THESIS

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by

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THE CHEMISTRY OF THE SOYASAPOGENOLS

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

The author wishes to record his gratitude to Professor F. S. Spring, F.R.S., for his keen interest and inspiring leadership throughout the past years. He is also indebted to Mr. J. M. Smith, D.R.C.S.T., for invaluable help and encouragement during the major part of the work.

C O N T E N T S

HISTORICAL

I. PENTACYCLIC TRITERPENOIDS: : CLASSIFICATION AND INTER-RELATION

Triterpenes are a class of naturally-occurring compounds, the molecules of which contain 30 carbon atoms arranged in a regular or irregular combination of six isoprene units. Recently, the more comprehensive term triterpenoid has been adopted to include related compounds with 31 carbon atoms in the molecule. Triterpenoids may be classified into three main groups.

(I) The Squalenoid Group, containing the aliphatic hydrocarbon squalene, the tricyclic alcohol ambrein, and the symmetrical diol onocerin.

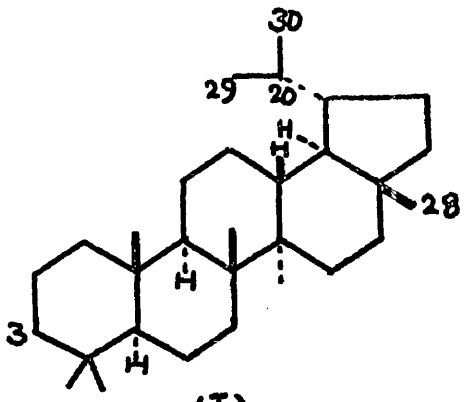
(II) The Tetracyclic Group, which are structurally related to the steroids, and include such compounds as agnosterol, butyrospermol, eburicoic acid, euphol, lanosterol and tirucallol.

(III) The Pentacyclic Group, which are the most abundant, and include such compounds as α- and β-amyrin, lupeol, taraxasterol and taraxerol. The chemistry of members of this group is adequately summarised in a number of excellent reviews which cover work done up to 1953.[1-8] Since this thesis is concerned with a group of pentacyclic triterpenoids which occur in nature as saponins, a brief review is given below of the chemistry of pentacyclic triterpenoid sapogenols.

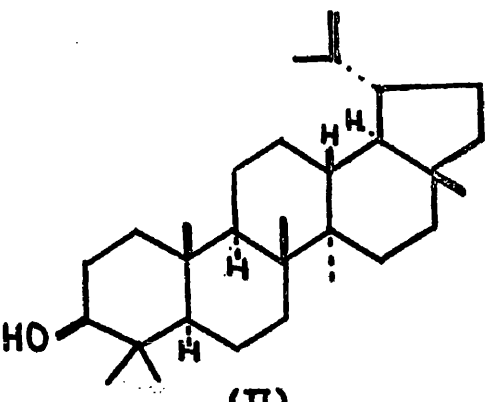
The Lupane Series.

All five members of this group have structures based on that of Lupane (I), and may be considered as hydroxyl and carboxyl

derivatives of lupeol (II), the first member of the series.

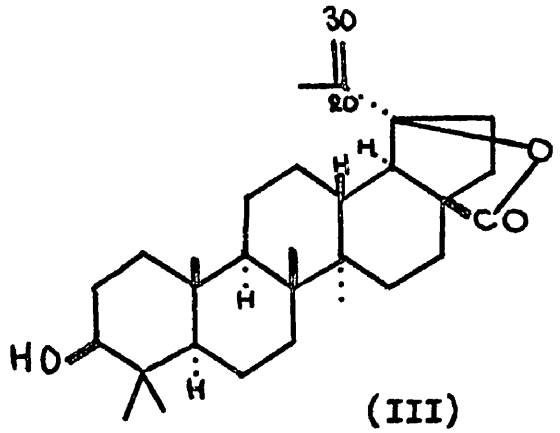


(I)



(II)

Lupeol is widely distributed in nature, but occurs as the saponin only in the giant cactus Lopocereus schottii.[6] Elucidation of the structure is due mainly to Jones and his associates, who have inter-related lupeol with olean-13(18)-ene[7] and with germanicol.[8] Betulin is the 28-hydroxyl derivative of lupeol and has been converted into α-lupene[9] and moradiol.[10] Djerassi has recently isolated betulin from the saponin of Lemaireocereus griseus,[11] but it normally occurs as the free alcohol in birch bark.[6] Three other members of this group are present as saponins in Lemaireocereus stellatus. Betulinic acid, the 28-carboxy-derivative of lupeol, has been characterised by Robertson, who carried out a partial synthesis from betulin.[12] Thurberogenin (III), a triterpenoid lactone, was identified by Djerassi and his collaborators, who reduced the 30-nor-20-ketone with calcium in liquid ammonia to give a known betulinic acid derivative.[13]

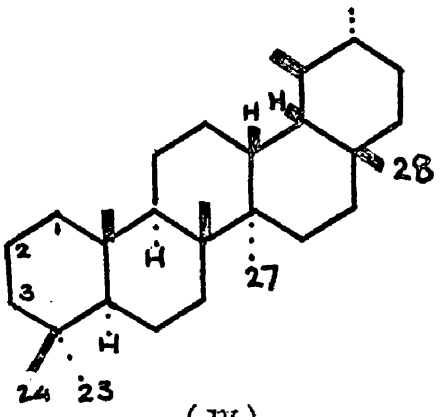


(III)

Stellatogenin is the last member of the series, and has been shown to be 20-hydroxy-dihydro-thurberogenin, by mild dehydration of the 3-monoacetate to give thurberogenin acetate.[14]

The Ursane Series.

