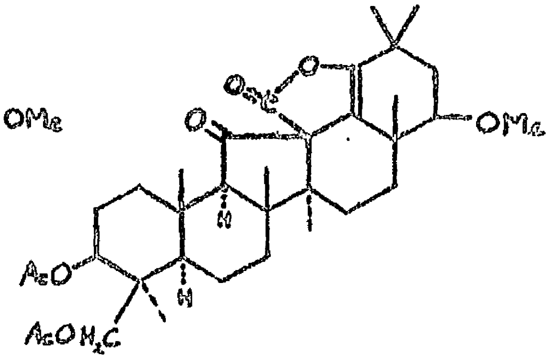
	$[\alpha]_D$	M_D	ΔM_D
δ-amyrin acetate	− 35.3°	−155.5°	−386.5°
derived αβ-unsaturated ketone	−112.5°	−542.0°	
Soyasapogenol D diacetate	− 44°	−245°	−388°
derived αβ-unsaturated ketone	−110.5°	−633°	



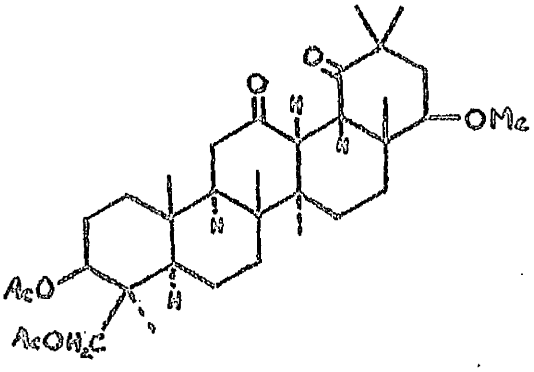
(LXV)



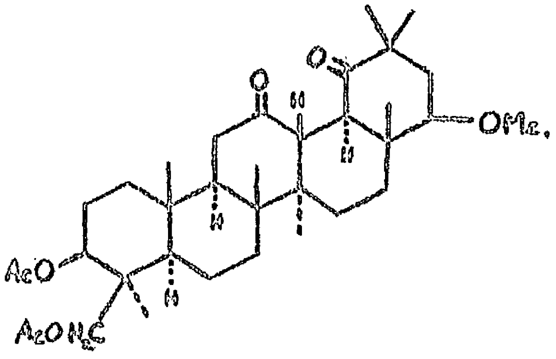
(LXVI)



(LXVII)



(LXVIII)



(LXIX)

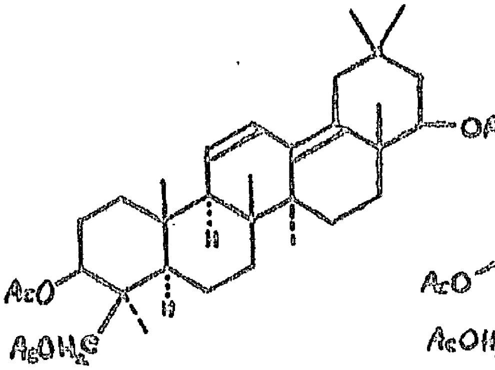
The second of these compounds showed ultraviolet absorption at 2260 Å ($\mathcal{E}$, 4,000) and bands in the infrared spectrum at 1787 and 1748 cm.$^{-1}$ Its light absorption properties are almost identical to those of the O_5-acetate in the β-amyrin series[68] and since analysis shows that its molecular formula is $C_{33}H_{50}O_5$ the sapogenol D derivative has been assigned the structure (LXVII). Further evidence for the location of the double bond at the 13(18)-position in sapogenol D is thus provided by these two compounds.

Two further compounds were obtained from the chromic acid oxidation. Both of these products showed strong absorption in the carbonyl region of their infrared spectra but neither of them exhibited any selective absorption in the ultraviolet region. Neither of these compounds has been further investigated but in the light of present evidence could be formulated as the epimeric diketones (LXVIII) and (LXIX).

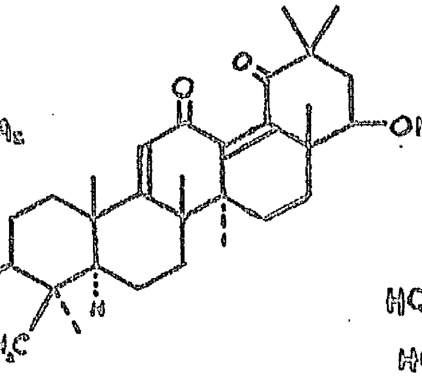
Additional evidence is presented below which confirms the position of the double bond and establishes the nature of the ether linkage. In earlier work the ether was considered as a five or higher membered oxide ring mainly because of the band at 1100 cm.$^{-1}$ in the infrared spectrum and the ease with which the ether linkage was split.[79] The author has shown independently, by a method similar to that published by Arigoni <u>et al</u>.,[68] that the ether in soyasapogenol D is, in fact, present as a methoxyl group which is readily replaced by chlorine on treatment with hydrogen chloride thus yielding the chloro-compound (LII). Similarly the ether linkage in soyasapogenol D is readily split by treatment with boron trifluoride in acetic anhydride to yield the triacetate (LIII) which can also be prepared from the chloro-derivative (LII) by the action of acetic anhydride and sodium acetate, or from the 11,13(18)-dienyl derivative (LXX) of soyasapogenol B triacetate by hydrogenation. The fact that the soyasapogenol B derivative (LIII) can be prepared directly from soyasapogenol D and also by way of the chloro-compound (LII) indicates that the methoxyl group in sapogenol D has the same configuration (β) as the ring E hydroxyl group in sapogenol B.

Chemical evidence for the presence of one methoxyl group in sapogenol D was initially provided by a Zeisel determination and completely confirmed by the regeneration of sapogenol D from the chloro-compound (LII) by treatment of the latter with a boiling solution of sodium methoxide. In addition, soyasapogenol B (XLIXa) is readily converted into sapogenol D (La) by boiling it under reflux with methanolic hydrochloric acid, conditions used to hydrolyse the soya bean saponin.

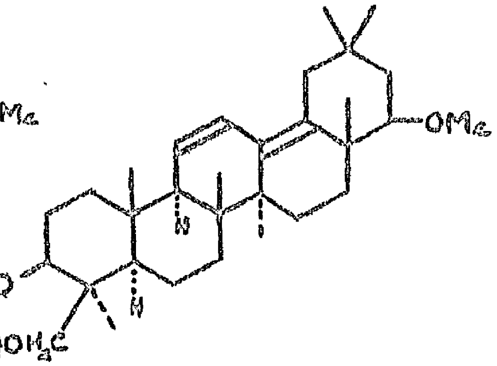
The ready interconversion of ring E substituents in derivatives of soyasapogenols B and D is only explicable if there is a nearby source of activation. It is therefore quite conceivable that the 22-position is activated by a source such as the 13(18)-double bond. Experimental evidence bears out this supposition since both the αβ-unsaturated ketone (LXVI) and the dioxodione (LXXI) derived from sapogenol D diacetate are recovered unchanged[80] after treatment with hydrochloric acid in acetic acid at 100°, conditions which quickly convert sapogenol D diacetate to the corresponding chloro-compound (LII). Similarly, soyasapogenol B is readily converted into soyasapogenol D by boiling methanolic hydrochloric acid whereas the dioxodionyl derivative (LIX) of the former is unaffected under the same conditions. It is obvious from these results that the electron withdrawing effect of the ketone groups at positions- 12 and 19 prevents activation of position-22 by the 13(18)-double bond.



(LXX)



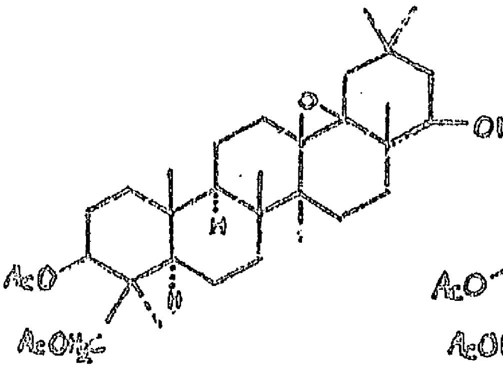
(LXXI)



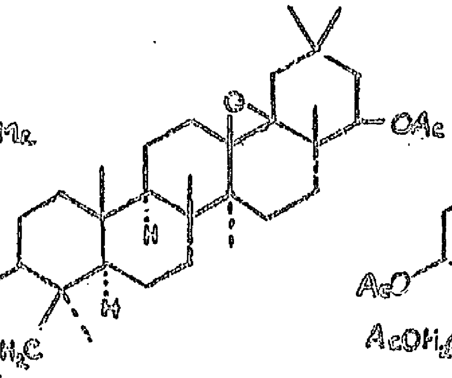
(LXXII)

A further significant fact is that the 11,13(18)-diene (LXXII) cannot be formed directly from sapogenol D, an olean-13(18)-enyl derivative, by oxidation with selenium dioxide. When treated with this reagent in boiling acetic acid, soyasapogenol D diacetate gave a small amount of the pure dioxodienyl derivative (LXXI) and an inseparable mixture whose ultraviolet absorption spectrum indicated that it contained some of the 11,13(18)-dienyl derivative (LXXII). More prolonged treatment under the same conditions gave two derivatives of soyasapogenol B, namely the 11,13(18)-diene (LXX) and the dioxodiene (LIX).[69] Again this illustrates the ease with which the 22-substituent can be replaced provided the 13(18)-double bond is present and is not deactivated by neighbouring carbonyl groups. Thus the 11,13(18)-dienyl derivative (LXX) of sapogenol B must be formed initially from the corresponding derivative (LXXII) of sapogenol D by solvolysis of the methoxyl group. Further oxidation of (LXX) then provides the dioxodienyl derivative (LIX) of sapogenol B.

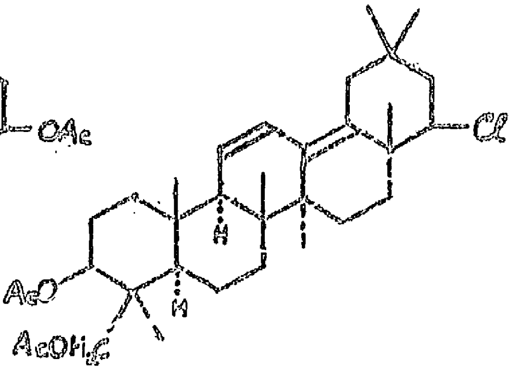
Further support for the hypothesis that the 13(18)-double bond is the source of activation has been provided by the following series of reactions. Oxidation of sapogenol D diacetate (Ib) with performic acid yielded a 13(18)-epoxide (LXXIII). This reaction is completely analogous to those of δ-amyrin acetate (XXXVII) and the 13(18)-isomer (LIII) of sapogenol B triacetate which yield the corresponding compounds (XXXVIII)[86] and (LXXIV). Under acid conditions these oxides (XXXVIII) and (LXXIV) rearrange to give the heteroannular dienes (XXXVI) and (LXX) respectively. However, treatment of sapogenol D diacetate oxide (LXXIII) with hydrochloric acid in acetic acid gave a compound which contained chlorine and exhibited the characteristic ultraviolet spectrum of the 11,13(18)-diene system. This compound has been formulated as 3β,24-diacetoxy-22-chloro-oleana-11,13(18)-diene (LXXV) which on treatment with a refluxing solution of sodium methoxide gave the 11,13(18)-dienyl derivative (LXXII) of sapogenol D. The structure of the latter compound was confirmed by acetylation and hydrogenation to yield soyasapogenol D diacetate (Lb), identified by mixed melting point and comparison of its infrared spectrum with that of an authentic sample.



(LXXIII)

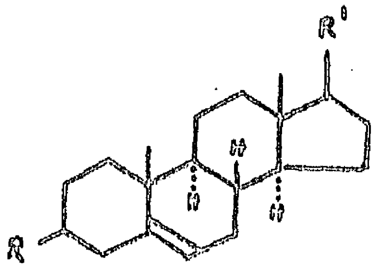


(LXXIV)



(LXXV)

An interesting comparison can be made between derivatives of sapogenol D and cholesterol. When cholesteryl chloride (LXXVIa) is heated to 120° with methanol it affords the corresponding methyl ether (LXXVIb).[90] Similarly the chloro-compound (LII) derived from soyasapogenol D is readily converted to the parent methoxyl compound (La) by treatment with methanol[88] at 120° or with sodium methoxide. It is significant that the double bond is in the same position, $\beta - \gamma$, relative to the chlorine atom in both of the compounds (LXXVIa) and (LII). Also, in both cases the environment of these groups is very similar. In conjunction with other evidence described above this favours structure (La) for soyasapogenol D.



(LXXVI) (a) R = Cl; R = C_8H_{17}
(b) R = OMe; R = C_8H_{17}

EXPERIMENTAL

SECTION I.

All melting points were uncorrected and were measured using a Kofler apparatus. Specific rotations were determined in chloroform solution unless otherwise stated using a one decimeter tube at room temperature. Ultraviolet spectra were carried out in absolute ethanol solution with either a Unicam SP500 or a Hilger H700.307 spectrophotometer. Infrared spectra were determined in Nujol mull unless otherwise stated using a Grubb-Parsons double beam spectrophotometer. Light petroleum refers to the fraction of b.p. 60-80° and Grade II alumina was used throughout.

Oxidation of 3β,22β,24-Triacetoxy-12,19-dioxo-oleana-9(11),13(18)-diene with Chromic Acid. - A solution of soyasapogenol B dioxodienyl triacetate, m.p. 272-274°, (2 g.) in glacial acetic acid (40 c.c.) was treated with chromium trioxide (2 g.) in acetic acid (90%, 45 c.c.), added during 10 minutes. The mixture was maintained at 90° for 1.5 hr., then methanol (5 c.c.) was added and the reaction mixture was poured into water and extracted with ether. The product, a resinous gum, (1.9 g.) was dissolved in benzene (50 c.c.) and chromatographed on alumina (50 g.). Elution with benzene-ethe (9:1, 1.5 l.) gave a fraction (1.1 g.) which crystallised from chloroform-light petroleum to give 3β,22β,24-triacetoxy-12,19-dioxo-13(18)-epoxy-olean-9(11)-ene as clusters of needles, m.p. 220-222°, $[\alpha]_D$ + 51° (c,0.8), $\lambda_{max.}$ 2560 Å. (ϵ = 12,000). The infrared spectrum showed bands at 1730, 1240 cm.$^{-1}$ (OAc), 1681 cm.$^{-1}$ (αβ-unsaturated ketone). (Found: C,68.9; H,8.2. $C_{36}H_{50}O_9$ requires C,69.0; H,8.0%).

Alkaline Treatment of 3β,22β,24-Triacetoxy-12,19-dioxo-13(18)-epoxy-olean-9(11)-ene. - (a) The above compound, m.p. 220-222°, (407 mg.) was treated with methanolic potassium hydroxide (10%, 10 c.c.) in an autoclave at 150° for 7 hr. The acidic and neutral components were separated in the usual way. The acidic fraction (91 mg.) failed to give any tractabl material even after acetylation and chromatography on silica gel. The ultraviolet spectrum of the gum showed maxima at 2100 and 2440 Å. Its infrared spectrum (in chloroform) showed bands at 3378, 1712, 1664, 1605 cm.$^{-1}$.

The neutral fraction (193 mg.) could not be crystallised and was acetylated in the usual way. Chromatography of the product failed to yield any crystalline material. The infrared spectra (in chloroform) of most of the fractions showed bands at 1670 and 1605 cm.$^{-1}$ as well as a band at 1730 cm.$^{-1}$ (OAc).

(b) A solution of the above compound, m.p. 220-222°, (190 mg.) in amyl alcohol (5 c.c.) was added to freshly prepared sodium amyloxide (from sodium, 250 mg., and amyl alcohol, 5 c.c.) and the mixture was refluxed for 90 minutes. The reaction mixture was diluted with water and steam-distilled to remove excess amyl alcohol. From the non-volatile residue the acidic (59 mg.) and neutral (145 mg.) fractions were separated in the usual manner. Again both fractions were completely intractable.

Treatment of Soyasapogenol B with Acetone and Sulphuric Acid. - A solution of soyasapogenol B (3 g.) in acetone (250 c.c.) was treated with concentrated sulphuric acid (8 c.c.) in dry ether (800 c.c.) at 17° for 48 hr. The product, isolated in the usual way, was dissolved in benzene-light petroleum

(1:1, 200 c.c.) and chromatographed on alumina (60 g.).

Fractions eluted with benzene (1 l.) and benzene-ether (9:1, 2 l.) were crystallised from aqueous methanol to give 22β-hydroxy-3β:24-isopropylidenedioxy-olean-12-ene, m.p. 173-177°, $[\alpha]_D$ + 72° (c, 1.76).

The infrared spectrum was identical with that of an authentic specimen.

Oxidation of the Isopropylidene Derivative. - The complex prepared from chromium trioxide (1 g.) and pyridine (10 c.c.) was added to a solution of the isopropylidene derivative of soyasapogenol B (1.1 g.) in pyridine (10 c.c.) and the mixture kept at 17° for 20 hr. The product (0.93 g.), isolated by means of ether, was dissolved in light petroleum (70 c.c.) and chromatographed on alumina (20 g.). Elution with light petroleum gave a fraction (0.69 g.) which was crystallised from aqueous methanol to give 22-oxo-3β:24-isopropylidenedioxy-olean-12-ene as rods, m.p. and mixed m.p. 208-209°, $[\alpha]_D$ - 13.2° (c, 2.2). The infrared spectrum was identical with that of an authentic sample.

Reduction of 22-Oxo-3β:24-isopropylidenedioxy-olean-12-ene. A solution of the ketone (0.69 g.) in ether (150 c.c.) was boiled under reflux with a suspension of lithium aluminium hydride (0.7 g.) for 3 hr. The product, isolated by means of ether but without using mineral acid, was dissolved in benzene-light petroleum (1:1, 100 c.c.) and chromatographed on alumina (25 g.).

Fractions (270 mg.) eluted with benzene and benzene-ether (4:1) were crystallised from aqueous methanol to give, 22α-hydroxy-3β:24-isopropylidenedioxy-olean-12-ene as needles, m.p.128-134°, at this point the material solidified and melted again at 183-185°, $[\alpha]_D$ + 58.5° (c, 1.25). The infrared spectrum was identical with that of an authentic sample.

Hydrolysis of 22α-Hydroxy-3β:24-isopropylidenedioxy-olean-12-ene. - A solution of 22α-hydroxy-3β:24-isopropylidenedioxy-olean-12-ene (270 mg.) in methanol (120 c.c.) was treated with concentrated hydrochloric acid (40 c.c.) and the mixture was refluxed for 30 minutes. Isolation of the product in the usual way followed by acetylation with acetic anhydride-pyridine (1:1, 10 c.c.) at 100° for 1 hr. gave a product (258 mg.) which was dissolved in benzene-light petroleum (3:2, 100 c.c.) and chromatographed on alumina.

Fractions eluted with the same solvent mixture were crystallised from chloroform-methanol to give 3β,22α,24-triacetoxy-olean-12-ene as prisms, m.p. 217-218°, $[\alpha]_D$ + 64.8° (c, 0.95). The infrared spectrum was identical with that of an authentic specimen.

4

Vigorous Oxidation of 3β,22α,24-Triacetoxy-olean-12-ene with Selenium Dioxide. - A solution of the epimeric sapogenol B triacetate, m.p. 217-218°, (200 mg.) in benzyl acetate (10 c.c.) was boiled under reflux with selenium dioxide (200 mg.) for 24 hr. Metallic selenium was removed by filtration and the benzyl acetate was evaporated under vacuum. A solution of the product in benzene-light petroleum (1:1, 50 c.c.) was chromatographed on alumina (6 g.).

Elution with benzene-ether (9:1) gave a fraction which crystallised from aqueous methanol to give 3β,22α,24-triacetoxy-12,19-dioxo-oleana-9(11),13(18)-diene as needles (133 mg.), m.p. 240-241°, $[\alpha]_D$ - 56.4° (*c*, 2.34). The infrared spectrum was identical with that of an authentic sample.

Reduction of 3β,22α,24-Triacetoxy-12,19-dioxo-oleana-9(11),13(18)-diene with Zinc in Ethanol. - A solution of the dioxodienyl triacetate (133 mg.) in ethanol (15 c.c.) was refluxed for 5 hr. with freshly activated zinc dust (1.4 g.) to which had been added a trace of zinc bromide. The solution was filtered and evaporated to dryness to give a product which was dissolved in benzene-light petroleum (1:2, 15 c.c.) and chromatographed on alumina.

Fractions eluted with benzene-ether (9:1, 4:1) were crystallised from chloroform-light petroleum to give 3β,22α,24-

-*triacetoxy*-12,19-*dioxo*-*olean*-9(11)-*ene* as plates, m.p. 234-236°, $[\alpha]_D$ + 90.4° (*c*,1.01), $\lambda_{max.}$ 2450 Å.($\mathcal{E}$ = 12,930). The infrared spectrum showed bands at 1730, 1241 cm.$^{-1}$ (OAc), 1667 cm.$^{-1}$ (αβ-unsaturated ketone), 1705 cm.$^{-1}$ (ketone). (Found: C,70.85; H,8.8. $C_{36}H_{52}O_8$ requires C,70.6; H,8.6%).

Hydrolysis of 3β,22α,24-*Triacetoxy*-12,19-*dioxo*-*olean*-9(11)-*ene*. - A solution of the dihydro dioxodienyl triacetate, m.p. 234-236°, (41.6 mg.) in methanol (45 c.c.) was treated with potassium hydroxide (1.5 g.) in water (5 c.c.) and the mixture was boiled under reflux for 2.5 hr. in an atmosphere of nitrogen. Extraction of the product in the usual manner gave a white solid (34.6 mg.) which was crystallised from methanol-light petroleum to give 3β,22α,24-*trihydroxy*-12,19-*dioxo*-18α-*olean*-9(11)-*ene* as plates, m.p. 350-352°, $[\alpha]_D$ + 85.9° (*c*,1.03) $\lambda_{max.}$ 2420 Å. ($\mathcal{E}$ = 12,500). The infrared spectrum showed bands at 3279 cm.$^{-1}$ (OH), 1705 cm.$^{-1}$ (ketone), 1680 cm.$^{-1}$ (αβ-unsaturated ketone). (Found: C,73.9; H,9.4. $C_{30}H_{46}O_5$ requires C,74.0; H,9.5%).