The parent hydrocarbon of this group is ursane (IV). Although α-Amyrin (V) has not been isolated from a naturally-occurring saponin, it is included in this section since the two known ursane sapogenins are hydroxy- and carboxy-derivatives of this compound. Its structure has been largely established by the elucidation of pyrolytic fission products,[15] and also by its partial synthesis from glycyrrhetic acid.[16]



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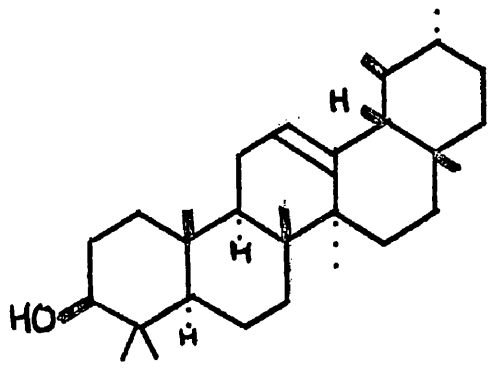
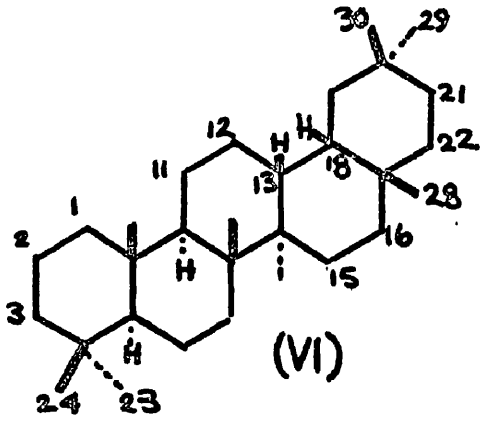


(V)

Asiatic acid, from the saponin of Hydrocotyle asiatica, is 2:24-dihydroxy-28-carboxy-α-amyrin. Structural elucidation is due to Mme. Polonsky, who has degraded the acid into the unsaturated hydrocarbon, 23-norurs-12-ene.[17] Quinovic acid or 27:28-dicarboxy-α-amyrin, is obtained from cinchona bark,[18] but it has not been directly related to ursane. The two carboxyl groupings have, however, been located at positions 27 and 28 by a series of extensive degradations.[19]

The Oleanane Series

This group is by far the largest, with some 26 members, all structurally related to the oleanane molecule (VI)



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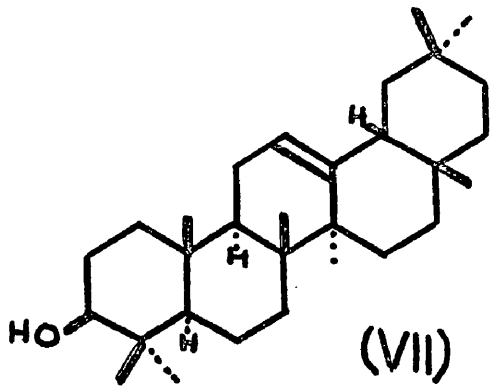

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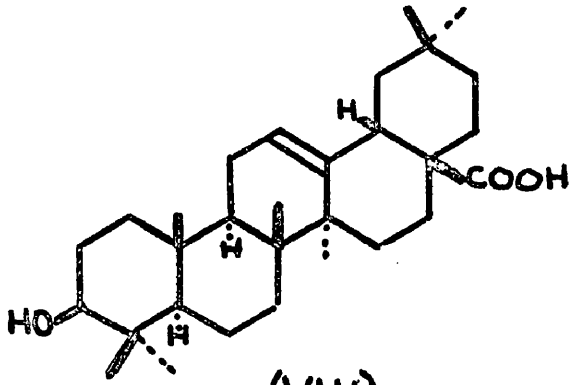

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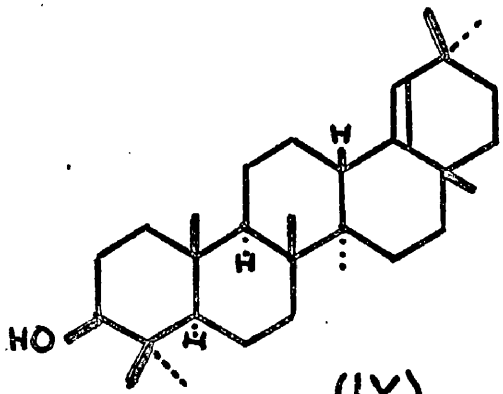


(IX)

For simplicity, members of this series may be sub-divided into three sub-groups, based on the structures of β-amyrin (VII), oleanolic acid (VIII) and germanicol (IX).

Table I : The β-Amyrin Sapogenins.

Name	Plant Source	Additional Functional Groups		Inter-relation with other triterpenoids
		Hydroxyl	Others	
Erythrodiol	Lemaireocereus longispinus etc.	28		Oleanolic acid[20-22]
Gummosogenin	Machaereocereus gummosus	16β	28-CHO	Maniladiol and Longispinogenin[25]
Maniladiol	Escontria chiotilla	16β		β-Amyrin and echinocystic acid[24]
Genin A	Primula offinalis	16α:28		Echinocystic acid[25]
Longispinogenin	L.longispinus etc.	16β:28		Echinocystic acid, gummosogenin.[25]
Myrtillogenic acid	Myrtillocactus cochal	16β:28	30-COOH	Longispinogenin[26]
Aescigenin	Aesculus hippocastanum	22β:24:28	16:21 ⟩C=O	Olean-12-ene[27]
Barringtogenol	Barringtonia racemosa	2α:23:28		Arjunolic acid[28]
Chichipegenin	L.chichipe	16β:21α:28		Longispinogenin[29]
Glycyrrhetic acid	Glycyrrhiza glabra	-	11⟩C=O,	β-Amyrin, queretaroic acid[30,31]

Table II : The Oleanolic Acid Sapogenins

Name	Plant Source	Additional Functional Groups		Inter-relation with other triterpenoids
		Hydroxyl	Others	
Oleanolic acid	Lemaireocereus longispinus etc			β-Amyrin[21,22]
Barringtogenic acid	Barringtonia racemosa	2α	23-COOH	Arjunolic acid, barringtogenol[20]
Cochalic acid	Myrtillocactus cochal	16β		Echinocystic acid, longispinogenin[32]
Echinocystic acid	Echinocystis fabacea	16α		Oleanolic acid[33]
Hederagenin	Hedera helix	23		Gypsogenin, β-amyrin, α-boswellic acid[34]
Machaerinic acid	Machaereocereus gummosus	21β		Oleanolic, rehmannic and machaeric acids[35]
Medicagenic acid	Medicago sativa	2β	23-COOH	Arjunolic acid[36]
Queretaroic acid	L.queretaroensis	30		Oleanolic acid[31]
Quillaic acid	Quillaja bark	16β	23-CHO	Echinocystic acid, hederagenin[37,38]
Bassic acid	Bassii Parkii	1:24		
Dumortierigenin	L.Dumortieri	15:21β	15→28 lactone	Erythrodiol[39]
Treleasegenic acid	L.Treleasei	21β:30		Machaeric, queretaroic acids[40]
Gypsogenin	Gypsophila paniculata		23-CHO	Oleanolic acid, hederagenin[41]
Machaeric acid	Machaereocereus gummosus		21>CO	Oleanolic, rehmannic acids[35]

Morolic acid, the $C_{(28)}$-acid derived from germanicol (IX), is the only member of the third Sub-Group. It is present as the saponin in the heart-wood of Mora excelsa, has been converted into germanicyl acetate,[42] and synthesised from siaresinolic acid.[43]

Triterpenoid Sapogenins of Unknown Structure

Many of these have been reported in the literature.[8] The more prominent are listed in the accompanying Table. Several of the molecular formulae quoted are possibly inaccurate, and future investigation into the chemistry of this group may well prove rewarding.

Table III : Unknown Triterpenoid Sapogenins

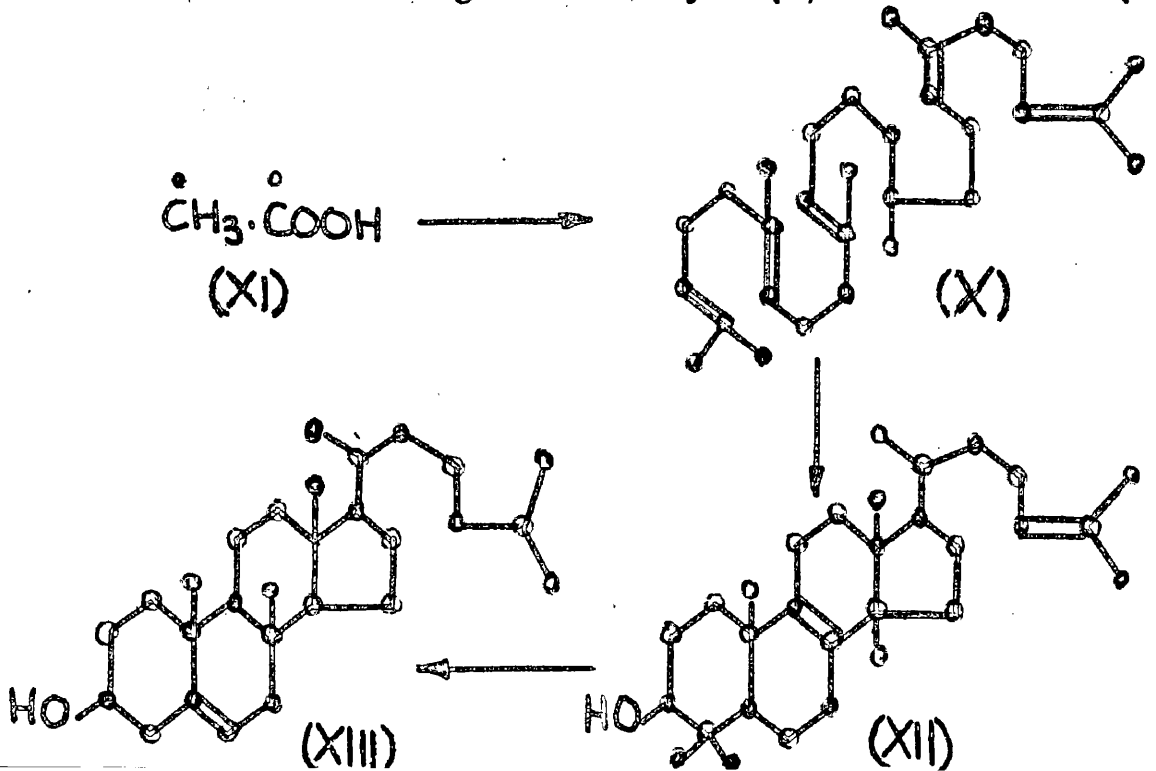
Name	Plant Source	Formula	Functional Groups
Aralidin	Aralia japonica		
Arasapogenin	A.bipinnatifada	$C_{29}H_{52}O_3$	
Adzukisapogenin	Phaseolus radiatus	$C_{30}H_{48}O_5$	2(OH), >CO
Camellia sapogenol	Camellia japonica	$C_{29}H_{44}O_5$	2(OH), >CO
Caulosapogenin	Caullophyllum thalictroides		2(OH)
Caulophyllosapogenin	C.thalictroides		6(OH)
Caryocarsapogenin	Caryocar glabrum	$C_{28}H_{44}O_4$	OH, COOH
Castanogenin	Castanospermum australe	$C_{30}H_{46}O_6$	2(OH),2(COOH), F.
Chenopodium sapogenin	Chenopodium ambrosioides	$C_{30}H_{48}O_4$	2(OH), COOH