3β,22α,24-*Triacetoxy*-12,19-*dioxo*-18α-*olean*-9(11)-*ene*. - The above triol (32 mg.) was treated with pyridine-acetic anhydride (1:1, 4 c.c.) at 100° for 1 hr. The product (40.6 mg.), isolated in the usual manner, was dissolved in benzene-light petroleum (1:1, 10 c.c.) and chromatographed on alumina

(10 g.). A fraction eluted with benzene-ether (9:1) was crystallised from chloroform-light petroleum to give 3β,22α,24-triacetoxy-12,19-dioxo-18α-olean-9(11)-ene as needles, m.p. 215-217°, $[\alpha]_D$ + 74.5° (c,1.20), $\lambda_{max.}$ 2420 Å. (ϵ = 11,230). The infrared spectrum showed bands at 1730, 1241 cm.$^{-1}$ (OAc), 1703 cm.$^{-1}$ (ketone) 1670 cm.$^{-1}$ (αβ-unsaturated ketone). (Found: C,70.3; H,8.7. $C_{36}H_{52}O_8$ requires C,70.6; H,8.6%).

Hydrolysis of 3β,22β,24-Triacetoxy-12,19-dioxo-olean-9(11)-ene. - Treatment of the dioxodienyl derivative with zinc in ethanol gives 3β,22β,24-triacetoxy-12,19-dioxo-olean-9(11)-ene as blades m.p. 265-267°, $[\alpha]_D$ + 125° (c,1.30)

A solution of this compound (200 mg.) in methanol (45 c.c.) was treated with potassium hydroxide (1.5 g.) in water (5 c.c.) and the mixture was refluxed for 3 hr. in a nitrogen atmosphere.

The product was isolated by means of ether and was crystallised from methanol to give 3β,22β,24-trihydroxy-12,19-dioxo-18α-olean-9(11)-ene as needles, m.p. 315-316°, $[\alpha]_D$ + 66.5° (c,0.68), $\lambda_{max.}$ 2420 Å. (ϵ = 10,800). The infrared spectrum showed bands at 3344 cm.$^{-1}$ (OH), 1701 cm.$^{-1}$ (ketone in six membered ring), 1672 cm.$^{-1}$ (αβ-unsaturated ketone). (Found: C,73.8; H,9.55. $C_{30}H_{46}O_5$ requires C,74.0; H,9.5%).

3β,22β,24-Triacetoxy-12,19-dioxo-18α-olean-9(11)-ene. - A solution of the above triol (75 mg.) in pyridine (2.5 c.c.)

was treated with acetic anhydride (2.5 c.c.) and the mixture was kept at 100° for 2 hr. Extraction with ether gave a product (89.4 mg.) which was crystallised from chloroform-methanol to give 3β,22β,24-*triacetoxy*-12,19-*dioxo*-18α-*olean*-9(11)-*ene* as prismatic needles, m.p. 305-307°, $[\alpha]_D$ + 53.5° (*c*,0.91), $\lambda_{max.}$ 2420 Å. (ε = 12,800). The infrared spectrum showed bands at 1730, 1244 cm.$^{-1}$ (OAc), 1701 cm.$^{-1}$ (ketone), 1678 cm.$^{-1}$ (αβ-unsaturated ketone).

Examination of the Soyasapogenols for Methoxyl Groups. Analyses of the acetates of soyasapogenols A, B, and C showed that none of these compounds contained a methoxyl group. However, analysis of soyasapogenol D diacetate showed the presence of one methoxyl group. (Found: OMe = 5.8%. $C_{35}H_{56}O_5$ requires for 1 O-Me group 5.6%).

Treatment of Soyasapogenol D Diacetate with Hydrochloric Acid. - Concentrated hydrochloric acid (1 c.c.) was added to a suspension of soyasapogenol D diacetate (150 mg.) in glacial acetic acid (35 c.c.) and the resultant solution was kept at 100° for 20 minutes. Evaporation of the solvent under reduced pressure gave a product which was crystallised from chloroform-methanol to give 3β,24-diacetoxy-22-chloro-olean-13(18)-ene as needles, m.p. and mixed m.p. 206-208°, $[\alpha]_D$ - 43° (*c*,0.95). The infrared spectrum was identical with that of an authentic specimen and showed no band at 1100 cm.$^{-1}$.

Treatment of 3β,24-Diacetoxy-22-chloro-olean-13(18)-ene with Sodium Methoxide. - A solution of the chloro-compound (50 mg.) in dry methanol (7 c.c.) was treated with a solution of sodium methoxide (from sodium, 5 mg., and methanol, 8 c.c.) and the mixture was boiled under reflux for 16 hr. The product was isolated by means of ether and was crystallised from methanol to give soyasapogenol D as prisms, m.p. and mixed m.p. 287-292°, $[\alpha]_D$ - 59° (c,1.37). The infrared spectrum was identical with that of authentic soyasapogenol D and showed a band at 1100 cm.$^{-1}$.

The above compound, m.p. 287-292°, was treated with pyridine-acetic anhydride (1:1, 4 c.c.) at 100° for 1 hr. Working up in the usual way and crystallisation of the product from chloroform-methanol gave soyasapogenol D diacetate as plates, m.p. and mixed m.p. 192-194°, $[\alpha]_D$ - 44° (c,1.9). The infrared spectrum was identical with that of authentic soyasapogenol D diacetate.

Treatment of Soyasapogenol B with Mineral Acid. - A solution of soyasapogenol B, m.p. 255-258°, $[\alpha]_D$ + 90°, (2 g.) in methanol (240 c.c.) was treated with concentrated hydrochloric acid (40 c.c.) and the mixture was refluxed for 48 hr. The solution was allowed to cool and the material which separated out was filtered off and recrystallised from chloroform-methanol to give soyasapogenol D as prisms, m.p. and mixed m.p. 297-300°, $[\alpha]_D$ - 59° (*c*, 1.2). The infrared spectrum was identical with that of soyasapogenol D obtained from soya beans.

The identity of the soyasapogenol D prepared above was confirmed by preparation of the diacetate, m.p. and mixed m.p. 192-194°, $[\alpha]_D$ - 43.6° (*c*, 1.35). The infrared spectrum was identical with that of an authentic specimen.

Treatment of the dioxodienyl derivative of soyasapogenol B triacetate in the same way followed by acetylation gave unchanged starting material, identified by mixed m.p. and infrared spectrum.

Oxidation of δ-Amyrin Acetate with Chromium Trioxide. - A solution of δ- amyrin acetate (1 g.) in stabilised acetic acid (600 c.c.) was treated with chromium trioxide (900 mg.), in stabilised acetic acid (60 c.c.) at room temperature for 20 hr. The product, isolated in the usual manner, was dissolved in light petroleum and chromatographed on alumina (32 g.).

Fractions (230 mg.) eluted with light petroleum were crystallised from aqueous methanol as fine needles, m.p. 199-203°,

$[\alpha]_D$ - 105° (c,0.52), $\lambda_{max.}$ 2560 Å. ($\mathcal{E}$ = 6,800). The infrared spectrum showed bands at 1736, 1248 cm.$^{-1}$ (OAc), 1670 cm.$^{-1}$ (αβ-unsaturated ketone). This compound is probably impure 3β-acetoxy-19-oxo-olean-13(18)-ene for which Ruzicka[64] gives m.p. 219°.

Oxidation of Soyasapogenol D Diacetate with Chromium Trioxide. - Chromium trioxide (700 mg.) in a minimum of water and acetic acid (50 c.c.) was added to a solution of soyasapogenol D diacetate (1.06 g.) in stabilised acetic acid (50 c.c.). The mixture was allowed to stand at room temperature for 20 hr. then the excess of chromic acid was destroyed with methanol and the product was isolated by means of ether. A solution of the product (1.02 g.) in benzene-light petroleum (1:4) was chromatographed on alumina (25 g.).

Elution with benzene-light petroleum (1:1) gave a fraction (215 mg.) which crystallised from chloroform-methanol to give 3β,24-diacetoxy-22-methoxy-19-oxo-olean-13(18)-ene as plates m.p. and mixed m.p. 229-231°, $[\alpha]_D$ - 110.5° (c,1.8), $\lambda_{max.}$ 2550 Å. ($\mathcal{E}$ = 6,950). The infrared spectrum showed bands at 1735, 1250 cm.$^{-1}$ (OAc), 1672 cm.$^{-1}$ (αβ-unsaturated ketone), 1090 cm^{-1} (OMe group). (Found: C,73.5; H,9.3. $C_{35}H_{54}O_6$ requires C,73.6; H,9.5%).

A fraction eluted with benzene-light petroleum (2:1) was crystallised from methanol to give the O_8-*diacetate* as prisms (23 mg.), m.p. 296-298°, $[\alpha]_D$ + 8° (*c*,0.5), $\lambda_{max.}$ 2260 Å. (ε = 4,000). The infrared spectrum showed bands at 1735, 1242 cm.$^{-1}$ (OAc), 1748 cm.$^{-1}$ (five membered ring ketone), 1787 cm.$^{-1}$ (γ-lactone), 1092 cm.$^{-1}$ (O-Me group). (Found: C,70.3; H,8.35. $C_{35}H_{50}O_8$ requires C,70.2; H,8.4%).

Elution with benzene yielded a fraction which crystallised from chloroform-methanol to give 3β,24-*diacetoxy*-22-*methoxy*-12,19-*dioxo*-*oleanane* as blades (165 mg.), m.p. 237-239°, $[\alpha]_D$ + 50° (*c*,0.5), $\lambda_{max.}$ 2070 (ε = 4,000). This compound gave no colour with tetranitromethane and showed bands in the infrared at 1736, 1235 cm^{-1} (OAc), 1715 cm.$^{-1}$ (ketone in six membered ring), 1098 cm.$^{-1}$ (O-Me group). (Found: C,71.8; H,9.5. $C_{35}H_{54}O_7$ requires C,71.6; H,9.3%).

A further fraction was eluted with benzene-ether (19:1) and was crystallised from chloroform-methanol to give 3β,24-*diacetoxy*-22-*methoxy*-12,19-*dioxo*-18α-*oleanane*, as needles (110 mg.), m.p. 291-292°, $[\alpha]_D$ ± 0° (*c*,0.5), $\lambda_{max.}$ 2070 (ε = 3,500). This compound gave no colour with tetranitromethane and showed bands in the infrared spectrum at 1733, 1239 cm.$^{-1}$ (OAc), 1712 cm.$^{-1}$ (ketone in six membered ring), 1098 cm.$^{-1}$ (O-Me group). (Found: C,71.85; H,9.2. $C_{35}H_{54}O_7$ requires C,71.6; H,9.3%).

Oxidation of 3β,22β,24-Triacetoxy-olean-13(18)-ene with Performic Acid. - A solution of 3β,22β,24-triacetoxy-olean-13(18)-ene (380 mg.) in ethyl acetate (16 c.c.) was treated at 45° with hydrogen peroxide (100 vol., 3 c.c.) in formic acid (98-100%, 14 c.c.), added over 2.5 hr. The solution was kept at the same temperature for 2.5 hr. then the volume of the solution was reduced to approximately 12 c.c. The solid which separated on cooling was filtered off and recrystallised from methanol to give 3β,22β,24-*triacetoxy*-13(18)-*epoxy*-*oleanane* as rods (204 mg.), m.p. 228-230°, $[\alpha]_D$ + 8.7° (*c*,1.59). This compound gave no colour with tetranitromethane and was transparent to ultraviolet light. The infrared spectrum showed bands at 1724 and 1238 cm.$^{-1}$ (OAc). (Found: C,72.3; H,9.2. $C_{36}H_{56}O_7$ requires C,72.0; H,9.4%).

Treatment of 3β,22β,24-Triacetoxy-13(18)-epoxy-oleanane with Mineral Acid. - A solution of the above epoxide triacetate m.p. 228-230°, (100 mg.) in glacial acetic acid (15 c.c.) was treated with concentrated hydrochloric acid (0.5 c.c.) and the mixture was maintained at 100° for 30 minutes. Evaporation of the solvent and crystallisation of the residue from methanol gave needles, m.p. 248-250°, $\lambda_{max.}$ 2420, 2510, 2600 Å. (ε=24,000; 27,500; 18,300). Identity with 3β,22β,24-triacetoxy-oleana-

-11,13(18)-diene was shown by mixed m.p. and infrared comparison.

Oxidation of Soyasapogenol D Diacetate with Performic Acid. - Sapogenol D diacetate (532 mg.) in ethyl acetate (20 c.c.) was treated with hydrogen peroxide (100 vol., 3.5 c.c.) and formic acid (98-100%, 17 c.c.), added with stirring over 2.5 hr. at 45°. After a further 2.5 hr. at the same temperature the solution was evaporated to approximately 15 c.c. and allowed to crystallise. Recrystallisation of the product from chloroform-methanol gave 3β,24-*diacetoxy*-22-*methoxy*-13(18)-*epoxy-oleanane* as rods, m.p. 232-234°, $[\alpha]_D$ - 8.3° (c,1.21). This compound gave no colour with tetranitromethane and showed no selective ultraviolet absorption. The infrared spectrum showed bands at 1735, 1242 cm.$^{-1}$ (OAc) and 1100 cm.$^{-1}$ (O-Me group). (Found: C,73.5; H,9.7. $C_{35}H_{56}O_6$ requires C,73.4; H,9.85%).

Treatment of Soyasapogenol D Diacetate Oxide with Mineral Acid. - A solution of the epoxide (140 mg.) in glacial acetic acid (29 c.c.) was treated with concentrated hydrochloric acid (1 c.c.) and kept at 100° for 0.5 hr. The solution was evaporated to dryness under reduced pressure and the product was crystallised from chloroform-methanol to give 3β,24-*diacetoxy*-22-*chloro*-*oleana*-11,13(18)-*diene* as needles, m.p. 212-214°, $[\alpha]_D$ - 49° (*c*, 0.71), $\lambda_{max.}$ 2440, 2520, 2610 Å. (ε = 29,000; 34,500; 22,400). The infrared spectrum showed bands at 1730, 1253, 1237 cm.$^{-1}$ (OAc). (Found: C,73.3; H,9.4; Cl,6.0. $C_{34}H_{51}O_4Cl$ requires C,73.0; H,9.2; Cl6.35%).

3β-24-*Dihydroxy*-22-*methoxy*-*oleana*-11,13(18)-*diene*. - A solution of the above chlorodienyl acetate (60 mg.) in dry methanol (10 c.c.) was treated with a solution of sodium methoxide (from sodium, 500 mg., in methanol 10 c.c.) and the solution was refluxed for 16 hr. The product, isolated by means of ether, was crystallised from chloroform-methanol to give 3β,24-*dihydroxy*-22-*methoxy*-*oleana*-11,13(18)-*diene* as plates (44 mg.), m.p. 296-298°, $[\alpha]_D$ - 79° (*c*, 0.71), $\lambda_{max.}$ 2440, 2520, 2610 Å. (ε = 27,300; 31,600; 19,500). The infrared spectrum showed bands at 3390 cm.$^{-1}$ (OH), 1087 cm.$^{-1}$ (O-Me group). (Found: C,77.5; H,10.4 $C_{35}H_{54}O_5.0.5CH_3OH$ requires C,77.7; H,10.7%).

Hydrogenation *of* *the* 11,13(18)-*Diene*.- The diene-diol, m.p. 296-298°, (18 mg.) was acetylated with pyridine and acetic anhydride (1:1, 4 c.c.) at room temperature. The product (21 mg.), isolated with ether, was dissolved in analar glacial acetic acid (25 c.c.) and shaken with hydrogen in the presence of platinum (from platinum oxide, 20 mg.) for 15 hr. Working up in the usual way and crystallisation of the product from chloroform-methanol gave soyasapogenol D diacetate m.p. and mixed m.p. 188-191°, $\lambda_{max.}$ 2080 Å. (ε = 9,140). A comparison of the infrared spectra, in carbon tetrachloride, confirmed that the product was sapogenol D diacetate.

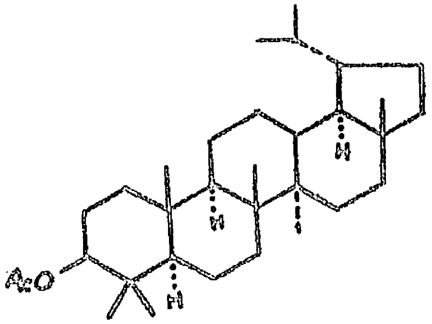
THEORETICAL

SECTION II

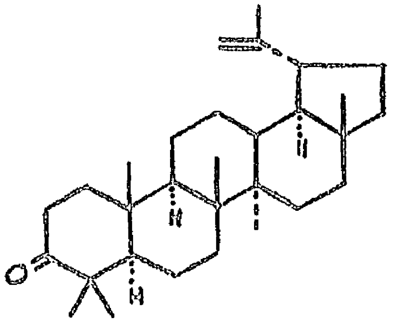
In 1946 Biedebach[91,92] investigated the action of mercuric acetate on two members of the lupane group of triterpenes, namely lupeol and betulin, and also on several other triterpenes of the oleanane and ursane groups. Biedebach reported that whereas α-amyrin, β-amyrin, and lupanyl acetate (LXXVII) are unchanged after treatment with mercuric acetate in chloroform-acetic acid solution, lupeol and betulin derivatives give dehydrogenated products. In the case of lupeol (VIIa) the product was described as an alcohol of molecular formula, $C_{30}H_{48}O$ and it was further characterised by the preparation of an acetate, $C_{32}H_{48}O_2$, and of a benzoate, $C_{37}H_{52}O_2$. The products from betulin and betulin diacetate were not characterised.

Mercuric acetate has had a very limited use in the triterpenoid field although it has been frequently employed for dehydrogenation of certain steroids.[93,94,95] In the latter series, unsaturated compounds are dehydrogenated with mercuric acetate to give derivatives which have a new double bond in conjugation with the original unsaturated system. This, however, is not the case with lupeol derivatives and the fact that these compounds are affected by mercuric acetate is apparently due to the reactive nature of the double bond in the side chain exocyclic to ring E.

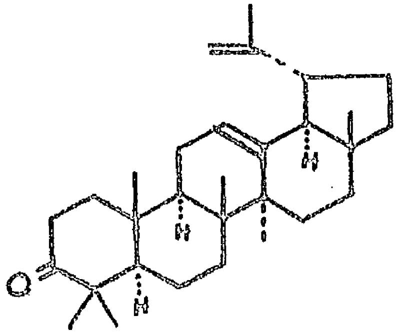
The author has reinvestigated the mercuric acetate dehydrogenation of lupeol derivatives and has obtained the same alcohol, acetate and benzoate described above although analyses of these compounds indicated the molecular formula $C_{30}H_{48}O$ for the parent alcohol. The corresponding ketone and hydrocarbon derivatives have also been prepared by dehydrogenation of lupenone (XVII) and α-lupene (VIIf) with mercuric acetate.



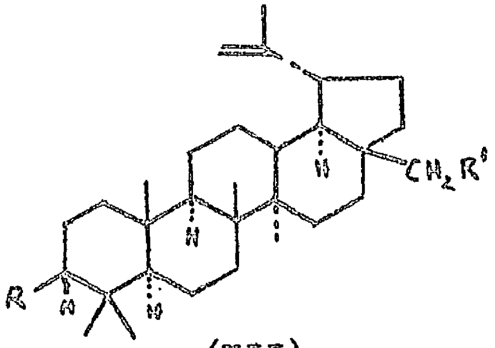
(LXXVII)



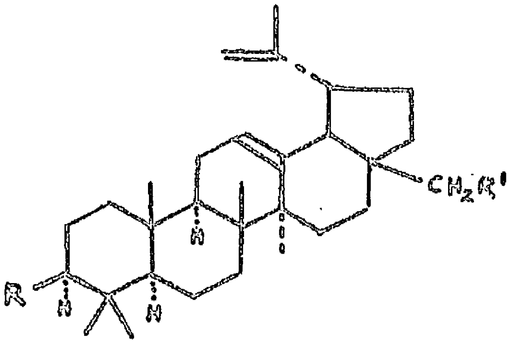
(XVII)



(LXXIX)



(VII)



(LXXVIII)

(a) R=OH; R' = H, (b) R=OH=R'. (a)R=OH; R'=H. (b) R =OH = R'
(c) R=OAc; R' = H, (d) R=OAc=R'.(c)R=OAc; R'=H. (d) R=OAc=R'
(e) R=OBz; R' = H, (f) R=H=R'. (e)R=OBz; R'=H. (f) R=H=R'.

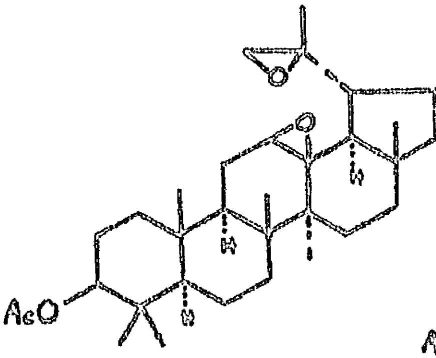
On the basis of evidence presented below these compounds have been formulated as non-conjugated dienes (LXXVIII a, c, e, f) and (LXXIX) with molecular formula derived from the hydrocarbon $C_{30}H_{48}$, (LXXVIIIf). All of these compounds are chromatographically homogeneous and have been interrelated by the following standard procedures. Alkaline hydrolysis of both the non-conjugated dienyl acetate and benzoate yielded the di-unsaturated alcohol from which the original acetate (LXXVIIIc) was regenerated by acetylation. Oxidation of the alcohol with the Kiliani reagent[98] furnished the corresponding ketone identical with dehydrolupenone (LXXIX) and this was readily reduced by the Wolff-Kischner method [cf. 97] to the hydrocarbon identical with dehydro-α-lupene (LXXVIII f). In all of these compounds the analytical and spectroscopic evidence indicated the presence of two non-conjugated double bonds in a pentacyclic system.