Table III (continued)

Name	Plant Source	Formula	Functional Groups.
Cyclamiretin	Cyclamen europaeum	$C_{30}H_{46}O_5$	2(OH), >CO, >O
Equisetogenin	Equisetum arvense	$C_{27}H_{48}O_6$	
Githagenin	Agrostemma githago	$C_{30}H_{46}O_4$	OH, COOH, >CO
Gratiogenin	Gratiola officinalis	$C_{30}H_{48}O_4$	3(OH), >CO, ⊨.
Japoaescigenol	Aesculus turbinata	$C_{30}H_{52}O_6$	4(OH), >O.
Jegosapogenol	Styrax japonicum	$C_{30}H_{50}O_5$	5(OH)
Phillyrigenin	Pittosporum phillyraeoides	$C_{30}H_{48}O_4$	2(OH), lactone
Pittosapogenin	P. undulatum	$C_{30}H_{50}O_7$	2(OH)
Platycodigenin	Platycodon grandiflorum	$C_{30}H_{48}O_7$	4(OH), ⊨.
Polysciassapogenin	Polyscias nodosa	$C_{26}H_{44}O_4$	Lactone
Sanguisorbigenin	Sanguisorba officinalis	$C_{30}H_{46}O_3$	OH, COOH, ⊨.
Sapindus sapogenin	Sapindus makurosi		
Senegenin	Polygala senega	$C_{30}H_{46}O_8$	2(OH), 2(COOH), ⊨, lactone
Spinach sapogenin	Spinacia oleracea	$C_{32}H_{56}O_5$	2(OH), COOH
Sasanqua sapogenin	Camellia sasanqua	$C_{29}H_{44}O_6$	3(OH), >CO.
Theasapogenin	Thea sinensis	$C_{22}H_{32}O_6$	OH.
Vanguerigenin	Vanguera edulis	$C_{30}H_{46}O_3$	OH, COOH, ⊨.

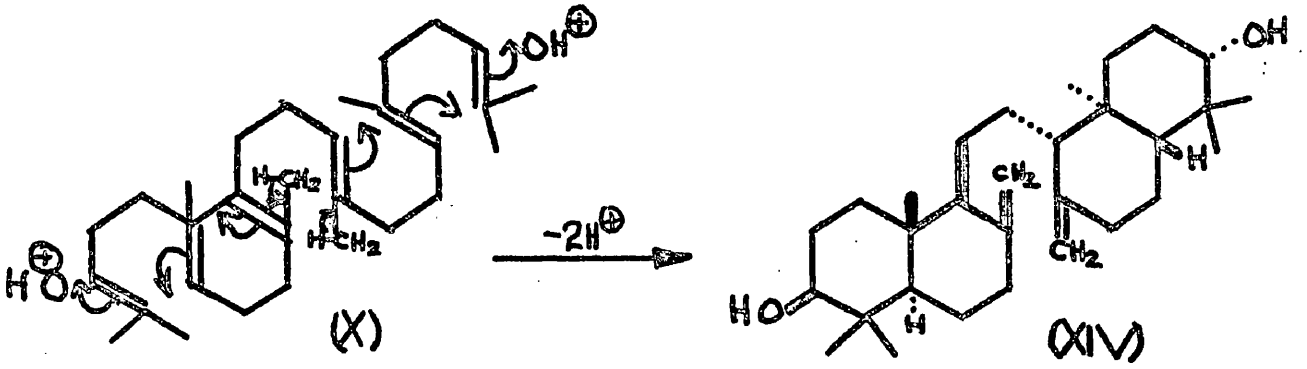
II. THE BIOGENESIS OF STEROIDS AND TRITERPENOIDS

In 1934, Robinson observed that the unsaturated hydrocarbon squalene contains the intact molecular skeleton of cholesterol with three additional carbon atoms.[44] On the basis of earlier work by H. J. Channon,[45] involving the feeding of squalene to rats, Robinson postulated that squalene might well be the biogenetic precursor of cholesterol. Recent work in Britain and America has confirmed this view, and shown that the biogenetic pathway involves triterpenoids as intermediates.[46,47] Using isotopic tracer techniques, and working with rat, mouse, and hog liver tissue, squalene (X) has been synthesised from both carbon atoms of labelled acetic acid (XI) and subsequently converted into lanosterol (XII) and thence cholesterol (XIII).[46] Degradative tracer studies have firmly established the cyclisation pattern to be as shown, and have located individual atoms in the molecule as arising from "methyl" (•) or "acetate" (○) carbons.[48]



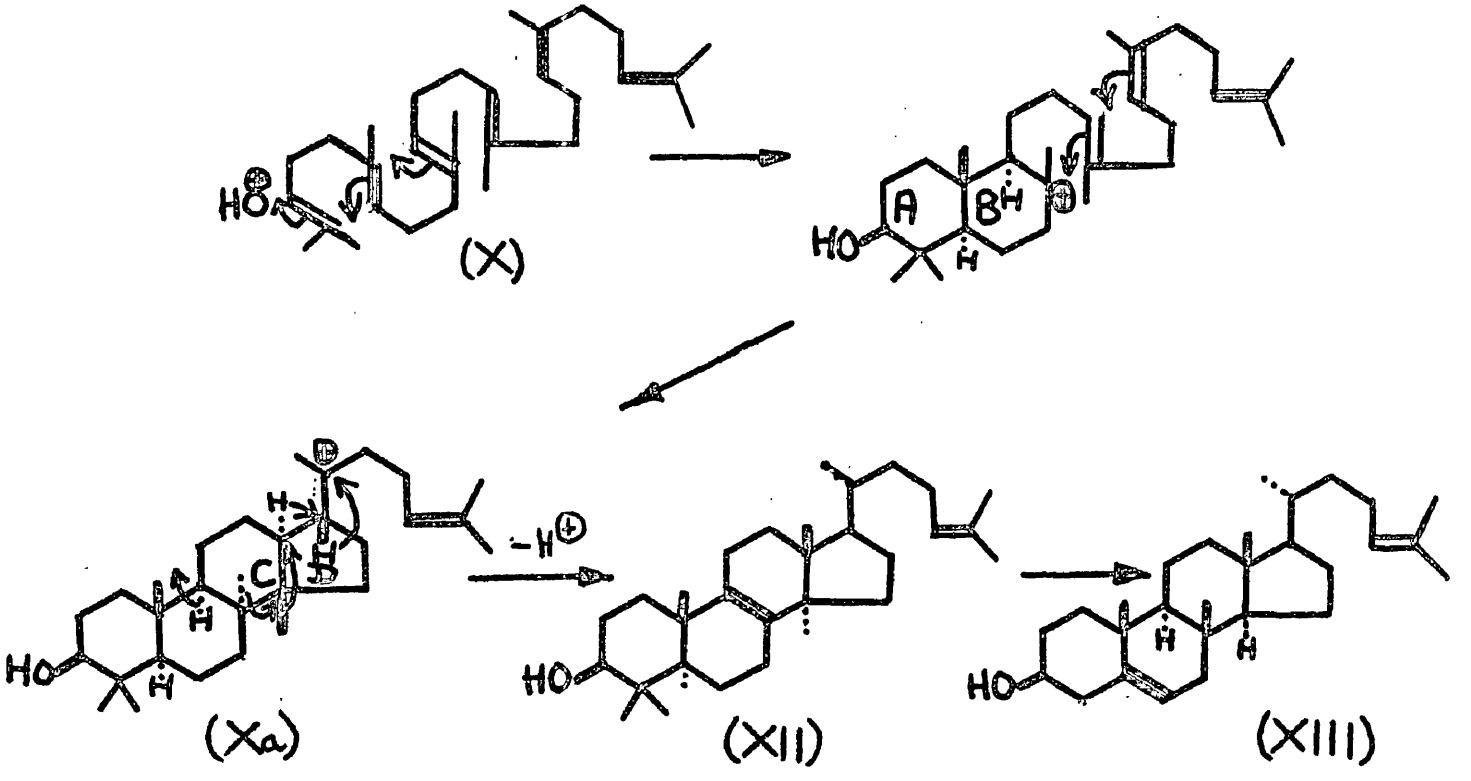
With the knowledge that the squalene molecule possesses a fully transoid structure,[49] it is now possible to set out a rational biogenetic pathway to triterpenoids and steroids.[50] This pathway assumes a fully concerted cyclisation for squalene, rather than a discrete stepwise mechanism involving individual rings, and leading to a non-natural cis-stereochemistry.[51,52] The process is known to be aerobic, requiring the presence of reduced pyridine nucleotide, and the divalent manganese or other metal ion.[47]

If cyclisation occurs at both ends of the squalene molecule, the squalenoid group of triterpenoids is produced. A typical example of the group is the symmetrical diol, α-onocerin (XIV).