Since the above compounds have been interconverted, the double bonds must occupy the same positions in each carbon skeleton. Therefore the reactions of the acetate (LXXVIIIc) are typical of the group. Each member of the group shows maximal ultraviolet absorption at 2050 Å. (ε, 7,500). This intensity is considerably higher than that of the corresponding lupeol derivatives from which they are generated (ε, 3,500).

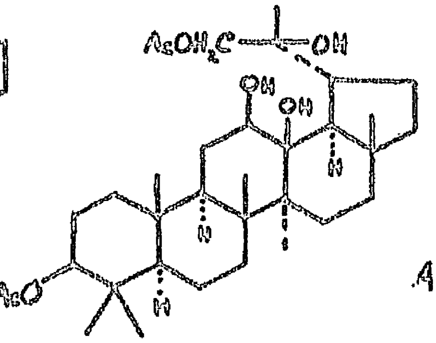
Furthermore, infrared evidence indicates the presence of a vinylidene group in all the dehydrogenation products.

Proof of the presence of two ethylenic linkages was forthcoming from several standard reactions. Treatment of the dienyl acetate (LXXVIIIc) with either performic acid or perbenzoic acid yielded a compound, $C_{32}H_{30}O_4$, which gave no colour with tetranitromethane and which was transparent to ultraviolet light. In addition, its infrared spectrum showed no bands which could be assigned to a carbonyl function. This compound has therefore been formulated as the diepoxide (LXXX). Lithium aluminium hydride reduction of the osmate ester, derived from the dienyl acetate by the action of osmium tetroxide, yielded a crude pentahydroxy compound which was acetylated and characterised as the saturated 3β,30-diacetoxy-12β,13β,20-trihydroxylupane (LXXXI). The structure of the pentol diacetate was deduced from a consideration of infrared, ultraviolet and analytical data and affords evidence for the existence of a diene system in the original acetate.

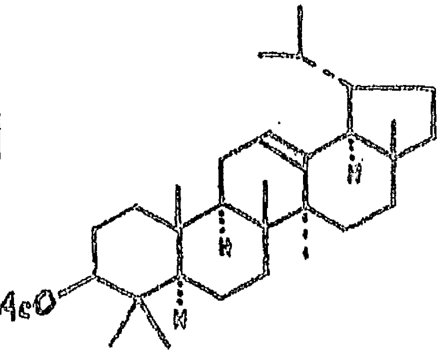
Catalytic hydrogenation of the acetate (LXXVIIIc) resulted in the uptake of one mole of hydrogen and gave a product, $C_{32}H_{52}O_3$, (LXXXII) which exhibited ultraviolet absorption at 2050 Å. ($\mathcal{E}$, 3,500). This ultraviolet absorption is typical of a trisubstituted double bond[90] and since the infrared spectrum of the compound provides no evidence for the existence of a vinylidene group it was concluded that hydrogenation of the non-conjugated diene affected only the more reactive vinylidene group.



(LXXX)



(LXXXI)



(LXXXII)

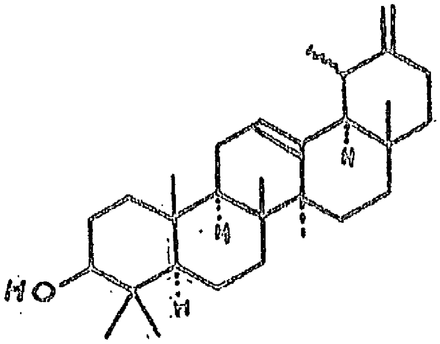
Treatment of a solution of the dehydro-lupenyl acetate (LXXVIIIe) in glacial acetic acid with concentrated hydrochloric acid gave a mixture of conjugated dienyl acetates which was readily resolved by chromatography on alumina into two well defined components. These products did not supply direct evidence for the structural elucidation of the mercuric acetate dehydrogenation compounds but they are of interest and their structures will be discussed later.

Location of the Double Bonds in Dehydro-lupeol Derivatives.

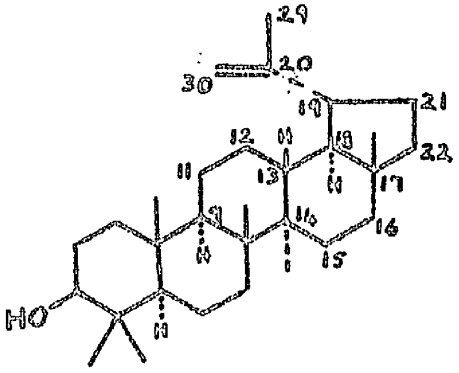
On the basis of infrared evidence it has already been shown that all the compounds obtained from lupeol derivatives by dehydrogenation with mercuric acetate contain a vinylidene group. Confirmation of this fact was readily obtained by chemical means when ozonolysis of the non-conjugated dienyl acetate gave formaldehyde in good yield. Similarly, lead tetra-acetate treatment of the pentahydroxy compound derived from the dienyl acetate by the action of osmium tetroxide also yielded formaldehyde. Unfortunately neither of these experiments afforded a pure norketone, presumably because of reaction at the other centre of unsaturation.

On the assumption that no rearrangement had taken place during the dehydrogenation it seemed most likely that the vinylidene group was present in the same environment as in lupeol i.e. in an isopropenyl side chain. Members of the lupeol group of triterpenes may isomerise in the presence of acid with enlargement of ring E[99,100] and since the dehydrogenation reactions were carried out in a glacial acetic acid medium, products of the type (LXXXIII) were also considered possible. However, the latter has been ruled out on the basis of convincing evidence obtained from compounds derived from betulin and betulinic acid by the action of mercuric acetate and the presence of a five membered ring E and isopropenyl side chain has been clearly established.

The conclusion that the vinylidene group was in the same environment as in lupeol made easier the location of the second double bond in the dehydrogenated series. The presence of a reactive double bond is obviously necessary to initiate the reaction with mercuric acetate since there is no reaction with α-amyrin (XXXIIIa), β-amyrin (XXXIIa), and lupanyl acetate (LXXVII).[91,92] From this observation it was deduced that the second double bond was introduced at a position close to the vinylidene group. Proximity of the double bonds might also be expected since dehydrogenation of certain steroids with mercuric acetate results in the formation of conjugated dienes.[93-95] Therefore in the dehydro-lupeol derivatives the most probable location of the second double bond would be in rings C, D or E.



(LXXXIII)



(VIIa)

The nature of the ultraviolet spectra of the non-conjugated dienes also indicates that the two unsaturated centres are situated close to each other. Besides showing a higher intensity at 2050 Å. these spectra had a distinct inflexion in the 2,300 Å. region (fig.1). This tendency to shift the absorption to higher wavelengths is apparently caused by interaction between the electrons of the double bonds.

Fig. 1.

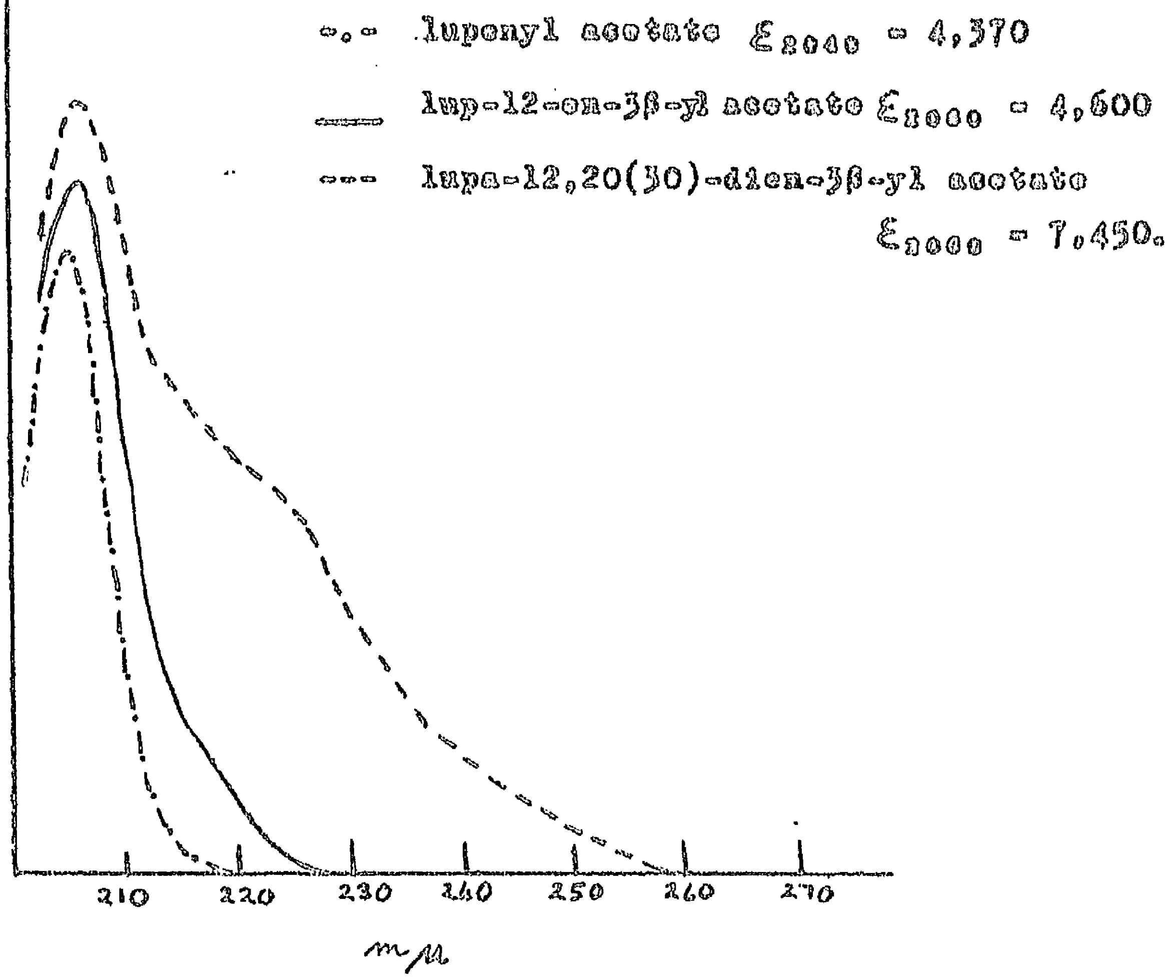


Table III gives details of the ultraviolet spectra of several derivatives of lupeol, betulin and betulinic acid. The absorption values shown are for the vinylidene group, the diene and the newly introduced double bond.

TABLE III

Compound	ε at. 2050 A.	Non-conjugated Diene Derivative.		Product from hydrogenation of diene. ε at 2050 A.
		ε at 2050 A.	ε at 2250 A.	
Lupeol	4,100	6,500	2,240	4,150
Lupenyl acetate	4,375	8,100	2,820	3,600
Betulin	4,050	8,300	2,610	
Betulin diacetate	3,900	8,450	2,810	
Methyl acetyl betulinate	4,180	7,290	3,050	4,300
Acetyl betulinic acid	4,400	7,550	2,850	3,900

Assuming that the vinylidene group in the dehydrogenated compounds is in the same position as in the parent lupeol derivatives we can limit the number of possible locations for the second double bond as follows. Positions 18(19) and 19(21) may be excluded as these would constitute a conjugated system with the 20(30)-double bond. Treatment of the non-conjugated dienyl acetate (LXXVIIIc) with hydrogen in the presence of a platinum catalyst caused reduction of the vinylidene group but did not affect the second double bond. Therefore the latter cannot be at positions 11(12), 15(16) or 21(22) since these are disubstituted and are readily reduced in other triterpenes.[80,88,113] The only other possible locations of the double bond in rings C, D or E are 9(11), 12(13), and 13(18) unless there has been methyl group migration during the mercuric acetate treatment. Such a rearrangement is extremely unlikely in view of the fact that the reaction medium is chloroform and acetic acid.

Of these three possibilities, the 9(11)-position was considered unlikely in view of its distance from the vinylidene group and although subsequent reactions involving the lupeol derivatives did not differentiate between this position and the 12(13)-position it can be excluded on the grounds of evidence presented below in the section dealing with betulinic acid derivatives.

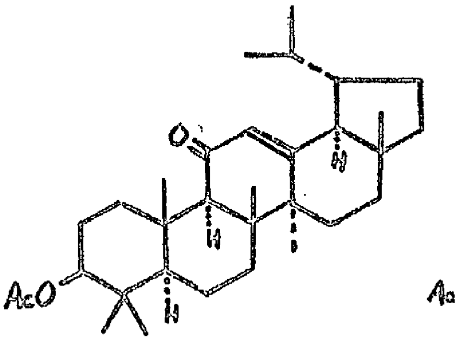
Differentiation between positions 12(13) and 13(18) was made in the following manner. The ultraviolet evidence given in Table III shows clearly that the new double bond has maximal absorption at 2050 Å. (ϵ, 4000). Derivatives of the olean-12-one type usually absorb at approximately 2050 Å. (ϵ,4000) whereas compounds of the olean-13(18)-ene type generally exhibit maximal absorption at about 2080 Å. (ϵ, 10,000). This provides evidence for the location of the double bond, introduced into the lupeol molecule by dehydrogenation, in a trisubstituted rather than a tetrasubstituted position i.e. the evidence favours the 12(13)-position.

Proof of the environment of the less reactive double bond was afforded by examination of the reactivity of the mono-ethylenic acetate (LXXXII) obtained by hydrogenation of dehydro-lupenyl acetate (LXXVIIIc). Oxidation of the acetate (LXXXII) with chromium trioxide in acetic acid gave an αβ-unsaturated ketone (LXXXIV) which showed selective absorption in the ultraviolet at 2440 Å. ($\mathcal{E}$, 13,000) and a band in the infrared spectrum at 1685 cm.$^{-1}$ Catalytic hydrogenation of the αβ-unsaturated ketone (LXXXIV) regenerated the original olefin (LXXXII). These reactions are characteristic of the trisubstituted 12(13)-double bond,[62,101] in such compounds as α-amyrin and β-amyrin.

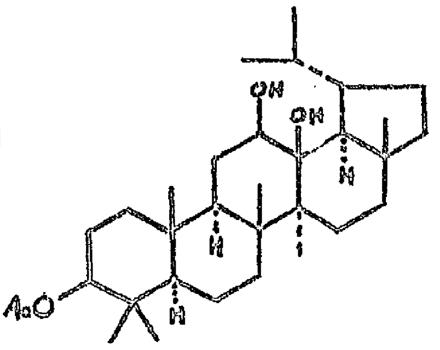
Mention has already been made of the saturated compound obtained from the dienyl acetate (LXXVIIIc) by the action of osmium tetroxide followed by reduction with lithium aluminium hydride and acetylation. The product was characterised as a pentol diacetate, $C_{34}H_{56}O_7$, which was transparent to ultraviolet light, gave no colour with tetranitromethane and showed bands in its infrared spectrum at 3509 cm.$^{-1}$ (hydroxyl), 1739 cm.$^{-1}$ (unbonded acetate), and 1712 cm.$^{-1}$ (bonded acetate). From this evidence the pentol diacetate has been formulated as (LXXXI). At first sight this conclusion may appear to be erroneous since the hydroxyl group attached to position 12 is secondary and might therefore be expected to form an acetyl derivative. The existence of a 13:18-glycol system would also explain the formation of a pentol diacetate since the 13, 18, and 20 hydroxyl groups would be tertiary and therefore unacylable.

However, from the study of a molecular model of the pentol it is obvious that the hydroxyl group at position 12 is very strongly hindered by the side chain and would not be acetylated under normal conditions and probably not even under forcing conditions. In fact, the hydroxyl group is apparently more strongly hindered than that in 11β-hydroxy steroids which do not give acetyl derivatives under normal conditions.[102]

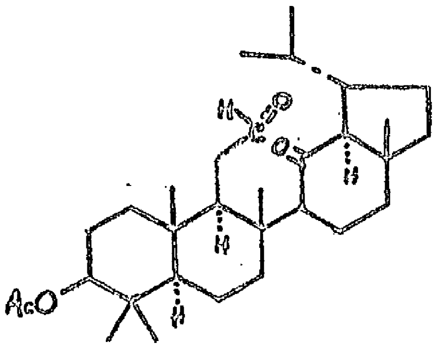
Similarly, treatment of the dihydro-compound (LXXXII) with osmium tetroxide followed by reduction with lithium aluminium hydride and acetylation of the product at room temperature gave a triol mono-acetate, $C_{32}H_{54}O_4$, (LXXXV). Two bands in the carbonyl region of its infrared spectrum at 1739 cm.$^{-1}$ and 1712 cm.$^{-1}$ indicated that there was intermolecular interference between the 3-acetate group and the 12:13 glycol system.[103] Another peculiarity of the triol mono-acetate was that, although it was beautifully crystalline and chromatographically homogeneous, it melted over a wide range, 215-245°. The wide melting range may be due to dehydration of the glycol group near the melting point or to the compound being a mixture of stereo-isomeric glycols.



(LXXXIV)



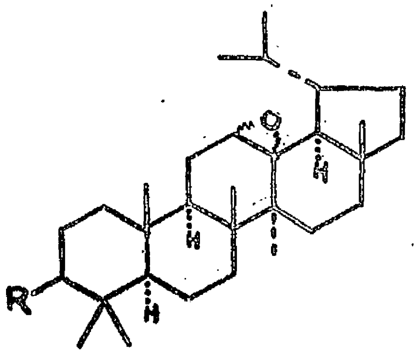
(LXXXV)



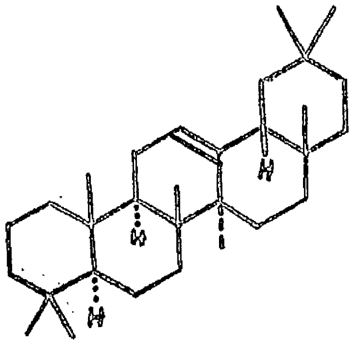
(LXXXVI)

Convincing proof of the environment of the glycol group in compound (LXXXV) and therefore of the second double bond in the non-conjugated dienes was obtained by treatment of the triol mono-acetate with lead tetra-acetate. Fission of the glycol in this way gave a compound, m.p. 186-188°, the infrared spectrum of which showed no bands attributable to a hydroxyl group but which contained a strong band in the carbonyl region. Using a calcium fluoride prism it was possible to resolve this carbonyl band and the spectrum thus obtained showed peaks at 1736, 1242 cm.$^{-1}$ (acetate), 2820, 1721 cm.$^{-1}$ (aldehyde) and 1706 cm.$^{-1}$ (ketone in a six membered ring). The compound has therefore been formulated as the keto-aldehyde (LXXXVI) since fission of a 13:18 glycol in the same way would give rise to a diketone.[104] Thus the triol mono-acetate must have structure (LXXXV).

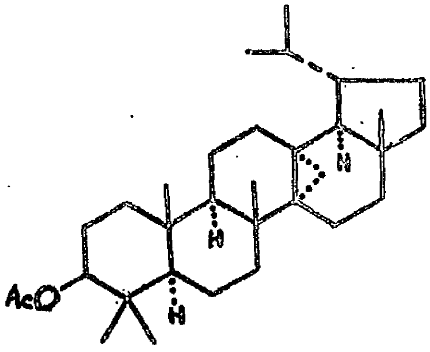
A further attempt was made to show that the nuclear double bond in dehydrolupenyl acetate had similar reactivity to that shown by derivatives of olean-12-ene (LXXXVII). Oxidation of the dihydro alcohol (LXXXIIa) and of its acetate (LXXXIIb) with perbenzoic acid gave the corresponding oxides which may be represented by the structures (LXXXVIIIa) and (LXXXVIIIb) respectively. Neither of these compounds showed any selective absorption in the ultraviolet nor gave any colour with tetranitromethane. In neither case did the infrared spectrum show any bands which could be assigned to a ketone group. Analyses of the oxides were consistent with the molecular formulae, $C_{30}H_{50}O_2$ and $C_{32}H_{52}O_3$ respectively.



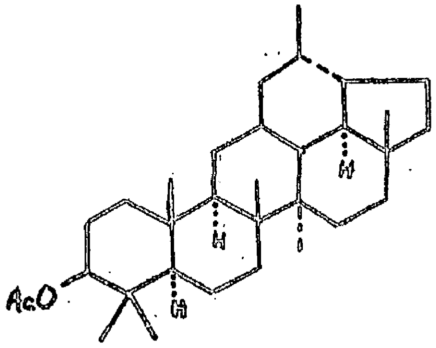
(LXXXVIII)(a) R = OH
(b) R = OAc



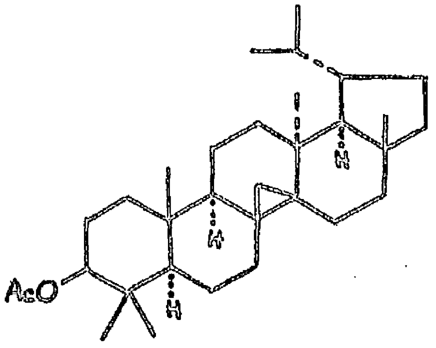
(LXXXVII)



(LXXXIX)



(XC)



(XCI)

Treatment of the alcohol oxide (LXXXVIIIa) with concentrated hydrochloric acid in acetic acid yielded a saturated acetate, m.p. 272-274°, $[\alpha]_D$ + 24°, which was transparent to ultraviolet light, gave no colour with tetranitromethane, showed only acetate bands in its infrared spectrum and was formulated by analysis as $C_{32}H_{52}O_2$. This saturated acetate was also obtained as a by-product in the chromium trioxide oxidation of the dihydro acetate (LXXXIIb). From this reaction there was also isolated a second saturated acetate, m.p. 242-244°, $[\alpha]_D$ + 36.5°, which was apparently isomeric with the first compound, m.p. 272-274°, and had very similar light absorption properties. A third isomeric acetate, m.p. 226-228°, $[\alpha]_D$ - 16°, was isolated in low yield from the perbenzoic acetate oxidation of the unsaturated acetate (LXXXIIb).