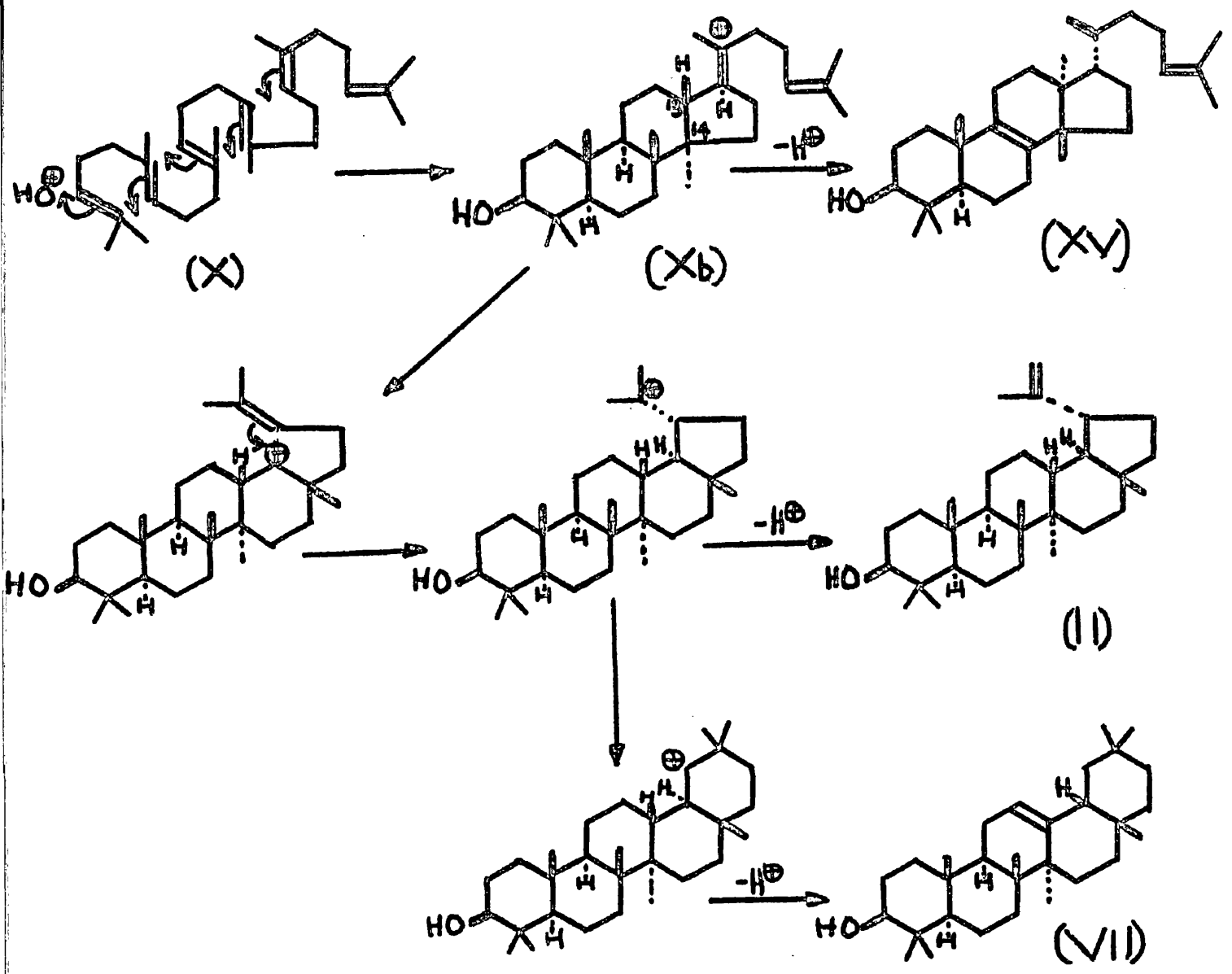


The biogenesis of steroids and of the pentacyclic triterpenoids is considered to involve an initial cyclisation at one of the terminal double bonds, motivated by electrophilic attack from an $OH^{\oplus}$ cation derived from molecular oxygen. Synchronous cyclisation may now proceed by one of two routes.

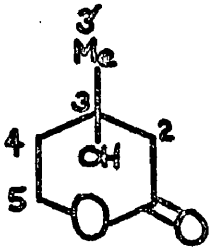
(1) Concerted formation of rings A and B, immediately followed by concerted closure of rings C and D, gives the carbonium ion (Xa), precursor of lanosterol (XII) and cholesterol (XIII).[52]



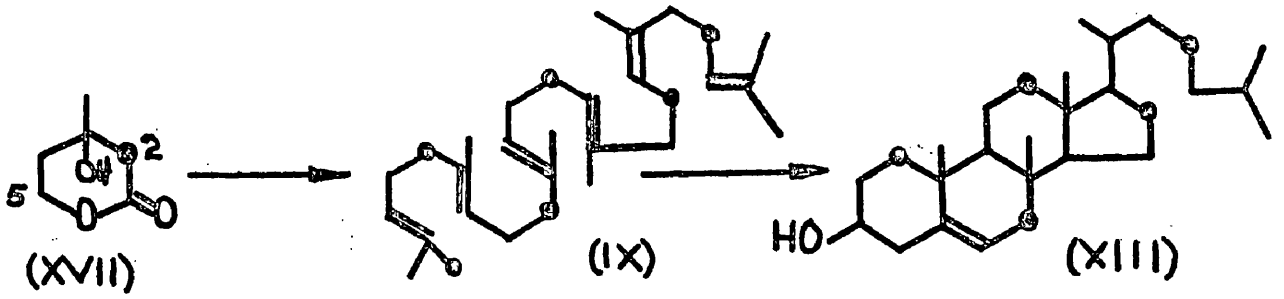
(2) Concerted closure of all four rings, giving the carbonium ion (Xb), which is the precursor of tetracyclic and pentacyclic triterpenoids such as tirucallol (XV), lupeol (II), and β-amyrin (VII).[50]



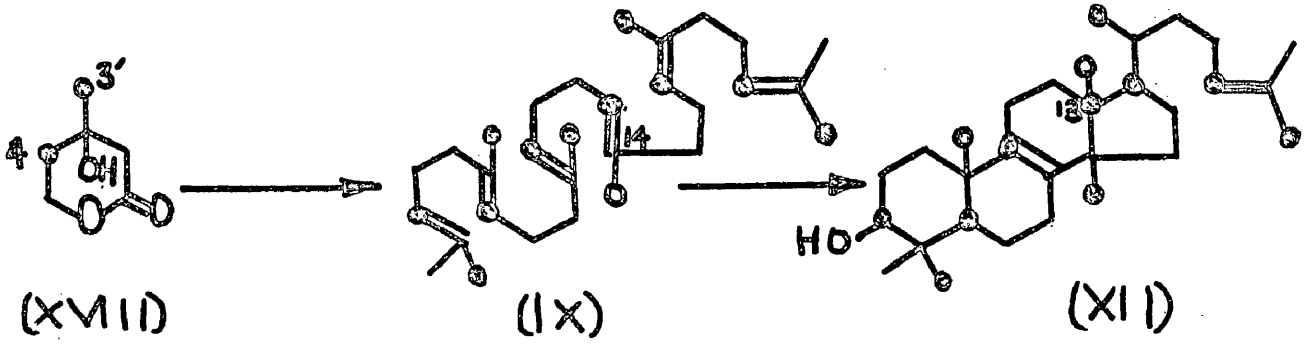
Current work in this field now centres around the use of mevalonolactone, (3-hydroxy-3-methyl-pentano-5-lactone, XVI), first isolated by Folkers and his collaborators in 1956 from distillers soluble residues.[53]