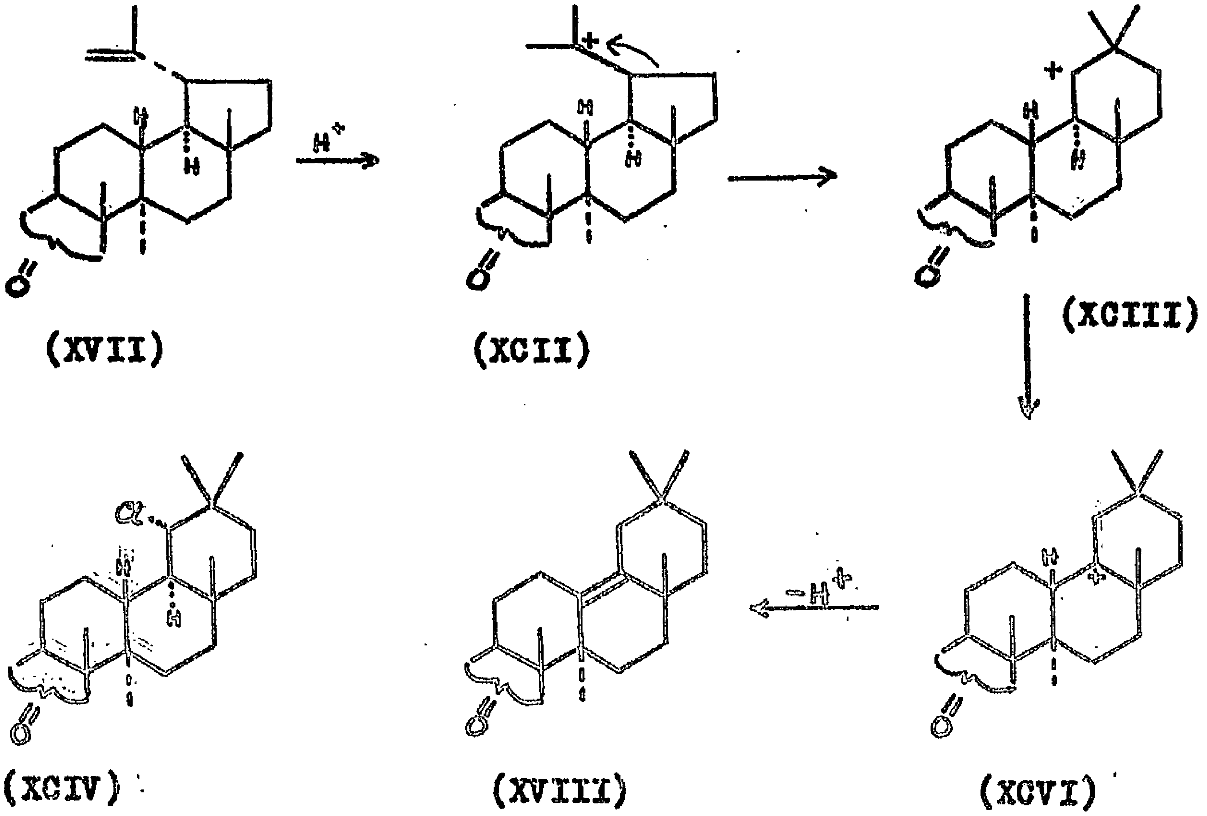
It is difficult to explain the formation of these compounds. The analyses figures, do not, of course, exclude a molecular formula of $C_{32}H_{54}O_2$ but it is impossible to see how reduction could occur in the oxidising conditions used. Alternatively, to accommodate a molecular formula of $C_{32}H_{52}O_2$ for these saturated acetates their structures must necessarily be hexacyclic. A possible method of formation of these compounds would involve the 12(13)-double bond and the side chain or a neighbouring methyl group as in other cyclopropane derivatives.[108] Possible structures for these compounds are (LXXXIX), (XC), and (XCI). The structures of the saturated acetates were not further investigated.

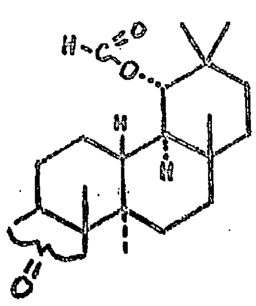
From the evidence presented above it was decided that the compounds obtained by dehydrogenation of lupeol derivatives with mercuric acetate were indeed the 12,20(30)-dienes (LXXVIIIa, c, e, f) and (LXXIX). Confirmation of this conclusion was forthcoming from the reactions on betulinic acid derivatives described below.

The Acid Isomerisation of Lupeol and the Dehydro-compounds.

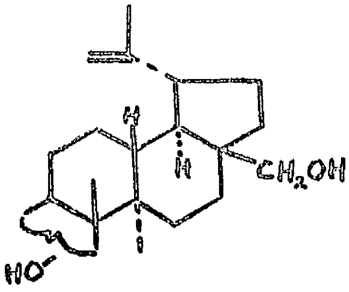
The classical isomerisation of lupenone (XVII) to δ-amyrenone (XVIII) carried out by Ames, Halsall, and Jones[22] is represented by the mechanism shown below in Table IV.

TABLE IV

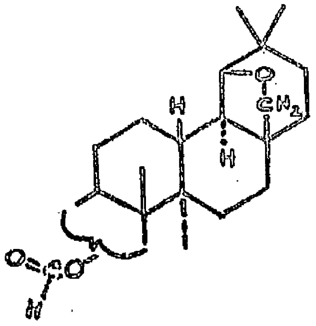




(XCV)



(VIIb)



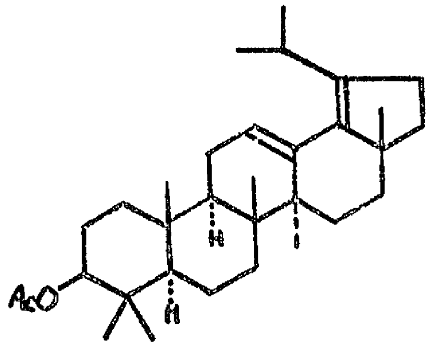
(XCVII)

Protonation of the double bond in the side chain is followed by fission of the bond between $C_{(19)}$ and $C_{(21)}$ with subsequent ring enlargement to give the intermediate carbonium ion (XCIII). Stabilisation of this ion may occur by several different means. For instance, treatment of lupenone with formic acid results in the formation of the 19α-formyloxy derivative (XCV) of 18α-olean-3-one. Similarly, treatment of lupenone (XVII) with dry hydrogen chloride yields the 19α-chloro-compound (XCIV) and when betulin is treated with formic acid the 28:19-ether (XCVII) is produced by loss of a proton from the alcohol group attached to $C_{(28)}$.

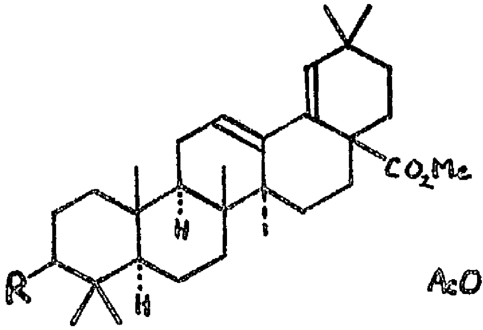
Attempts to obtain analogous results with the non-conjugated dienes were unsuccessful. Dehydrolupenyl acetate (LXXVIIIc) was isomerised by treatment with concentrated hydrochloric acid in acetic acid to a mixture of conjugated dienyl acetates. Chromatography of the mixture on alumina furnished two well defined components. The first of these compounds exhibited maximal absorption in the ultraviolet region at 2340 Å. ($\mathcal{E}$, 10,000) and showed a very high positive specific rotation, ($[\alpha]_D$ + 260°). Also, it gave a dark brown colour with tetranitromethane and had a band in its infrared spectrum at 1630 cm.$^{-1}$ It was therefore formulated as the cisoid diene (XCVIII) since its properties proved to be completely analogous to those of methyl isodehydro-oleanolate[106] (XCIXa), $[\alpha]_D$ + 214°, $\lambda_{max.}$ 2370 Å. ($\mathcal{E}$, 10,200).

The other compound isolated from this reaction had a negative specific rotation ($[\alpha]_D$ - 77°) and its ultraviolet spectrum contained three well defined maxima at 2420, 2510, 2600 Å. ($\mathcal{E}$, 23,000; 26,000; 19,000). Again a weak band a 1630 cm.$^{-1}$ was evident in the infrared spectrum and the compound gave a brown colour with tetranitromethane. The ultraviolet spectrum and specific rotation were closely analogous to those of the transoid heteroannular -11,13(18)-dienes found in the oleanane[106,62] and ursane[107] series. For example, oleana-11,13(18)-dienyl acetate (XXXVI) has $[\alpha]_D$ - 67° and $\lambda_{max.}$ 2440, 2520, 2610 Å. ($\mathcal{E}$, 26,000; 30,000; 21,000). Structure (C) was therefore proposed for the lupeol derivative.

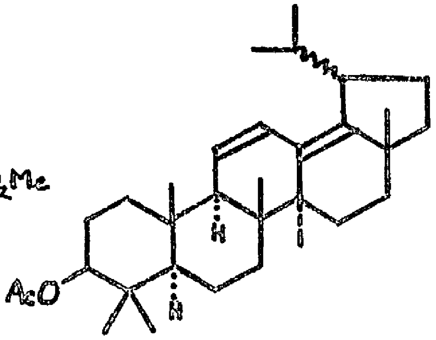
Hydrogenation of both conjugated dienes (XCVIII) and (C) yielded the same tetrasubstituted olefin, $\lambda_{max.}$ 2060 Å. ($\mathcal{E}$, 10,000), which was assigned the structure (CI). The constitutions ascribed to the dienes (XCVIII) and (C), and also to the olefin (CI) are



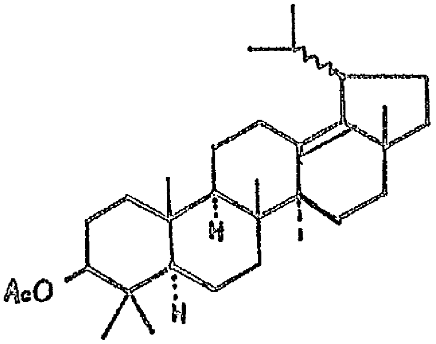
(XCVIII)



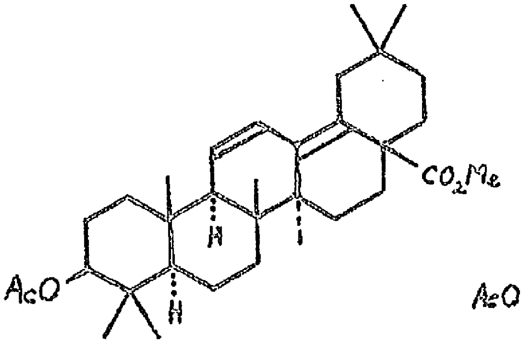
(XCIX) (a) R = OH
(b) R = OAc



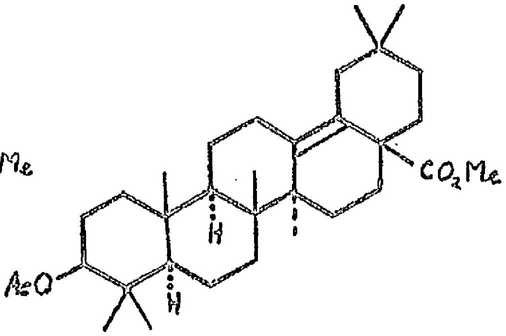
(C)



(CI)



(CII)



(CIII)

supported by the molecular rotation changes which occur on hydrogenation of the dienes. It is evident from Table V that the rotational changes involved are similar to those which occur when the analogous dienes, methyl dehydro-oleanolate acetate (CII) and methyl isodehydro-oleanolate acetate (XCIXb) are hydrogenated[100,66] to the olefin, methyl olean-13(18)-enolate acetate (CIII).

It is noteworthy that the two conjugated dienes from lupeol can be interconverted by the action of mineral acid i.e. treatment of either of these compounds with hydrochloric acid yields an equilibrium mixture of both. In the oleanane series, methyl isodehydro-oleanolate acetate (XCIXb) is converted, almost quantitatively, into methyl dehydro-oleanolate (CII) by hydrogen chloride.

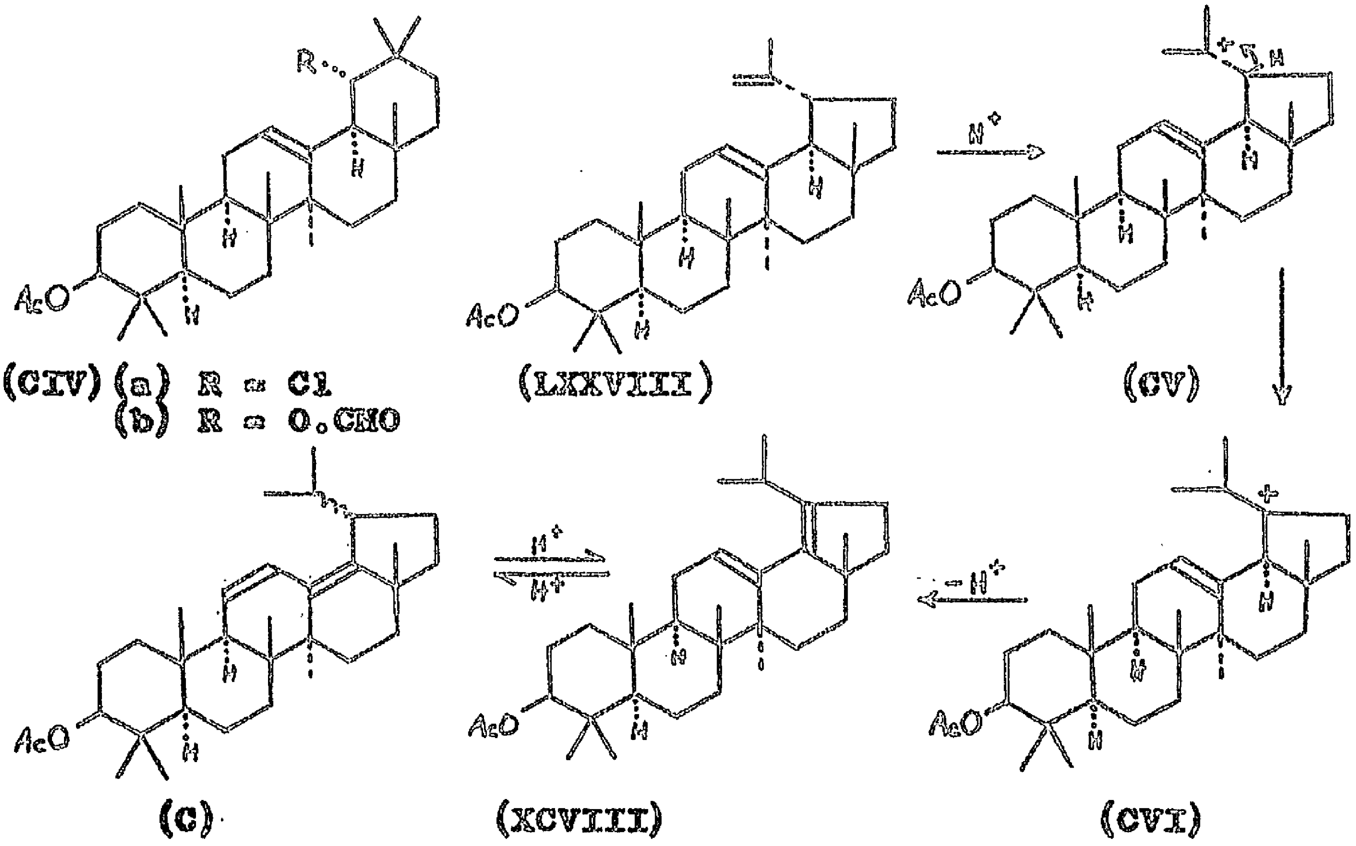
TABLE V

Molecular Rotation Changes Accompanying Hydrogenation

	$[\alpha]_D$	M_D	ΔM_D
Methyl isodehydro-oleanolate acetate (XCIX)	+209°	+1065°	
			-1410°
Methyl olean-13(18)-enolate acetate (CIII)	- 67.5°	- 345°	
			+ 305°
Methyl dehydro-oleanolate acetate (CII)	-127°	- 650°	
3β-Acetoxylupa-12,18-diene (XCVIII)	+263°	+1230°	
			-1324°
3β-Acetoxylup-13(18)-ene (CI)	- 20°	- 94°	
			+253.5°
3β-Acetoxylupa-11,13(18)-diene (C)	- 74.5°	-347.5°	

However, in the latter case the reaction is irreversible. This slight difference in the relative stabilities of the dienes in the two series may be due to the presence of a five membered ring in the lupeol derivatives.

Further attempts to prepare the 19α-chloro derivative (CIVa) and the 19α-formyloxy derivative (CIVb) of 18α-olean-12-one from dehydrolupenyl acetate (LXXVIIIc) by the action of dry hydrogen chloride and of formic acid, respectively, were unsuccessful and simply gave the mixture of conjugated dienes (XCVIII) and (C), described above. The 11,13(18)-dienyl derivative (C) of lupeol was not identical with 3β-acetoxyoleana-11,13(18)-diene (XXXVI) therefore the isomerisation of the non-conjugated diene (LXXVIIIc) cannot proceed by enlargement of ring E as in other lupeol type compounds (cf. Table IV) since this would have yielded the oleanane type of dione (XXXVI). A more probable mode of formation of the dienes (XCVIII) and (C) is outlined below.



(CIV) (a) R = Cl
(b) R = O.CHO

(LXXVIII)

(CV)

(C)

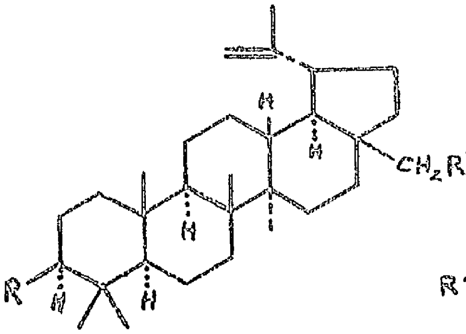
(XCVIII)

(CVI)

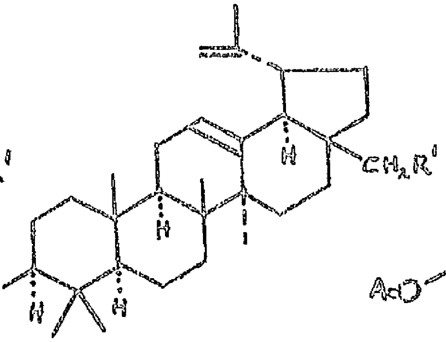
In this case formation of the carbonium ion (CV) is followed, not by ring enlargement, but by hydride shift to give the carbonium ion (CVI) which is stabilised by loss of a proton from $C_{(18)}$ to form the cisoid-type diene (XCVIII) and thence an equilibrium mixture of both dienes (XCVIII) and (C).

The Reactions of Betulin and Betulinic Acid with Mercuric Acetate.

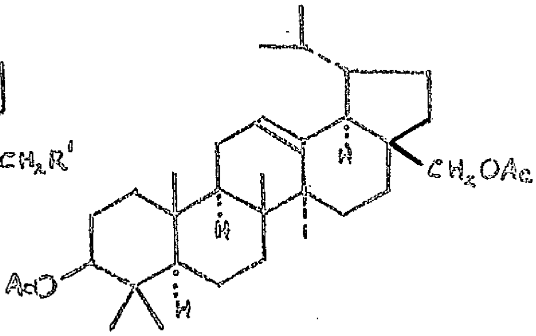
While the foregoing work on lupeol and related compounds was in progress it was decided to investigate the reaction of mercuric acetate on derivatives of betulin. Biedebach[91,92] reported that mercuric acetate reacted with betulin and with its diacetate but he did not characterise the products. An investigation of these reactions was carried out in this department by G. R. Taylor[109] to whom the author is indebted for supplying much of the information described below. A description of the details of this work is included at this stage as it is very closely linked with the author's investigation of betulinic acid derivatives.



(VII)



(LXXVIII)



(CVII)

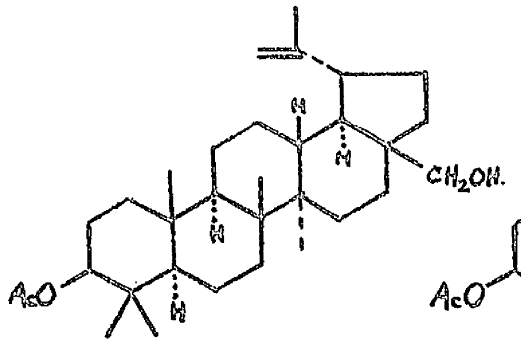
(b) R = R' = OH
(d) R = R' = OAc

(b) R = R' = OH
(d) R = R' = OAc

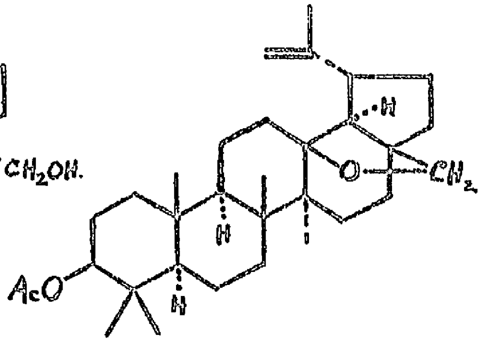
Dehydrogenation of betulin diacetate (VIId) with mercuric acetate in acetic acid furnished the expected diene-diacetate (LXXVIIId) which was hydrolysed by alkali to the diene-diol (LXXVIIIb). Acetylation of the diol regenerated the parent diacetate (LXXVIIId). Hydrogenation of the diacetate, with the uptake of one mole of hydrogen, gave an unsaturated compound, $C_{34}H_{54}O_4$, (CVII), $\lambda_{max.}$ 2060 Å. (ε, 7,900), which showed no bands in the infrared spectrum attributable to a vinylidene group. The above reactions and products show complete analogy to those in the lupeol series.

However, treatment of betulin (VIIb) itself with mercuric acetate in acetic acid gave a product which was not identical with the diene-diol (LXXVIIIb) although there were bands in its infrared spectrum which could be assigned to hydroxyl and vinylidene groups. Furthermore, acetylation of this compound gave an acetate, the analysis of which indicated a molecular formula, $C_{32}H_{50}O_{3}$, consistent with a mono-acetyl dehydro-derivative of betulin. From an examination of the infrared spectrum of the mono-acetate it was evident that there was no free hydroxyl group present in the compound. That the vinylidene group was the only centre of unsaturation in the mono-acetate was shown by hydrogenation (1 mol.) to a saturated compound $C_{32}H_{52}O_{3}$. The unsaturated mono-acetate, $C_{32}H_{50}O_{3}$, was also prepared by dehydrogenation of betulin 3-mono-acetate (CVIII) with mercuric acetate.