This compound is now considered to be the direct and irreversible precursor of squalene, since on incubation with rat liver homogenate, both squalene and cholesterol can be isolated in high yield.[54] Condensation of the lactone is known to take place by the linking of $C_{(5)}$ from one residue onto $C_{(2)}$ of the next,[55] as evidenced by the skeletal distribution in squalene (X) and cholesterol (XIII) synthesised in vivo from the [2 -^{14}C]--isotope (XVII).[56,57]



Also, by means of the [3':4-^{13}C]-isotope (XVIII), Cornforth and his associates have confirmed the postulated 14→13 methyl group migration in the biosynthesis of lanosterol (XII) from squalene.[58]



Mention must also be made of the elegant work of Arigoni in Zurich, who has now extended biogenetic study to the pentacyclic triterpenoids.[89] Degradative experiments with soyasapogenol A, biosynthesised in soyabeans from labelled mevalanolactone, enable the [2 - ^{14}C]-atoms to be located in the molecule, in full agreement with the general mechanism discussed above.

This thesis is concerned with the elucidation of the structures of four triterpenoid sapogenols isolated from soya beans. Since soyasapogenols A, B, C and D are closely related to β-amyrin, it is pertinent at this juncture to outline the chemistry of this important triterpenoid alcohol

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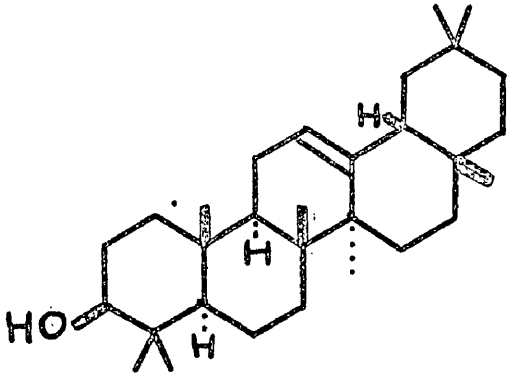
III. ASPECTS OF THE CHEMISTRY OF β-AMYRIN.

The structure (VII) for β-amyrin, first suggested in 1937 by Haworth,[60] has since been confirmed by the work of Ruzicka, Spring, and their associates.[61] Elaboration of the stereochemistry is due to the elegant researches of Barton,[62] supported by optical rotation evidence by Klyne,[63] and crystallographic studies by Carlisle.[64]

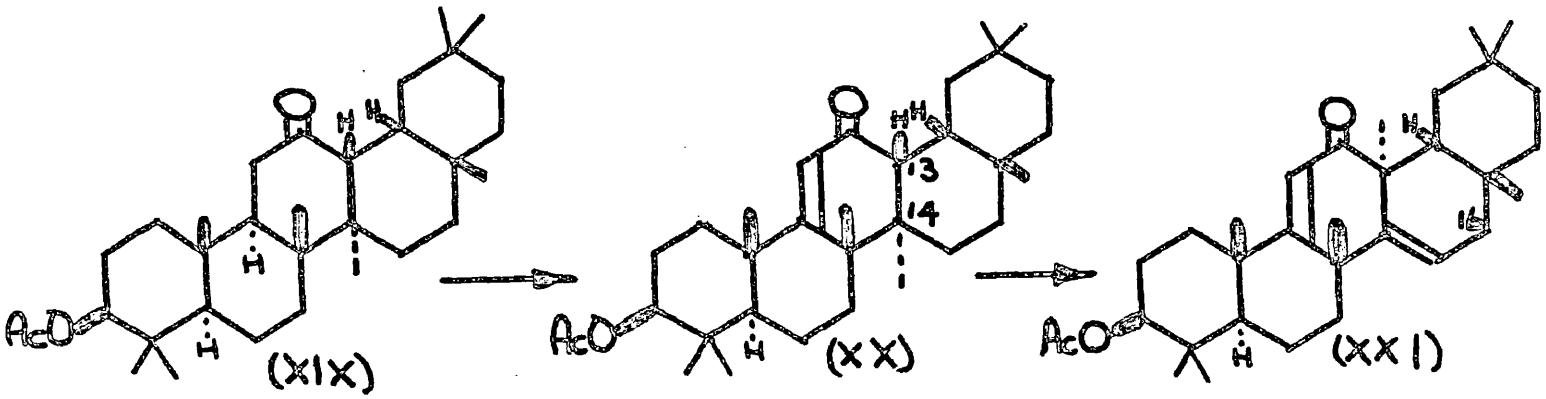


(VII)