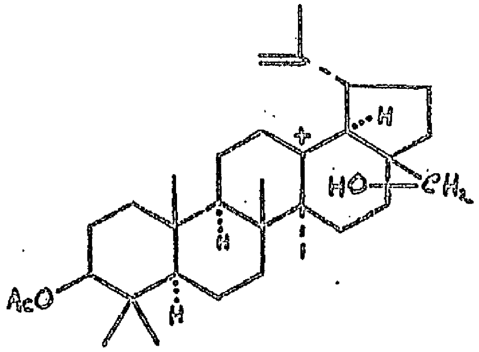
From a consideration of these facts it was obvious that the oxygen function attached to $C_{(28)}$ was no longer present as a primary hydroxyl group nor as a carbonyl function. To accommodate a molecular formula of $C_{32}H_{52}O_{3}$ for the saturated acetate, the structure must be hexacyclic. Thus it seemed most



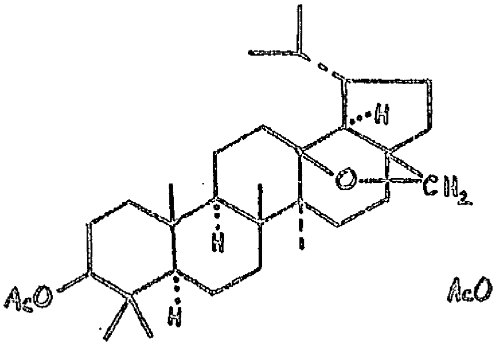
(CVIII)



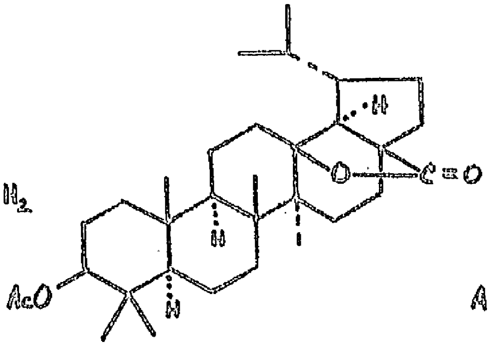
(CIX)



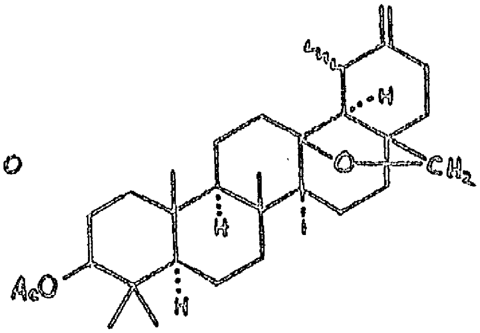
(CX)



(CXI)



(CXII)



(CXIII)

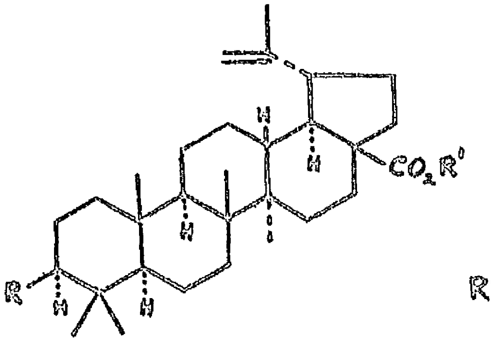
likely that the primary alcohol group had reacted to form an ether in a manner similar to that described by Henbest and Nicholls[110] for the cyclisation of the γδ-unsaturated alcohol, bicyclo [2:2:1] hept-5-en-2α-ylmethanol in the presence of mercuric acetate. Since the dehydrogenation of lupeol described above resulted in the introduction of a double bond at the 12(13)-position it was attractive to consider that an ether linkage between $C_{(28)}$ and $C_{(13)}$ had been formed in this case by way of the carbonium ion (CX). Thus the unsaturated acetate, $C_{32}H_{50}O_3$, described above may be represented by structure (CIX) and its dihydro derivative by (CXI).

These structures have been substantiated by oxidation of the saturated ether (CXI) to a γ-lactone which has also been obtained directly from acetyl betulinic acid (VIIIb) by dehydrogenation with mercuric acetate and hydrogenation of the resultant unsaturated lactone (XIb). The saturated γ-lactone must therefore be represented by structure (CXII).

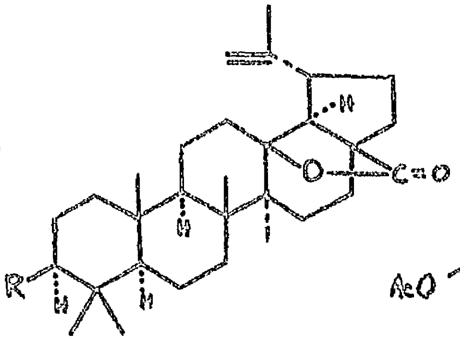
Nuclear magnetic resonance studies carried out on the unsaturated acetyl ether (CXI) indicated that ring E was, as postulated, five membered with an isopropenyl side chain, thus excluding the alternative structure (CXIII) for the unsaturated ether. The main features of this spectrum were:- weak bands at + 63c/s and + 89 c/s due to $C-CH_2-O-$ protons, and peaks at +140 c/s and +150 c/s due to CH_3-C- protons. There was no evidence of a 7 c/s doublet peak in the CH_3-C- region which would be expected from the the CH_3-CH methyl group in the alternative structure (CXIII).

The Reactions of Betulinic Acid Derivatives.

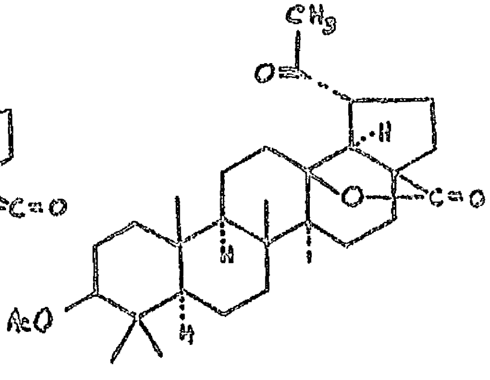
The results obtained from the mercuric acetate treatment of betulin indicated that betulinic acid, by analogy, cf[110] would give a lactone under the same conditions. Acetyl betulinic acid (VIIIb) was therefore treated with mercuric acetate and a neutral product was obtained from the reaction mixture. This compound showed a band in its infrared spectrum at 1786 cm.$^{-1}$ which was attributed to a γ-lactone[111,112] and it had bands at 1630 and 915 cm.$^{-1}$ attributable to the vinylidene group although the latter band is apparently displaced from the more usual position at 890 cm.$^{-1}$ One unusual feature of this compound was that it showed only low intensity absorption in the ultraviolet at 2050 Å. (ε, 2000) and gave no colour with tetranitromethane. In spite of this unconvincing physical evidence for the existence of a vinylidene group, the acetyl lactone underwent reactions typical of this unsaturated centre in lupeol derivatives and it has consequently been formulated as (XIb).



(VIII)



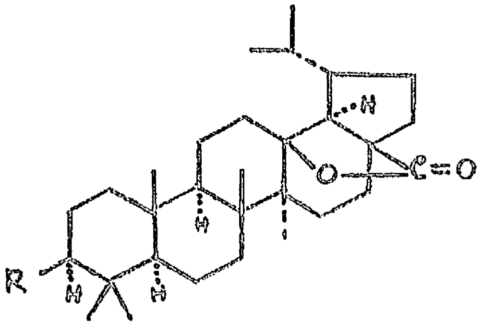
(XI)



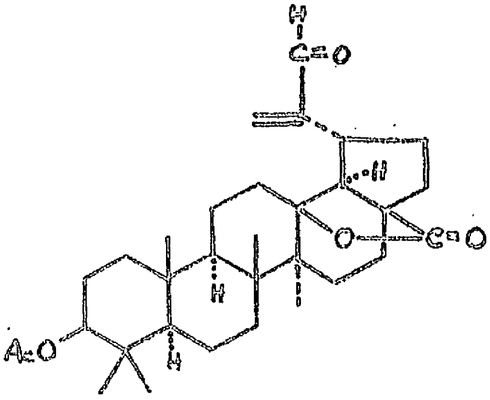
(CXIV)

(b) R =OAc; R' = H
(c) R =OAc; R' = Me

(a) R =OH
(b) R =OAc



(CXV)



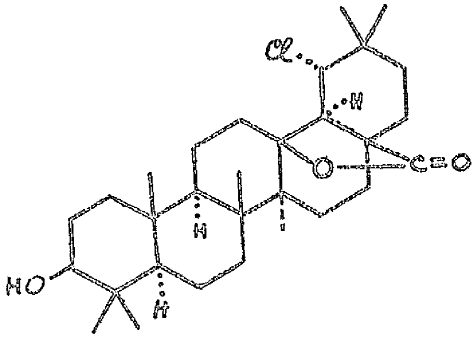
(CXVI)

(a) R =OH
(b) R =OAc

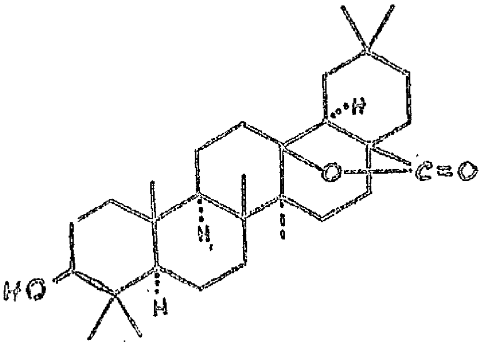
Catalytic hydrogenation of the acetyl lactone (XIb) gave a dihydro-derivative (CXVb) thus indicating the presence of a reactive double bond. Fission of this double bond by low temperature ozonolysis of the unsaturated lactone yielded a 30-nor-20-ketone (CXIV) and formaldehyde in good yield. Treatment of the unsaturated lactone (XIb) with selenium dioxide in boiling glacial acetic acid afforded an αβ-unsaturated aldehyde (CXVI) which showed bands in its infrared spectrum at 1779 cm.$^{-1}$ (γ-lactone), 1739 cm.$^{-1}$ (acetate), and 1692 cm.$^{-1}$ (αβ-unsaturated aldehyde).[13] The aldehyde has a characteristic ultraviolet spectrum with maximal absorption at 2220 Å. (ε, 7000).

These three reactions show that the unsaturated lactone

(XI) contains a vinylidene group and the product obtained from the selenium dioxide oxidation is specific for a vinylidene group of the lupeol type i.e. in an isopropenyl side chain. Only this type of double bond gives rise to an αβ-unsaturated aldehyde showing ultraviolet absorption at 2220 Å. cf.[13,44] This is further proof that ring E in the dehydrogenation products is the same as that in the starting material. However, an attempt to enlarge ring E to a six membered ring cf.[99] by acid isomerisation was unsuccessful. It was hoped that treatment of the unsaturated lactone (XIb) with hydrogen chloride would give, by analogy with lupeol,[99] a 19α-chloro-lactone (CXVII) which could then have been hydrogenated to the known compound, 18α-oleanolic lactone (CXVIII). Although the product from this reaction gave a positive Beilstein test it was identified as slightly impure hydroxy-lactone (XIa) by mixed melting point and comparison of its infrared spectrum with that of an authentic sample.



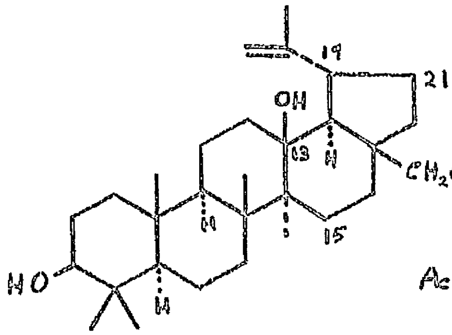
(CXVII)



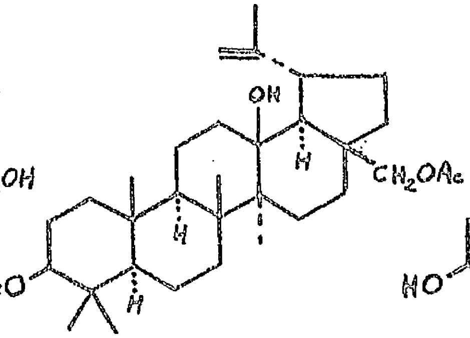
(CXVIII)

The position of the lactone ring in compound (XI) was deduced by consideration of the evidence presented below. Since the lactone is derived from betulinic acid (VIIIa) the carbonyl group of the lactone ring must be at $C_{(28)}$ and in view of the infrared evidence which indicates that it is a γ-lactone the other bridge must link $C_{(28)}$ with either $C_{(13)}$, $C_{(15)}$, $C_{(19)}$, or $C_{(21)}$.

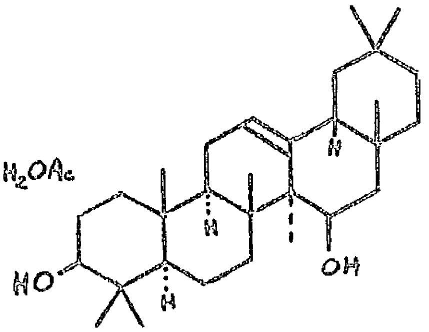
Fission of the lactone ring with lithium aluminium hydride yielded a triol (CXIX) which was acetylated using pyridine and acetic anhydride at 100° to give a diacetyl derivative (CXX). By means of phosphoryl chloride in pyridine the triol diacetate was readily dehydrated to furnish the dienyl diacetate (LXXVIIId), identical in all respects with the compound obtained by mercuric acetate dehydrogenation of betulin diacetate. In the triol one of the hydroxyl groups must be at position 3 and therefore the other two hydroxyl groups must be attached to the terminal carbon atoms of the lactone ring. Since the carbonyl group of the lactone is located at $C_{(28)}$, reduction with lithium aluminium hydride must therefore have produced a primary alcohol at that position. Hydroxyl groups at positions 3 and 28 are readily acetylated in other closely related compounds such as betulin therefore the unacetylated hydroxyl group in the triol diacetate (CXX) must mark the other terminal carbon atom of the lactone ring. Both the hydroxymethyl group and the hydroxyl group produced by fission of the lactone ring are considered to have the β-configuration since the carboxyl group in betulinic acid has this configuration and the derived lactone system can only be accommodated on the β-face of the molecule.



(CXIX)



(CXX)



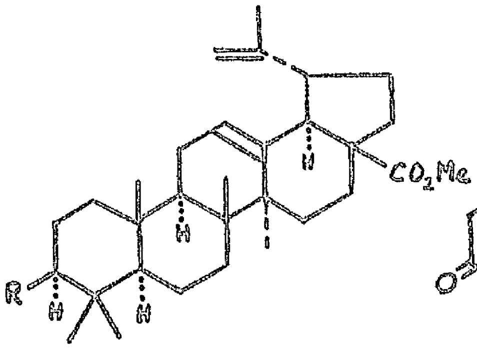
(CXXI)

Of the four possible positions $C_{(13)}$, $C_{(18)}$, $C_{(19)}$ and $C_{(21)}$ for the unacetylated hydroxyl group that at $C_{(21)}$ can be excluded because the 21β-hydroxyl group would be secondary and is not strongly hindered, thus it would be readily acetylated as is the case in derivatives of soyasapogenol A.[88] On the above evidence the 15β-position may also be ruled out: In a taraxerol derivative (CXXI) the 15β hydroxyl group is not acetylated under normal condition but unlike the triol diacetate (CXX) this 15β-hydroxy compound cannot be readily dehydrated.[14,113]

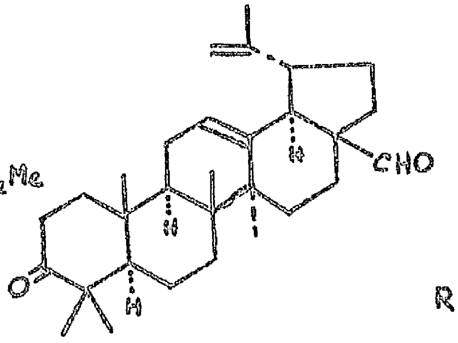
The two remaining positions, $C_{(18)}$ and $C_{(19)}$ would give rise to tertiary alcohols which would not acetylate under normal conditions although they would probably be readily dehydrated. A decision between these two possibilities was readily made because thurberogenin[18] has been shown to be a 28:19β-lactone (X) of the lupeol sub-group of triterpenoids and neither it nor its acetate was identical with the corresponding derivatives of the lactone obtained from betulinic acid by the action of mercuric acetate. In addition, the dehydration of the triol diacetate (CXX) yielded a non-conjugated diene which is not in agreement with the product expected from dehydration of a tertiary hydroxyl group at $C_{(19)}$ i.e. the vinylidene group would be αβ to the hydroxyl. Thus, the lactone, prepared by the action of mercuric acetate on acetyl betulinic acid must have the structure (XIb) and the derived triol and triol diacetate must be represented by structures (CXIX) and (CXX).

Further evidence for the above structures was obtained from the reactions carried out on derivatives of methyl acetyl betulinate (VIIIc). Dehydrogenation of the ester (VIIIc) with mercuric acetate in acetic acid gave a non-conjugated diene which showed light absorption properties very similar to those of the dienes obtained from derivatives of lupeol and betulin. It has therefore been formulated as (CXXIIb). Reduction of this compound with lithium aluminium hydride furnished a diene-diol (LXXVIIIb) which was identical with that compound previously prepared by alkaline hydrolysis of dehydrobetulin diacetate (LXXVIIId). Attempts to oxidise the diene-diol (LXXVIIIb) to the corresponding keto-aldehyde (CXXIII) were unsuccessful and even very mild conditions gave largely an acidic fraction which was not further investigated. It had been intended to reduce the keto-aldehyde (CXXIII) by the Wolff-Kishner method to give lupa-12,20(30)-diene (LXXVIIIf) and thus relate the diene from methyl acetyl betulinate with those of the lupeol series. Lack of material prevented further investigation of the oxidation of the diene-diol.

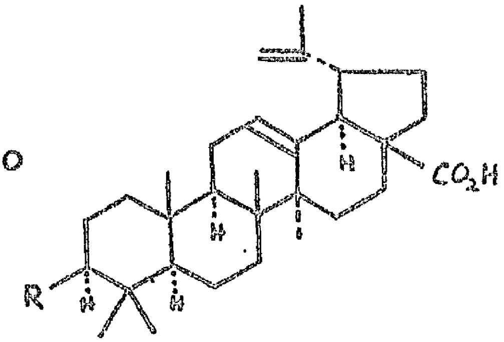
Hydrolysis of the diene (CXXIIb) under normal conditions (3% methanolic potassium hydroxide) gave the hydroxy methyl ester (CXXIIa). However, the methyl ester group in this compound can be hydrolysed using conditions similar to those employed for the hydrolysis of methyl-18α-oleanolate[114] and also used successfully on methyl acetyl betulinate (VIIIc). Thus treatment of the methyl ester diene (CXXIIb) with sodium ethoxide in a sealed tube at high temperature gave a hydroxy acid (CXXIVa) which was further characterised as its acetate (CXXIVb) and as its dihydro derivative (CXXVa). Methylation of the acetoxy-dienyl acid (CXXIVb) with ethereal diazomethane regenerated the original acetyl methyl ester (CXXIIb) and thus demonstrated that no molecular rearrangement had taken place during the high temperature alkaline treatment.



(CXXII)



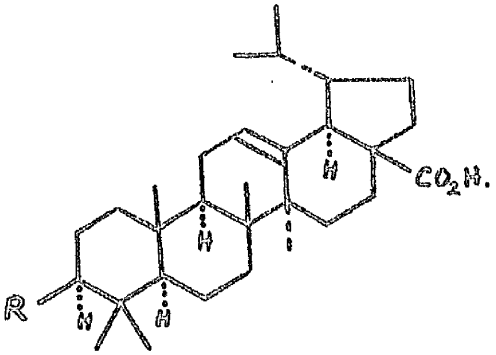
(CXXIII)



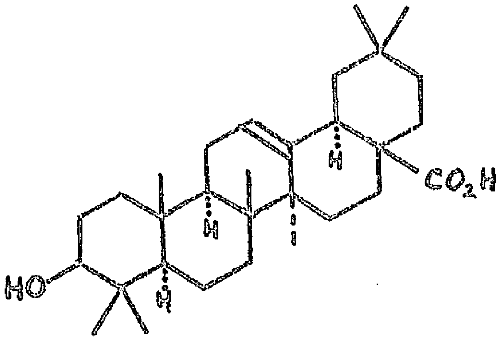
(CXXIV)

(a) R = OH
(b) R = OAc

(a) R = OH
(b) R = OAc



(CXXV)



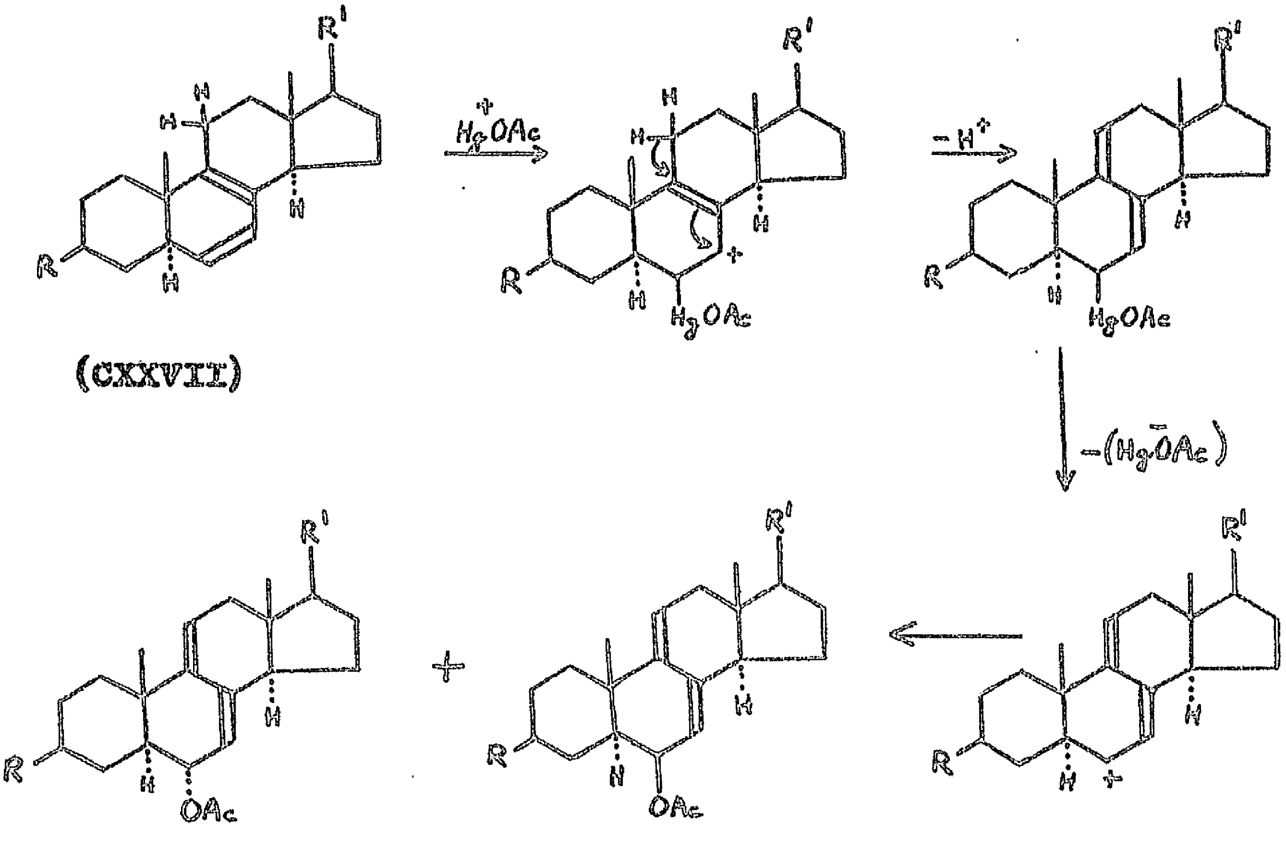
(CXXVI)

(a) R = OH
(b) R = OAc

The conversion of the methyl ester diene (CXXIIb) into the diene diol (LXXVIIIb) of the dehydrobetulin series has been described previously. A further relationship between the methyl ester diene (CXXIIb) and the lactone (XI) was demonstrated as follows: Vigorous alkaline hydrolysis of the methyl ester diene (CXXIIb) gave dehydrobetulinic acid (CXXIVa) which was readily hydrogenated to the hydroxy acid (CXXVa). An ethanolic solution of the unsaturated acid (CXXVa) was treated with dry hydrogen chloride to give, in good yield, the saturated hydroxy lactone (CXVa) which had previously been prepared from the acetyl lactone (XIb) by hydrogenation followed by mild hydrolysis. The lactonisation of the hydroxy acid (CXXVa) is very closely analogous to that of 18α-oleanolic acid[124] (CXXVI) and thus offers further support for the formulation of the non-conjugated dienes as 12,20(30)-dienes and of the ether and lactone as 28→13 cyclic derivatives.

The Mechanism of the Mercuric Acetate Reaction

Mercuric acetate dehydrogenation of certain unsaturated steroids produces conjugated systems[93-95] and the type of mechanism postulated by Barton[94] for this reaction is shown below. Initial electrophilic attack by $\overset{+}{\mathrm{HgOAc}}$ on the unsaturated system (CXXVII) is followed by loss of a proton to form in this case a heteroannular diene system. The final stage is removal of $\overset{-}{\mathrm{HgOAc}}$ which is replaced by $\overset{-}{\mathrm{OAc}}$ to give a mixture of epimers (CXXVIII) and (CXXIX).

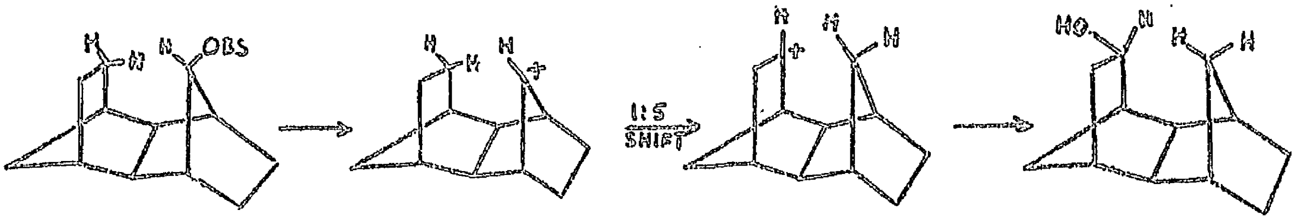


(CXXVII)

(CXXXVIII)

(CXXIX)

The mechanism of the action of mercuric acetate on lupeol, betulin, and betulinic acid is as yet uncertain but it is possible to make the following speculations, the first of which involves radical changes in the stereochemistry of the molecule, the D E ring junction passing from trans to cis fusion and the side chain at $C_{(19)}$ changing from the α-face to the β-face of the molecule. The second speculation involves a 1,5-hydride shift from $C_{(12)}$ to $C_{(20)}$ such as that described by Winstein[118] to explain the hydrolysis of the bromobenzene sulphonate (CXXX) to the alcohol (CXXXI). This alternative does not involve any radical stereochemical change.

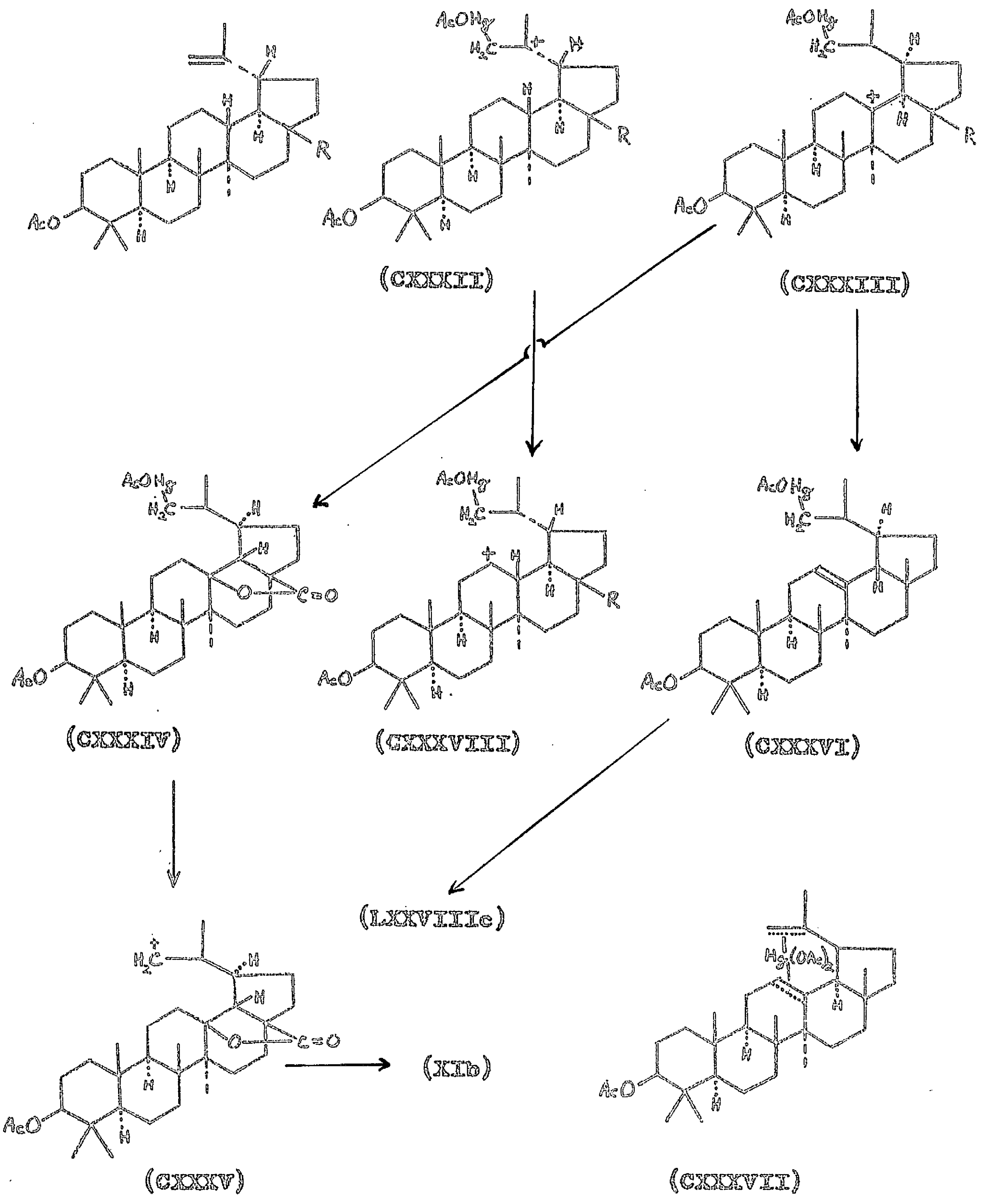


(CXXX)

(CXXXI)

(1) In the first case we may regard the reaction as being initiated by electrophilic attack of $\overset{+}{Hg}OAc$ on the double bond followed by hydride ion transfer from $C_{(18)}$ to $C_{(20)}$ by a concerted mechanism to form the carbonium ion (CXXXIII, $R = CH_3$ or CO_2H). In the case of acetyl betulinic acid (VIIIb) loss of a proton from the mercuricomplex (CXXXIII, $R = CO_2H$) results in the formation of the lactone (CXXXIV) which then loses $\overset{-}{Hg}OAc$ by combination with $\overset{+}{Hg}OAc$ to give mercurous acetate and the ion (CXXXV). Loss of a proton then leads to the lactone (XIb).

In the case of lupenyl acetate (VIIc) we may regard the ion (CXXXIII, $R = CH_3$) as losing a proton to form the mercuricomplex (CXXXVI), which is then transformed by loss of $\overset{-}{Hg}OAc$ followed by a proton to the dienyl acetate (LXXVIIIc). Probably


AcO
H
R
AcOHg
H2C
+
(CXXXII)
(CXXXIII)
O
C=O
(CXXXIV)
(CXXXVIII)
(CXXXVI)
(LXXVIIIc)
(CXXXV)
(XIb)
Hg(OAc)2
(CXXXVII)

the diene (LXXVIII) is formed in preference to isomeric dienes as its geometry is such that it can form a complex of type (CXXXVII) with an excess of mercuric acetate.

(2) An alternative mechanism involves formation of the mercuricomplex (CXXXII, R = CH_3 or CO_2H) which then forms the carbonium ion (CXXXVIII, R = CH_3 or CO_2H) as a result of a 1,5-hydrogen shift from $C_{(18)}$ to $C_{(20)}$. Loss of a proton from (CXXXVIII, R = CH_3) can lead to dehydrolupenyl acetate whereas in the case of acetyl betulinic acid proton loss is preceded by a 1,2-hydrogen shift, with ultimate formation of the lactone (XIb).

EXPERIMENTAL

SECTION II

Extraction of Lupeol from Ge-Tah Jelutong. - Powdered Ge-Tah Jelutong (1.25 Kg.) was heated under reflux with ethanol (7 l.) for 2.5 hr. The small amount of insoluble material was filtered off and the filtrate allowed to stand at room temperature overnight. A solution, in benzene (2.3 l.), of the yellow solid which separated out from the ethanol was treated with potassium hydroxide (800 g) in water (400 c.c.) and methanol (5 l.). After heating under reflux for 9 hr., approximately half of the solvent was evaporated off under reduced pressure. Addition of water (6 l.) precipitated the crude non-saponifiable fraction which was filtered off, washed well with water and carefully dried.

A solution of the dried solid (550 g.) in pyridine (1.5 l.) was treated with benzoyl chloride (400 c.c.), added over 30 minutes. The mixture was maintained at 100° for 2.5 hr. then treated cautiously with water (2.5 l.) and the resultant precipitate filtered off, washed with water and dried. The solid was shaken with ether (2.5 l.) then filtered to yield an ether insoluble fraction which was dried and crystallised several times from benzene-ethanol to give lupenyl benzoate as plates, m.p. 265-270°, $[\alpha]_D$ + 58.5° (c, 1.62). Further purification was effected by reduction with lithium aluminium hydride in ether, acetylation, and crystallisation of the resulting lupenyl acetate from chloroform-methanol as needles, m.p. and mixed m.p. 215-217°, $[\alpha]_D$ + 40° (c,0.79).

<u>Mercuric Acetate Dehydrogenation of Lupeol Derivatives.</u> - The mercuric acetate treatment of lupeol, lupenyl acetate, lupenyl benzoate, lupenone, α-lupene, betulin diacetate, acetyl betulinic acid, and methyl acetyl betulinate was carried out in the manner described below for lupenyl acetate.

A solution of lupenyl acetate, m.p. 215-217°, (10 g.) in chloroform (200 c.c.) was treated with a solution of mercuric acetate (120 g.) in glacial acetic acid (2 l.) at room temperature for 14 days or alternatively at 100° for 5 hr. The resultant precipitate of mercurous acetate was filtered off and the yellow filtrate diluted with water and extracted with chloroform. Careful washing of the combined chloroform layers followed by drying and evaporation of the solvent gave an orange solid which was dissolved in pyridine (300 c.c.) and treated with a stream of hydrogen sulphide until precipitation of mercuric sulphide was complete. A product, free from mercury, was obtained by filtration through kieselguhr and evaporation of the solvent under reduced pressure. A solution of this material (8.5 g.) in benzene-light petroleum (1:4) was filtered through a column of alumina (300 g.) and the product was crystallised from chloroform-methanol to give 3β-*acetoxylupa*-12,20(30)-*diene* as needles (1.7 g.), m.p. 229-231°, $[\alpha]_D$ + 47° (*c*, 1.3), $\lambda_{max.}$ 2070 Å., (ε = 7,000). This compound gave a deep yellow colour with tetranitromethane and showed bands in its infrared spectrum at 1736, 1248, 1626, and 891 cm.$^{-1}$ (Found: C, 82.4; H, 10.9. $C_{32}H_{50}O_2$ requires C, 82.3; H, 10.8%). Biedebach[91,92] gives m.p. 230-231° for this compound.

3β-*Hydroxylupa*-12,20(30)-*diene*. - Treatment of lupeol, m.p. 213-215°, (4 g.) as above, filtration of the product through alumina in a solution of benzene-ether (9:1) and crystallisation from chloroform-methanol gave 3β-*hydroxylupa*-12,20(30)-*diene* as needles (1.0 g.), m.p. 218-222°, $[\alpha]_D$ + 31° (*c*, 1.15), $\lambda_{max.}$ at 2070 Å. (ε = 6,500). This compound gave a deep yellow colour with tetranitromethane and showed bands in the infrared spectrum at 3509 cm.$^{-1}$ (hydroxyl), 1631, and 891 cm.$^{-1}$ (vinylidene). (Found: C, 84.8; H, 11.4. $C_{30}H_{48}O$ requires C, 84.8; H, 11.4%).

Hydrolysis of the above acetate with lithium aluminium hydride gave the same alcohol which regenerated the parent acetate, m.p. 229-231°, on treatment with acetic anhydride-pyridine.

Biedebach gives m.p. 228-229° for the alcohol.

3β-Benzoyloxylupa-12,20(30)-diene. – Lupenyl benzoate m.p. 260–265°, (17 g.) was treated with mercuric acetate as described above and a solution of the product in benzene-light petroleum (1:4) was filtered through alumina (500 g.) and crystallised from benzene-ethanol to give *3β-benzoyloxylupa-12,20(30)-diene* as rods (3.9 g.), m.p. 280–281°, $[\alpha]_D$ + 67.6° (*c*, 1.11), $\lambda_{max.}$ 2040, 2290 Å. (ε = 7,400; 4,450). It gave a deep yellow colour with tetranitromethane and showed bands in the infrared spectrum at 1733, 1279 cm.$^{-1}$ (ester), 890 cm.$^{-1}$ (vinylidene). (Found: C, 84.0; H, 10.2. $C_{37}H_{52}O_2$ requires C, 84.0; H, 9.9%).

Hydrolysis of the benzoate (400 mg.), m.p. 280–281°, with lithium aluminium hydride in benzene-ether (1:4, 250 c.c.) and crystallisation of the product from chloroform-methanol gave needles (260 mg.) m.p. 218–222°. The identity of this compound with 3β-hydroxylupa-12,20(30)-diene was shown by mixed m.p. and infrared comparison.

Biedebach gives m.p. 267–269° for the dienyl benzoate.

3-Oxolupa-12,20(30)-diene. –

(a) *By Mercuric Acetate Dehydrogenation of Lupenone.* Lupenone, m.p. 170–172°, (4 g.) was treated with mercuric acetate as in previous cases. The product (1.2 g.), eluted from alumina (120 g.) with light petroleum was crystallised from chloroform-methanol to give 3-oxolupa-12,20(30)-diene as rods (0.8 g.), m.p. 156-158°, $[\alpha]_D$ + 66.3° (c, 0.93), $\lambda_{max.}$ 2070 Å. ($\mathcal{E}$ = 7,750). This compound gave a dark yellow colour with tetranitromethane and showed bands in the infrared spectrum at 1701 cm.$^{-1}$ (ketone), 1623, 890 cm.$^{-1}$ (vinylidene). (Found: C, 85.3; H, 11.1. $C_{30}H_{48}O$ requires C, 85.2; H, 11.0%).

(b) By Kiliani Oxidation of 3β-Hydroxylupa-12,20(30)-diene. - A solution of 3β-hydroxylupa-12,20(30)-diene, m.p. 218-222°, (200 mg.) in acetone (15 c.c.) was treated dropwise with Kiliani reagent (0.174 g. sodium dichromate/ c.c.; 1 c.c.) and the reaction mixture was allowed to stand for 5 minutes. Careful dilution with water produced a crystalline precipitate which was recrystallised from chloroform-methanol yielding 3-oxolupa-12,20(30)-diene as rods, m.p. and mixed m.p. 156-158°.

Lupa-12,20(30)-diene. -

(a) By Mercuric Acetate Dehydrogenation of α-Lupene. -

α-Lupene, m.p. 163-165°, $[\alpha]_D$ + 28°, (4 g.) was treated with mercuric acetate as in the previous cases. The product, eluted with light petroleum from alumina (120 g.) was crystallised from chloroform-methanol to give lupa-12,20(30)-diene as rods (0.9 g.), m.p. 160-162°, $[\alpha]_D$ + 29.3° (c, 2.00). This compound depressed the m.p. of α-lupene. It gave a strong yellow colour with tetranitromethane and exhibited maximal absorption in the ultraviolet region at 2070 Å. (ε = 6,400). The infrared spectrum showed bands at 3075, 1626, 890 cm.$^{-1}$ (vinylidene), and was distinct from starting material. (Found: C, 88.4; H, 11.9. $C_{30}H_{48}$ requires C, 88.2; H, 11.8%).

(b) *By Wolff Kishner Reduction of* 3-*Oxolupa*-12,20(30)-*diene*. The ketone, m.p. 156-158°, (130 mg.) in ethanol (5 c.c.) was treated in an autoclave with sodium (150 mg.), ethanol (7 c.c.) and hydrazine hydrate (100%; 0.5 c.c.) at 195° for 6 hr. Isolation of the product in the usual way followed by purification by chromatography and crystallisation from chloroform-methanol gave lupa-12,20(30)-diene (70 mg.), m.p. and mixed m.p. 160-162°, $[\alpha]_D$ + 30° (*c*, 1.42). The material was identical with that obtained above.

Oxidation of 3β-*Acetoxylupa*-12,20(30)-*diene with Perbenzoic Acid*. - A solution of the dienyl acetate, m.p. 227-229°, (1 g.) in benzene (50 c.c.) was treated with a solution of perbenzoic acid (1.9N, 10 c.c.) and the reaction mixture was allowed to stand at room temperature for 48 hr. The product, extracted by means of benzene, was crystallised several times from chloroform-methanol to give 3β-*acetoxy*-12(13), 20(30)-*diepoxylupane* as needles (440 mg.), m.p. 275-277° (vac.),

$[\alpha]_D$ + 49° (c, 0.95). This compound gave no colour with tetranitromethane and showed no selective absorption in the ultraviolet region. Its infrared spectrum showed bands at 1739, 1250 cm.$^{-1}$ (OAc). (Found: C, 77.0; H, 10.2. $C_{32}H_{50}O_4$ requires C, 77.1; H, 10.1%).

<u>Treatment of 3β-Acetoxylupa-12,20(30)-diene with Osmium Tetroxide</u>. - The dienyl acetate (1 g.) in pyridine (10 c.c.) was treated with osmium tetroxide (1.6 g.) in ether (20 c.c.) and the mixture was kept in darkness at room temperature for 11 days. After dilution of the mixture with ether (100 c.c.), a suspension of lithium aluminium hydride (4 g.) in ether (50 c.c.) was added slowly to the boiling reaction mixture. When addition of the metal hydride suspension was complete, 0.5 hr., the mixture was allowed to reflux for a further hour. The product, isolated by extraction with ether, was a resinous gum (900 mg.) which showed only slight end absorption in the ultraviolet region and strong absorption at 3300 cm.$^{-1}$ (OH) in the infrared. This crude pentahydroxy derivative was not further purified.

<u>Treatment of the Pentahydroxy Derivative with Lead Tetra-acetate</u>. - A solution of the pentahydroxy derivative (244 mg.) in chloroform (3 c.c.) and glacial acetic acid (24 c.c.) was treated with lead tetra-acetate (1 g.). The

mixture was maintained at room temperature in a stoppered flask for 20 hr. then shaken thoroughly with water (50 c.c.) and extracted with chloroform (100 c.c.). The combined aqueous phase, after two further washes of the chloroform layer with water (2 x 50 c.c.), was treated with a saturated aqueous solution (50 c.c.) of dimedone (5,5-dimethylcyclohexan-1,3-dione) and allowed to stand at room temperature for 40 hr. after which, fine needles had separated. Crystallisation of this material from methanol gave the dimethone derivative of formaldehyde m.p. and mixed m.p. 192°. Its infrared spectrum was identical with that of an authentic specimen. (Found: C,69.6; H,8.0. Calc. for $C_{17}H_{24}O_4$: C,69.8; H,8.2%).

The product isolated from the chloroform extract could not be crystallised.

3β,30-Diacetoxy-12β,13β,20-trihydroxylupane. - Acetylation of the pentahydroxy derivative (650 mg.) with pyridine-acetic anhydride (1:1, 30 c.c.) at room temperature for 22 hr. and isolation of the product by means of ether yielded a brown solid which was dissolved in benzene-light petroleum (1:1, 150 c.c.) and chromatographed on alumina (25 g.). Elution with benzene-ether (4:1) gave fractions which crystallised from chloroform-light petroleum to give 3β,30-diacetoxy-12β,13β,20-trihydroxylupane, m.p. 239-240° (vac.), $[\alpha]_D$ + 4.6° (c, 2.64). The compound was transparent to ultraviolet light and gave no colour with tetranitromethane. The infrared spectrum contained bands at 3484 cm.$^{-1}$ (OH), 1736, 1712, 1244, 1212 cm.$^{-1}$ (OAc). (Found: C, 71.1; H, 9.8, and C, 70.7; H, 9.8. $C_{34}H_{56}O_7$ requires C, 70.8; H, 9.8%).

Treatment of 3β-Acetoxylupa-12,20(30)-diene with Mineral Acid. - A solution of the dienyl acetate, m.p. 227-229°, (2 g.) in glacial acetic (200 c.c.) at 100° was treated with concentrated hydrochloric acid (5 c.c.) and the mixture was kept at the same temperature for 15 minutes. Rapid evaporation of the solvent under reduced pressure gave a brown gum which was dissolved in light petroleum (200 c.c.) and chromatographed on alumina (80 g.).

3β-Acetoxylupa-12,18-diene. - The fractions (716 mg.) eluted with light petroleum (2 l.) were crystallised from chloroform-methanol to yield 3β-acetoxylupa-12,18-diene as needles m.p. 179-181°, $[\alpha]_D$ + 260° (c, 1.43), $\lambda_{max.}$ 2340 Å (ϵ = 9,500) The infrared spectrum showed bands at 1730, 1253 cm.$^{-1}$ (OAc) 1642 cm.$^{-1}$ (diene). (Found: C, 82.2; H, 11.0. $C_{32}H_{50}O_2$ requires C, 82.3; H, 10.8%).

3β-Hydroxylupa-12,18-diene. - Hydrolysis using lithium aluminium hydride in ether and crystallisation from chloroform-

-methanol yielded 3β-*hydroxylupa*-12,18-*diene* as needles, m.p. 208-211°, $[\alpha]_D$ + 282° (*c*, 0.82), $\lambda_{max.}$ 2340 Å. (ϵ = 8,800). Infrared absorption: - bands at 3584 cm.$^{-1}$ (OH), 1639 cm.$^{-1}$ (diene). (Found: C, 84.9; H, 11.7. $C_{30}H_{48}O$ requires C, 84.8; H, 11.4%).

Both compounds gave a brown colour with tetranitromethane.

3β-*Acetoxylupa*-11,13(18)-*diene*. - The fractions (250 mg.) eluted with benzene-light petroleum (1:4, 2 l.) crystallised from chloroform-methanol to give 3β-*acetoxylupa*-11,13(18)-*diene* as needles, m.p. 155-156°, $[\alpha]_D$ - 74.5° (*c*, 1.12), $\lambda_{max.}$ 2440, 2520, 2600 Å. (ϵ = 23,800; 27,000; 19,400). Infrared absorption bands at 1736, 1245 cm.$^{-1}$ (OAc), 1639 cm.$^{-1}$ (diene). (Found: C, 82.3; H, 10.9. $C_{32}H_{50}O_2$ requires C, 82.3; H, 10.8%).

3β-*Hydroxylupa*-11,13(18)-*diene*. - Hydrolysis using lithium aluminium hydride in ether and crystallisation from chloroform-methanol gave 3β-*hydroxylupa*-11,13(18)-*diene* as needles, m.p. 205-207°, $[\alpha]_D$ - 80° (*c*, 0.66), $\lambda_{max.}$ 2420, 2500, 2600 Å. (ϵ = 21,000; 24,400; 16,100). The infrared spectrum showed bands at 3448 cm.$^{-1}$ (OH), 1639 cm.$^{-1}$ (diene). (Found: C, 84.7; H, 11.75. $C_{30}H_{48}O$ requires C, 84.8; H, 11.4%).

Both compounds gave a brown colour with tetranitromethane.

Treatment of the Conjugated Dienyl Acetates with Mineral Acid. -

(a) 3β-Acetoxylupa-12,18-diene, m.p. 179-181°, (40 mg.) in glacial acetic acid (50 c.c.) and concentrated hydrochloric acid (1 c.c.) was heated on the steam bath for 1 hr. Evaporation of the solvent followed by chromatography of the residue in light petroleum (10 c.c.) on alumina (3 g.) gave a fraction (18 mg.) which crystallised from methanol to yield starting material, identified by m.p., mixed m.p., comparison of infrared spectra and ultraviolet absorption [$\lambda_{max.}$ 2350 Å. (ε = 8,700)].

A later fraction (15 mg.), eluted with light petroleum, crystallised from methanol to give 3β-acetoxylupa-11,13(18)-diene as needles m.p. and mixed m.p. 152-154°, $\lambda_{max.}$ 2440, 2510, 2600 Å.; (ε = 24,000; 26,800; 17,600). The infrared spectrum was identical with that of an authentic sample.

(b) 3β-Acetoxylupa-11,13(18)-diene, m.p. 154-155°, (84 mg.) in glacial acetic acid (50 c.c.) and concentrated hydrochloric acid (1 c.c.) was heated on the steam bath for 1 hr. Chromatography of the product resolved the mixture into the same two conjugated dienyl acetates as in (a). They were identified by m.p.'s, mixed m.p.'s, ultraviolet absorption spectra, and infrared spectra.

<u>Hydrogenation of</u> 3β-<u>Acetoxylupa</u>-12,18-<u>diene</u>. - 3β-Acetoxylupa-12,18-diene, m.p. 179-181°, (120 mg.) in glacial acetic acid (100 c.c.), containing platinum (from platinum oxide, 100 mg.), was shaken with hydrogen for 20 hr. The product, isolated in the usual manner, crystallised from methanol to give 3β-<u>acetoxylup</u>-13(18)-<u>ene</u> as fine needles (89 mg.), m.p. 184-185° (vac.), $[\alpha]_D$ - 20.7° (<u>c</u>,1.33), $\lambda_{max.}$ 2070 Å. ($\mathcal{E}$ = 10,100). This compound gave a yellow colour with tetranitromethane and showed bands in the infrared spectrum at 1730, 1241 cm.$^{-1}$ (OAc). (Found: C,80.6, H,11.2 and C,80.4; H,11.6. $C_{32}H_{52}O_2 . 0.5CH_3OH$ requires C,80.5; H,11.2%).

3β-<u>Hydroxylup</u>-13(18)-<u>ene</u>. - Hydrolysis using lithium aluminium hydride in ether and crystallisation of the product from methanol gave 3β-<u>hydroxylup</u>-13(18)-<u>ene</u> as clusters of blades, m.p. 193-196°, $[\alpha]_D$ - 39.4° (<u>c</u>,1.22), $\lambda_{max.}$ 2070 Å. ($\mathcal{E}$ = 9,300). This compound gave a yellow colour with tetranitromethane and showed a band at 3367 cm.$^{-1}$ in the infrared spectrum. (Found: C,84.1; H,11.9. $C_{30}H_{50}O$ requires C,84.4; H,11.8%).

<u>Hydrogenation of</u> 3β-<u>Acetoxylupa</u>-11,13(18)-<u>diene</u>. - A solution of 3β-acetoxylupa-11,13(18)-diene, m.p. 154-155°, (150 mg.) in glacial acetic acid (100 c.c.) was shaken with

hydrogen in the presence of platinum (from platinum oxide, 100 mg.) for 20 hr. After working up in the usual manner the product was crystallised from methanol to give 3β-acetoxylup-13(18)-ene as fine needles (105 mg.), m.p. and mixed m.p. 184-185° (vac.). The ultraviolet spectrum showed maximal absorption at 2080 Å. ($\mathcal{E}$ = 8,000) and the infrared spectrum was identical with that of the compound from the previous experiment.

Hydrogenation of 3β-*Acetoxylupa*-12,20(30)-*diene*. - A solution of 3β-acetoxylupa-12,20(30)-diene, m.p. 227-229°, (1.5 g.) in ethyl acetate-acetic acid (1:1, 600 c.c.) was shaken with hydrogen in the presence of platinum (from platinum oxide, 500 mg.) for 28 hr. Filtration and evaporation of the solvent gave a white solid which crystallised from chloroform-methanol to give 3β-*acetoxylup*-12-*ene* as blades, m.p. 249-251° (vac.), $[\alpha]_D$ + 9° (*c*, 1.40), $\lambda_{max.}$ 2060 Å. ($\mathcal{E}$ = 3,600). This compound gave a pale yellow colour with tetranitromethane and showed bands in the infrared spectrum at 1739, 1240 $cm.^{-1}$ (OAc). (Found: C, 81.65; H, 11.2. $C_{32}H_{52}O_2$ requires C, 82.0; H, 11.2%).

3β-*Hydroxylup*-12-*ene*. - Hydrolysis of the above acetate with lithium aluminium hydride in ether, or with alcoholic potassium hydroxide (3%), and crystallisation of the product from methanol gave 3β-hydroxylup-12-ene as needles, m.p. 225-227° (vac.), $[\alpha]_D$ - 12.5° (c, 1.21), $\lambda_{max.}$ 2060 Å. (ε = 6,000). This compound gave a pale yellow colour with tetranitromethane and showed a band in the infrared spectrum at 3448 cm.$^{-1}$ (OH). (Found: C, 84.2; H, 12.0. $C_{30}H_{50}O$ requires C, 84.4; H, 11.8%). Acetylation with pyridine-acetic anhydride regenerated the parent acetate.

Oxidation of 3β-Acetoxylup-12-ene with Chromium Trioxide. - The acetate (500 mg.) in stabilised acetic acid (150 c.c.) was treated at room temperature with a solution of chromium trioxide (200 mg.) in 90% acetic acid (70 c.c.) for 20 hr. After destroying excess chromic acid with methanol, the product was isolated by means of ether and chromatographed in light petroleum (100 c.c.) on alumina (20 g.).

Elution with light petroleum gave a product which crystallised from chloroform-methanol as plates (75 mg.), m.p. 272-274°, $[\alpha]_D$ + 24° (c, 1.1). This compound gave no colour with tetranitromethane, showed no absorption in the ultraviolet region and showed bands at 1736 and 1244 cm.$^{-1}$ in its infrared spectrum (Found: C, 81.8; H, 11.6 and C, 81.75; H, 11.4. $C_{32}H_{52}O_2$ requires C, 81.6; H, 11.2%).

A second saturated acetate was eluted with light petroleum-benzene (1:2) and crystallised from chloroform methanol as needles (85 mg.), m.p. 244-246°, $[\alpha]_D$ + 36.5°

(c, 0.7). This compound gave no colour with tetranitromethane and showed no selective absorption in the ultraviolet region. Again there were only acetate bands at 1736, 1247 cm.$^{-1}$ in the infrared spectrum. (Found: C, 81.6; H, 11.5. $C_{32}H_{52}O_2$ requires C, 82.0; H, 11.2%).

3β-Acetoxy-11-oxolup-12-ene. - Elution with benzene and benzene-ether (4:1) gave fractions which crystallised from chloroform-methanol to give 3β-acetoxy-11-oxolup-12-ene as plates (260 mg.), m.p. 290-292°, $[\alpha]_D$ - 61° (c, 0.9), $\lambda_{max.}$ 2440 Å. (ε = 13,500). This product gave a faint yellow colour with tetranitromethane and showed bands in the infrared at 1730, 1253 cm.$^{-1}$ (OAc), 1689 cm.$^{-1}$ (αβ-unsaturated ketone). (Found: C, 79.8; H, 10.75. $C_{32}H_{50}O_3$ requires C, 79.6; H, 10.4%)

Hydrogenation of 3β-Acetoxy-11-oxolup-12-ene. - The αβ-unsaturated keto-acetate, m.p. 290-292° (50 mg.) in glacial acetic acid (75 c.c.) was shaken with hydrogen in the presence of platinum (from platinum oxide, 40 mg.) for 48 hr. Isolation of the product in the usual way gave a white solid which crystallised from chloroform-methanol as plates (42 mg.), m.p. 248-250° (vac.), $[\alpha]_D$ + 9° (c, 1.68). Identity with 3β-acetoxylup-12-ene was shown by mixed m.p. with an authentic specimen and comparison of their infrared spectra.

Treatment of 3β-*Acetoxylup*-12-*ene with Perbenzoic Acid.* - A solution of 3β-acetoxylup-12-ene (150 mg.) in benzene (5 c.c.) was treated with a solution of perbenzoic acid in benzene (1N, 1.5 c.c.) at room temperature for 20 hr. The product, isolated in the usual way, was dissolved in light petroleum (25 c.c.) and chromatographed on alumina (15 g.)

The fraction (20 mg.), eluted with light petroleum (200 c.c.), crystallised from methanol as plates, m.p. 226-228°, $[\alpha]_D$ - 20.4° (*c*, 0.82). This compound gave no colour with tetranitromethane and showed no selective absorption in the ultraviolet. Infrared bands 1739, 1242 cm.$^{-1}$ (OAc). (Found: C, 81.5; H, 11.4. $C_{32}H_{52}O_2$ requires C, 82.0; H, 11.2%).

The fractions (91 mg.) eluted with benzene-light petroleum (1:3, 1 l.) crystallised from methanol to give 3β-*acetoxy*-12(13)-*epoxylupane* as blades, m.p. 236-238°, $[\alpha]_D$ + 32° (*c*, 0.69). This compound showed no absorption in the ultraviolet and gave no colour with tetranitromethane. Infrared bands 1736, 1242 cm.$^{-1}$ (OAc). (Found: C, 79.2; H, 10.7. $C_{32}H_{52}O_3$ requires C, 79.3; H, 10.8%).

Treatment of 3β-*Hydroxylup*-12-*ene with Monoperphthalic Acid.* - A solution of 3β-hydroxylup-12-ene, m.p. 225-227° (vac), (500 mg.) in ether (10 c.c.) was treated with excess monoperphthalic acid in ether for 5 days at room temperature. Isolation by means of ether gave a white solid (480 mg.) which crystallised from methanol to give 3β-hydroxy-12(13)-epoxylupane as needles, m.p. 234-236° (vac.), $[\alpha]_D$ + 28.2° (c, 0.92). This compound gave no colour with tetranitromethane and showed no selective absorption in the ultraviolet. The infrared spectrum showed only a hydroxyl band at 3509 cm.$^{-1}$. (Found: C, 81.3; H, 11.6. $C_{30}H_{50}O_2$ requires C, 81.4; H, 11.4%).

Treatment of 3β-Hydroxy-12(13)-epoxylupane with Mineral Acid. - The saturated alcohol, m.p. 234-236° (vac.), (55 mg.) in glacial acetic acid (25 c.c.) was treated with concentrated hydrochloric acid (1 c.c.) for 30 minutes at 100°. Rapid evaporation of the solvent under reduced pressure gave a saturated acetate which crystallised from chloroform-methanol as lustrous plates (22 mg.), m.p. 270-272°, $[\alpha]_D$ + 23.8° (c, 0.68). This compound gave no colour with tetranitromethane and was transparent to ultraviolet light. It did not depress the m.p. of the saturated acetate from chromic acid oxidation of 3β-acetoxylup-12-ene. The identity of these two saturated acetates was demonstrated by comparison of their infrared spectra which showed bands at 1739, 1245 cm.$^{-1}$ (OAc).

Treatment of 3β-Acetoxylup-12-ene with Osmium Tetroxide. - A solution of 3β-acetoxylup-12-ene (700 mg.) in pyridine (5 c.c.) and dry ether (10 c.c.) was treated with osmium tetroxide (700 mg.) in ether (10 c.c.). The mixture was kept in darkness at room temperature for 14 days and the product isolated in the usual manner as a yellow solid (620 mg.). This material was treated with pyridine-acetic anhydride (1:1, 20 c.c.) at room temperature for 20 hr., and the resulting crude acetate was dissolved in benzene (25 c.c.) and chromatographed on alumina (30 g.)

Elution with benzene (500 c.c.) and with benzene-ether (4:1, 1500 c.c.) gave a product which crystallised from chloroform-methanol to yield 3β-acetoxy-12β,13β-dihydroxylupane as well formed plates, m.p. 215-245°, $[\alpha]_D$ + 8.9° (c,0.79). This compound gave no colour with tetranitromethane and showed no selective absorption in the ultraviolet region. Infrared bands:- 3534 cm^{-1} (OH), 1712, 1269, 1255 cm.$^{-1}$ (OAc) (Found: C,76.6; H,10.8. $C_{32}H_{54}O_4$ requires C,76.4; H,10.8%).

Treatment of the above Triol-monoacetate with Lead Tetra-acetate. - A solution of the above triol mono-acetate (72.6 mg.) in chloroform (10 c.c.) and dry benzene (40 c.c.) was treated with lead tetra-acetate (180 mg.) at room temperature for 24 hr. During this period, a precipitate of orange solid settled out. Addition of ethylene glycol (2 drops) followed by filtration through kieselguhr gave a colourless filtrate which was evaporated to dryness. The white residual solid was crystallised from methanol to give 3β-acetoxy-12,13-seco-

-12,13-dioxolupane (LXXXVI), as plates, m.p. 186-188°, $[\alpha]_D$ + 39.5° (c,1.05). This compound showed low intensity absorption in the ultraviolet at 2800 Å. ($\mathcal{E}$ = 150), and it gave no colour with tetranitromethane. The infrared spectrum of this compound in Nujol mull and using a sodium chloride prism showed no hydroxyl band but showed a broad carbonyl band between 1730 and 1705 cm.$^{-1}$ which was not resolved. Determination of the infrared spectrum using a 10% solution of the compound in carbon tetrachloride and with a calcium fluoride prism gave excellent resolution of the following bands: 2882, 1717 cm.$^{-1}$ (aldehyde), 1739, 1240 cm.$^{-1}$ (OAc), 1706 cm.$^{-1}$ (ketone in a six-membered ring) (Found: C,76.5; H,10.4. $C_{32}H_{52}O_4$ requires C,76.75; H,10.5%).

The Isolation of Betulinic Acid.

The isolation of betulinic acid was carried out in approximately the same manner as that described by Bruckner, Kovacs and Koczka.[116] The method of purification of the crude acid thus obtained was modified considerably and resulted in a greatly increased yield (*ca.* 2.2% as compared with 0.7%).

Finely powdered bark (2 Kg.) from the London plane tree (Platanus acerifolia) was extracted with methanol (10 l.) in a Soxhlet apparatus for 9 hr. Concentration of the methanolic extract to approximately 2 litres, followed by cooling, yielded the crude betulinic acid as a brown solid (40 g.), m.p. 270-280°. Two further extractions yielded 32 g. and 16 g. respectively. Evaporation of the mother liquors from the three extractions afforded a further crop of crude acid (10 g.).

Attempted purification of the crude acid by crystallisation from methanol, after treatment with charcoal, proved to be unsatisfactory and extremely wasteful. For this reason the following procedure was adopted. The crude acid (90 g.) was partially purified by crystallisation from methanol and the solid thus obtained (60 g.) was treated with pyridine-acetic anhydride (1:1, 500 c.c.) at 100° for 2 hr. The reaction mixture was diluted with water, extracted with ether and the combined ethereal extract washed with dilute hydrochloric acid then with water. The ethereal solution was dried and evaporated to give a brown gum which was heated under reflux with aqueous methanol (5%, 1 l.) for 30 minutes to destroy any mixed anhydride of betulinic acid. Removal of the solvent under vacuum, followed by azeotropic distillation with benzene to remove traces of water, gave a brown solid (65 g.). A portion of this material (15 g.) was dissolved in benzene-light petroleum (1:1, 400 c.c.) and chromatographed on silica gel (1 kg.).

Elution with benzene (3 l.) and benzene-ether (99:1, 6 l.) gave a fraction (13.5 g.), crystallisation of which from chloroform-methanol gave acetyl betulinic acid (11 g.) as rods, m.p. 283-288° (decomp.), 296-297° (vac.), $[\alpha]_D$ + 22.2° (c, 1.24), $\lambda_{max.}$ 2040 Å. (ε = 4,700). The infrared spectrum showed bands at 1739, 1245 cm.$^{-1}$ (OAc), 1695 cm.$^{-1}$ (CO_2H), 1645, 885 cm.$^{-1}$ (vinylidene). (Found: C,75.7; H,10.3. Calc. for $C_{32}H_{50}O_4 \cdot \frac{1}{2}CH_3OH$: C,75.8; H,10.2%).

Ruzicka[117] gives m.p. 288-290° (decomp.), $[\alpha]_D$ + 20°, for this compound.

<u>Methylation of Acetyl Betulinic Acid.</u> - Acetyl betulinic acid, m.p. 283-288°, (10 g.) in ether (250 c.c.) was

treated with an ethereal solution of diazomethane at room temperature for 20 hr. Evaporation of the ether, filtration of the product in benzene solution through alumina (150 g.) and crystallisation of the resultant material from chloroform-methanol yielded methyl acetyl betulinate as rods, m.p. 202-203°, $[\alpha]_D$ + 18.9° (c, 2.49), $\lambda_{max.}$ 2050 Å. (ε = 4,180). The infrared spectrum showed bands at 1736, 1242, 1224 cm.$^{-1}$ (ester), 1645, 891 cm.$^{-1}$ (vinylidene). (Found: C, 77.1; H, 10.2. Calc. for $C_{33}H_{52}O_4$: C, 77.3; H, 10.2%).

Bruckner[116] gives m.p. 202-203°, $[\alpha]_D$ + 17° for this compound.

Reductive Hydrolysis of Methyl Acetyl Betulinate. - As a further check of identity, methyl acetyl betulinate (150 mg.) in ether (30 c.c.) was boiled under reflux with lithium aluminium hydride (50 mg.) for 2.5 hr. Working up in the usual way and crystallisation of the product from chloroform-methanol gave betulin, m.p. and mixed m.p. 252-255°, $[\alpha]_D$ + 20.8° (c, 1.58). Identity with an authentic specimen of betulin was confirmed by comparison of the infrared spectra.

Treatment of Acetyl Betulinic Acid with Mercuric Acetate. - A solution of acetyl betulinic acid (10 g.) in chloroform (300 c.c.) was treated with mercuric acetate (140 g.) in glacial acetic acid (2 l.) and the mixture was maintained either at room temperature for 14 days or alternatively at 100° for 5 hr. The crude product was isolated in the usual way and was purified by chromatography on alumina (300 g.). Elution of the column with benzene-light petroleum (1:1) gave a fraction (5.2 g.), crystallisation of which from chloroform-methanol gave 3β-*acetoxylup*-20(30)-*en*-28:13β-*olide* as blades, m.p. 315-317°, $[\alpha]_D$ + 60.3° (*c*, 2.54). The ultraviolet spectrum of this compound showed only low intensity absorption at 2040 Å. (ε=2000) and it gave no colour with tetranitromethane (cf. 13). The infrared spectrum contained bands at 1786 cm.$^{-1}$ (γ-lactone), 1739, 1245 cm.$^{-1}$ (OAc), 1630, 913 cm.$^{-1}$ (vinylidene). (Found: C, 77.1; H, 9.8. $C_{32}H_{48}O_4$ requires C, 77.4; H, 9.7%).

3β-*Hydroxylup*-20(30)-*en*-28:13β-*olide*. - Hydrolysis of the acetoxy lactone with methanolic potassium hydroxide (3%, 50 c.c.) for 3 hr. and working up in the usual way gave a product which was dissolved in ether and filtered through alumina. The resulting material crystallised from methanol to give 3β-*hydroxylup*-20(30)-*en*-28:13β-*olide* as needles, m.p. 308-309° (vac.), $[\alpha]_D$ + 57° (*c*, 1.0). The compound showed no appreciable absorption in the ultraviolet and gave no colour with tetranitromethane. Infrared bands:- 3436 cm.$^{-1}$ (OH), 1776 cm.$^{-1}$ (lactone), 1639, 893 cm.$^{-1}$ (vinylidene).

(Found: C, 79.0; H, 10.3. $C_{30}H_{46}O_3$ requires C, 79.2; H, 10.2%).

Hydrogenation of 3β-Acetoxylup-20(30)-en-28:13β-olide. - A solution of the unsaturated acetyl lactone, m.p. 315-317°, (150 mg.) in glacial acetic acid (100 c.c.) was shaken with hydrogen in the presence of platinum (from platinum oxide, 50 mg.) for 20 hr. Filtration and evaporation of the filtrate to dryness gave a product which crystallised from chloroform-methanol to give 3β-acetoxylupan-28:13β-olide as blades, m.p. 301-303° (vac.), $[\alpha]_D$ + 46° (c, 1.78). This compound showed no selective absorption in the ultraviolet region nor did it give any colour with tetranitromethane. The infrared spectrum showed bands at 1773 cm.$^{-1}$ (lactone), 1730, 1248 cm.$^{-1}$ (OAc) and was identical with that of the saturated lactone prepared by oxidation of the acetyl ether (CXI) derived from betulin. A mixed m.p. with this compound was undepressed, 301-303° (vac.). (Found: C, 76.85; H, 10.2. $C_{32}H_{50}O_4$ requires C, 77.1; H, 10.1%).

3β-Hydroxylupan-28:13β-olide. - Similar hydrogenation of the hydroxy lactone, m.p. 308-309 (vac.), gave 3β-hydroxylupan-28:13β-olide as needles, m.p. 314-315°, $[\alpha]_D$ + 40.7° (c, 1.06). This compound was transparent to ultraviolet light and gave no colour with tetranitromethane. Infrared bands:- 3571, 3472 cm.$^{-1}$ (OH), 1767 cm.$^{-1}$ (γ-lactone). (Found: C, 78.7; H, 10.6. $C_{30}H_{48}O_3$ requires C, 78.9; H, 10.6%).

Ozonolysis of 3β-*Acetoxylup*-20(30)-*en*-28:13β-*olide*. - A solution of the unsaturated acetyl lactone, m.p. 313-315°, (220 mg.) in chloroform (20 c.c.) was treated with a stream of ozonised oxygen at -45° for 45 minutes. When the solution had regained room temperature it was acidified with glacial acetic acid (4 c.c.) and treated with zinc dust (1 g.), added with stirring over 20 minutes. The solution was stirred for a further 40 minutes, filtered, diluted with chloroform (75 c.c.) and the chloroform layer was washed with water (3 x 50 c.c.).

The aqueous phase was separated carefully from chloroform and was then treated with a cold saturated aqueous solution of dimedone and allowed to stand for 48 hr. Filtration of the resultant precipitate and crystallisation from methanol gave formaldehyde dimethone as needles (58 mg., 45%), m.p. and mixed m.p. 192°.

The chloroform extract was dried and evaporated under reduced pressure to yield a white solid (195 mg.) which crystallised from ethanol to give 3β-*acetoxy*-30-*nor*-20-*oxolupan*-28:13β-*olide* as plates, m.p. 317-319° (vac.), $[\alpha]_D$ + 1.8° (*c*, 2.3). This compound was transparent to ultraviolet light and gave no colour with tetranitromethane. The infrared spectrum contained bands at 1786 cm.$^{-1}$ (γ- lactone) 1733, 1247 cm.$^{-1}$ (OAc) and 1715 cm.$^{-1}$ (ketone). (Found: C, 74.8; H, 9.5. $C_{31}H_{46}O_5$ requires C, 74.7; H, 9.3%).

Oxidation of 3β-Acetoxylup-20(30)-en-28:13β-olide with Selenium Dioxide. - The unsaturated acetyl lactone, m.p. 313-315°, (203 mg.) in analar glacial acetic acid (10 c.c.) was boiled under reflux with selenium dioxide (350 mg.) for 2 hr. Metallic selenium was removed by filtration and the filtrate evaporated to dryness under reduced pressure to give a reddish brown solid which was dissolved in benzene-light petroleum (1:1, 20 c.c.) and chromatographed on alumina (10 g.). Elution with the same solvent mixture yielded fractions (160 mg.) which crystallised from methanol to give 3β-acetoxy-29-oxolup-20(30)-en-28:13β-olide as fine needles, m.p. 304-305° (decomp.), 312-314° (vac.), $[\alpha]_D$ + 46.5° (c, 1.25), $\lambda_{max.}$ 2220 A. (ε = 6,800). This compound gave no colour with tetranitromethane and showed bands in its infrared spectrum at 1779 cm.$^{-1}$ (γ- lactone), 1730 and 1242 cm.$^{-1}$ (OAc), and 1692 cm.$^{-1}$ (αβ-unsaturated aldehyde). (Found: C, 75.3; H, 9.2. $C_{32}H_{46}O_5$ requires C, 75.3; H, 9.1%).

Reductive Hydrolysis of 3β-Acetoxylup-20(30)-en-28:13β-olide. - A solution of the unsaturated acetyl lactone, m.p. 313-315°, (2 g.) in benzene-ether (1:4, 500 c.c.) was refluxed with lithium aluminium hydride (2 g.) for 1.5 hr.

then worked up through ether (without using mineral acid) to yield a white solid which crystallised from aqueous methanol to give 3β,13β,28-*trihydroxylup*-20(30)-*ene* as prisms, m.p. 204-206°, $[\alpha]_D$ + 49° (*c*,1.75), $\lambda_{max.}$ 2040 Å. (ε = 3,500). This compound gave a pale yellow colour with tetranitromethane Its infrared spectrum exhibited bands at 3356 cm.$^{-1}$ (OH), 1647, 893 cm.$^{-1}$ (vinylidene). (Found: C,75.55; H,11.2 and C,75.9; H,11.0. $C_{30}H_{50}O_3 . CH_3OH$ requires C,75.9; H,11.1%).

3β,28-*Diacetoxy*-13β-*hydroxylup*-20(30)-*ene*. - Acetylation of the triol, m.p. 204-206°, (1.5 g.) in pyridine-acetic anhydride (1:1, 30 c.c.) at room temperature for 48 hr. followed by the usual working up and crystallisation from chloroform-methanol afforded 3β,28-*diacetoxy*-13β-*hydroxylup*-20(30)-*ene* as blades, m.p. 228-230°, $[\alpha]$ + 43.5° (*c*,1.93) $\lambda_{max.}$ 2050 Å. (ε = 3,900). This compound gave a yellow colour with tetranitromethane and its infrared spectrum showed bands at 3460 cm.$^{-1}$ (OH), 1739, 1712, 1254 cm.$^{-1}$ (OAc), 1645, 884 cm.$^{-1}$ (vinylidene). (Found: C,75.3; H,10.1. $C_{34}H_{54}O_5$ requires C,75.2; H,10.0%).

Treatment of the above triol diacetate (200 mg.) in pyridine-acetic anhydride (1:1, 10 c.c.) at 100° for 3 hr. and isolation of the product in the usual way gave unchanged starting material, m.p. and mixed m.p. 228-230°, $[\alpha]_D$ + 45°

(c, 1.05). Identity was confirmed by comparison of the infrared spectra.

Dehydration of 3β,28-Diacetoxy-13β-hydroxylup-20(30)-ene.- A solution of 3β,28-diacetoxy-13β-hydroxylup-20(30)-ene (1 g.) in dry pyridine (60 c.c.) was treated at 100° with phosphorus oxychloride (8 c.c.) for 3 hr. Excess phosphorus oxychloride was destroyed by cautious addition of crushed ice to the cooled solution then the product was isolated by means of ether and crystallised from ethanol as plates, m.p. 177-179°, $[\alpha]_D$ + 45° (c, 0.97). This compound was shown to be identical with 3β,28-diacetoxylupa-12,20(30)-diene (p.125) by comparison of their infrared spectra and by mixed m.p.

Treatment of 3β-Acetoxylup-20(30)-en-28:13β-olide with Hydrogen Chloride. - Dry ethanol (400 c.c.) was saturated at 0° with hydrogen chloride and was added in portions to a solution of 3β-acetoxylup-20(30)-en-28,13β-olide (1 g.) in dry chloroform (50 c.c.) and dry ethanol (300 c.c.). The mixture was kept at room temperature for 6 days then was poured into water and extracted with chloroform. The chloroform layer was washed with water (4 x 250 c.c.), dried and evaporated to dryness to give a dark yellow gummy solid which crystallised from methanol as needles, m.p. 298-303°, $[\alpha]_D$ + 44.7° (c, 0.90). This compound gave a positive Beilstein test for halogen but was apparently impure 3β-hydroxylup-20(30)-en-28,13β-olide since it did not depress the m.p. of this compound and the infrared spectra were identical.

Dehydrogenation of Methyl Acetyl Betulinate with Mercuric Acetate. Methyl acetyl betulinate, m.p. 202-203°, (10 g.) in chloroform (300 c.c.) was treated with mercuric acetate (140 g.) in acetic acid (2 l.). The mixture was maintained either at room temperature for 14 days or at 100° for 6 hr. The product, isolated in the manner described for lupeol derivatives, was dissolved in benzene-light petroleum (1:4, 500 c.c.) and chromatographed on alumina (200 g. The fractions (3.1 g.), eluted with the same solvent, crystallised from chloroform-methanol to give *methyl-3β-acetoxylupa-12,20(30)-dien-28-oate* as prisms, m.p. 217-220°, $[\alpha]_D$ + 60° (*c*, 3.75), $\lambda_{max.}$ 2060 Å. (ε=7,100). The infrared spectrum showed bands at 1739, 1253 cm.$^{-1}$ (ester), 1631, 885 cm.$^{-1}$ (vinylidene). (Found: C, 77.5; H, 10.2. $C_{33}H_{50}O_4$ requires C, 77.6; H, 9.9%).

Methyl-3β-hydroxylupa-12,20(30)-dien-28-oate. - A solution of the above methyl acetyl ester (200 mg.) in benzene (5 c.c.) was treated with methanolic potassium hydroxide (3%, 50 c.c.) and the mixture was refluxed for 3 hr. The product (173 mg.) isolated in the usual way, was dissolved in ether (20 c.c.) and filtered through alumina. Elution with the same solvent gave material which crystallised from slightly aqueous methanol to give *methyl-3β-hydroxylupa-12,20(30)dien-28-oate* as needles, m.p. 182-184°, $[\alpha]_D$ + 44.7° (*c*,1.10), $\lambda_{max.}$ 2070 Å. (ϵ = 6,320). This compound gave a yellow colour with tetranitromethane and showed bands in the infrared spectrum at 3571 cm.$^{-1}$ (OH), 1718 cm.$^{-1}$ (CO_2Me), 1637, 893 cm.$^{-1}$ (vinylidene). (Found: C,79.4; H,10.5. $C_{31}H_{48}O_3$ requires C,79.4; H,10.3%).

Hydrogenation of Methyl-3β-acetoxylupa-12,20(30)-dien-28-oate. - A solution of methyl-3β-acetoxylupa-12,20(30)-dien-28-oate (230 mg.) in ethyl acetate-glacial acetic acid (1:5, 150 c.c.) was shaken with hydrogen in the presence of platinum (from platinum oxide, 50 mg.) for 20 hr. Isolation of the product in the usual way and crystallisation from chloroform-methanol gave *methyl-3β-acetoxylup-12-en-28-oate* as blades, m.p. 215.5-217°, $[\alpha]_D$ + 19.3° (*c*,1.14), $\lambda_{max.}$ 2050 A. (ϵ= 5,300). This compound gave a yellow colour with tetranitromethane. The infrared spectrum showed bands at 1739, 1250 cm.$^{-1}$ (ester). (Found: C,77.6; H,10.2. $C_{33}H_{52}O_4$ requires C,77.3; H,10.2%).

Reductive Hydrolysis of Methyl-3β-acetoxylupa-12,20(30)-dien-28-oate. A solution of methyl-3β-acetoxylupa-12,20(30)-

dien-28-oate (300 mg.) in ether (50 c.c.) was boiled under reflux with lithium aluminium hydride (200 mg.) for 1.5 hr. The product, extracted by means of ether (without using mineral acid), was crystallised from methanol to give 3β,28-*dihydroxylupa*-12,20(30)-*diene* as rods. The melting point of this compound was rather indefinite, apparently due to the presence of solvent, but the analysis sample which was prepared by several crystallisations from methanol followed by drying under vacuum at 100° for 60 hr. had an initial m.p. 196-198°. At this point the material resolidified and showed a second m.p. 211-214°, $[\alpha]_D$ + 6.6°, +8° (*c*, 1.54, 1.48), $\lambda_{max.}$ 2070 Å. ($\mathcal{E}$ = 6,500). This compound gave a yellow colour with tetranitromethane and its infrared spectrum showed bands at 3257 cm.$^{-1}$ (OH), 1626, 889 cm.$^{-1}$ (vinylidene). (Found: C, 81.5; H, 11.1. $C_{30}H_{48}O_2$ requires C, 81.75; H, 11.0%).

The above diol was completely identical with that prepared by hydrolysis of 3β,28-diacetoxylupa-12,20(30)-diene which is derived from betulin diacetate by mercuric acetate oxidation.

Acetylation of the diol, m.p. 196-198° and 211-214°, (100 mg.) with pyridine-acetic anhydride (1:1, 5 c.c.) at 100° for 1 hr. and extraction in the usual way followed by crystallisation of the product from ethanol afforded 3β,28-diacetoxylupa-12,20(30)-diene as plates, m.p. and mixed m.p. 177-179°, $[\alpha]_D$ +46° (c,0.54), $\lambda_{max.}$ 2060 Å. (ϵ = 7,500). The infrared spectrum showed bands at:- 1736, 1238 cm.$^{-1}$ (OAc), 1629, 899 cm.$^{-1}$ (vinylidene), and was identical with that of the diacetate prepared from betulin diacetate by mercuric acetate dehydrogenation. (Found: C,77.8; H,10.2. $C_{34}H_{52}O_4$ requires C,77.8; H,10.0%).

Hydrolysis of Methyl-3β-acetoxylupa-12,20(30)-dien-28-oate with Sodium Ethoxide. - Methyl-3β-acetoxylupa-12,20(30)-dien-28-oate, m.p. 217-220°, (900 mg.) was heated in a sealed tube with a solution of sodium ethoxide (from sodium, 1 g., in absolute ethanol, 30 c.c.) at 200° for 19 hr. The cooled solution was poured into water and extracted with ether to remove any neutral impurity. Acidification of the aqueous phase and isolation of the product with ether gave an off-white solid, crystallisation of which from methanol yielded 3β-hydroxylupa-12,20(30)-dien-28-oic acid as needles (290 mg.), m.p. 289-291° (vac.), $[\alpha]_D$ + 61.2° (c,0.54), $\lambda_{max.}$ 2060 Å. (ϵ = 6,600). The infrared spectrum showed bands at 3436 cm.$^{-1}$ (OH), 1701, 1678 cm.$^{-1}$ (CO_2H), 1639, 891 cm.$^{-1}$ (vinylidene). This compound gave a yellow colour with tetranitromethane. (Found: C,78.0; H,10.2. $C_{30}H_{46}O_3 \cdot \frac{1}{2}CH_3OH$ requires C,77.8; H,10.3%).

3β-*Acetoxylupa*-12,20(30)-*dien*-28-*oic Acid*. - A solution of the above hydroxy acid (250 mg.) in pyridine (3 c.c.) was treated with acetic anhydride (3 c.c.) and the mixture was maintained at 100° for 1.5 hr. The product, isolated in the usual manner was crystallised first from aqueous acetic acid and then from aqueous methanol to give 3β-*acetoxylupa*-12,20(30)-*dien*-28-*oic acid* as plates, m.p. 280-282° (vac.), $[\alpha]_D + 68°$ (*c*,0.59), $\lambda_{max.}$ 2060 Å. ($\mathcal{E}$ = 7,440). The infrared spectrum showed bands at 1739, 1247 cm.$^{-1}$ (OAc), 1706 cm.$^{-1}$ (CO_2H), 1639, 891 cm.$^{-1}$ (vinylidene). This compound gave a yellow colour with tetranitromethane. (Found: C,76.4; H,9.9. $C_{32}H_{48}O_4 . \frac{1}{2}CH_3OH$ requires C,76.1; H,9.8%).

Treatment of the above acetyl acid (45 mg.) with ethereal diazomethane at 0° for 3 days followed by filtration of the product in benzene solution through alumina (2 g.) gave methyl-3β-acetoxylupa-12,20(30)-dien-28-oate as prisms, m.p. and mixed m.p. 217-220°, $[\alpha]_D + 63°$ (*c*,1.22). A comparison of its infrared spectrum with that of the original ester confirmed identity.

Hydrolysis of Methyl-3β-acetoxylup-12-en-28-oate with Sodium Ethoxide. - Methyl-3β-acetoxylup-12-en-28-oate, m.p. 215.5-217°, (170 mg.) was heated in a sealed tube with a solution of sodium ethoxide (from sodium, 200 mg., and dry ethanol, 7 c.c.) at 200° for 17 hr. The reaction mixture was poured into water and the acidic product, isolated in the usual way, was crystallised from methanol to give 3β-*hydroxylup*-12-*en*-28-*oic acid*.as needles, m.p. 287-289° (vac.), $[\alpha]_D$ + 10.7° (*c*,1.02), $\lambda_{max.}$ 2060 Å. (ε = 4,600) The infrared spectrum showed bands at 3425, 3268 cm.$^{-1}$ (OH), 1690 cm.$^{-1}$ (CO_2H). This compound gave a yellow colour with tetranitromethane. (Found: C,77.3; H,10.8. $C_{30}H_{48}O_3 . \frac{1}{2}CH_3OH$ requires C,77.5; H,10.7%).

3β-*Acetoxylup*-12-*en*-28-*oic Acid*. - The above hydroxy acid was acetylated in the usual manner and the product was crystallised from slightly aqueous methanol to give 3β-*acetoxylup*-12-*en*-28-*oic acid* as plates, m.p. 294-295° (vac.), $[\alpha]_D$ + 31.2° (*c*,0.67), $\lambda_{max.}$ 2060 Å., (ε = 4,900). This compound gave a pale yellow colour with tetranitromethane and showed bands in the infrared spectrum at 1739, 1245 cm.$^{-1}$ (OAc), 1690 cm.$^{-1}$ (CO_2H). (Found: C,77.2; H,10.3. $C_{32}H_{50}O_4$ requires C,77.1; H,10.1%).

Treatment of 3β-*Hydroxylup*-12-*en*-28-*oic Acid with Hydrogen Chloride*. - A solution of 3β-hydroxylup-12-en-28-oic acid, m.p. 287-289° (vac.), (20 mg.) in dry chloroform (10 c.c.) was treated with a stream of dry hydrogen chloride for

45 minutes. Working up in the usual way gave a neutral product which was dissolved in benzene-ether (1:1, 10 c.c.) and filtered through a column of alumina (2 g.). Elution with the same solvent gave a fraction (17 mg.) which crystallised from methanol to give 3β-hydroxylupan-28:13β-olide as needles, m.p. and mixed m.p. 313-315° (vac.), $[\alpha]_D$ + 41.5° (c, 0.36). The infrared spectrum was identical with that of the authentic saturated hydroxy lactone (p.117).

Treatment of Acetyl Oleanolic Acid with Mercuric Acetate. A solution of acetyl oleanolic acid (2 g.) in chloroform (30 c.c. was treated with mercuric acetate (10 g.) in glacial acetic acid (200 c.c.) and the mixture was maintained at 100° for 5 hr. The product, isolated in the usual way, was identified as unchanged starting material. The infrared spectrum showed no trace of a band due to a lactone.

Similarly, treatment of acetyl 18α-oleanolic acid, under the same conditions, gave unchanged starting material.

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