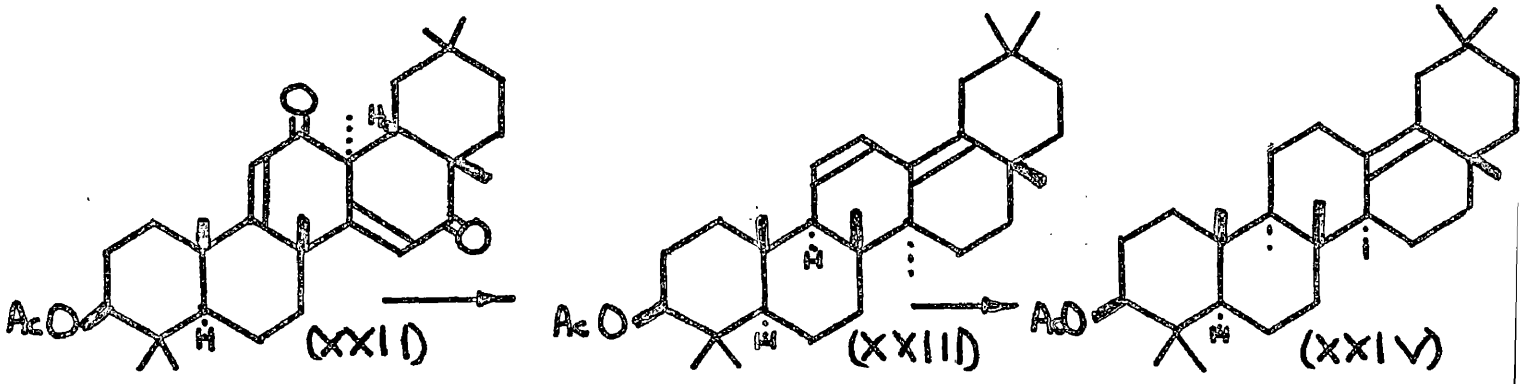
The Oxidation of β-Amyrin Acetate.

The 12:13 double bond in β-amyrin is unaffected by catalytic hydrogenation, but readily attacked by oxidising

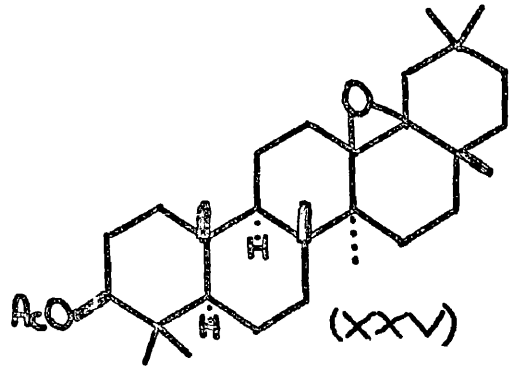
agents. For example, treatment of β-amyrin acetate with hydrogen peroxide in acetic or formic acids yields the saturated 12-ketone, β-amyranonyl acetate, or 3β-acetoxy-12-oxo-oleanane (XIX).[65]



Treatment of the 12-ketone with hydrobromic and acetic acids gives an unstable α-bromoketone, which readily dehydrobrominates to the αβ-unsaturated ketone, iso-β-amyrenonyl acetate (XX).[66] Oxidation of (XX) with selenium dioxide in acetic acid effects a retro-pinacolic α-methyl migration from $C_{(14)}$ to $C_{(13)}$, with the formation of the taraxerane derivative, iso-β-amyradienonyl acetate (XXI). More vigorous selenium dioxide oxidation attacks the $C_{(16)}$-methylene group and gives the dioxo-dienyl derivative, iso-β-amyradienedionyl acetate (XXII).



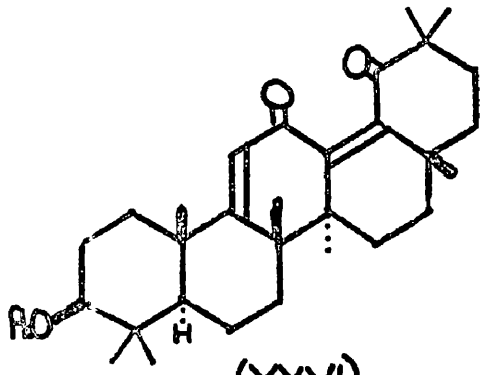
Clemmensen reduction of iso-β-amyradienonyl acetate eliminates the 12-oxygen function, and reverses the earlier 14 ⇒ 13 methyl group migration, affording the heteroannular 11:13(18)-diene, β-amyradienyl-II-acetate (XXIII).[67] The latter compound can also be obtained directly from β-amyrin acetate by selenium dioxide oxidation in acetic acid.[68] Catalytic hydrogenation of (XXIII) effects the saturation of the 11:12 double bond, and gives 3β-acetoxyolean-13(18)-ene, or δ-amyrin acetate (XXIV).[69] When heated with hydrogen peroxide in acetic acid, δ-amyrin forms the saturated 13(18)-epoxide (XXV). Brief mineral acid treatment of the epoxide regenerates the 11:13(18)-diene (XXIV)



(XXV)

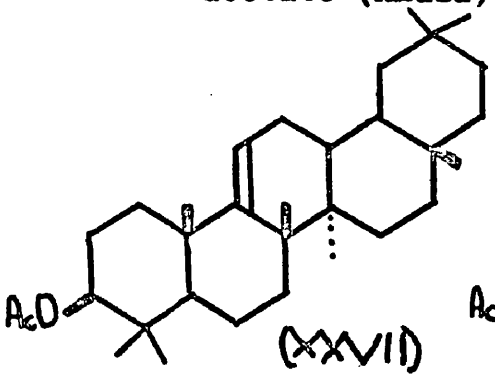
Reduction of Dioxo-β-amyradienyl Acetate.

On vigorous selenium dioxide oxidation in dioxan at 200°, β-amyrin acetate forms a dioxodienyl derivative, 3β-acetoxy--12:19-dioxo-oleana-9(11):13(18)-diene, (XXVI).[70]



(XXVI)

The dioxodiene has also been prepared by selenium dioxide oxidation of δ-amyrin acetate (XXIV), ε-amyrin acetate (XXVII), β-amyradienyl-I-acetate (XXVIII), β-amyradienyl-II--acetate (XXIII) and β-amyratrienyl acetate (XXIX).[68,69,71]



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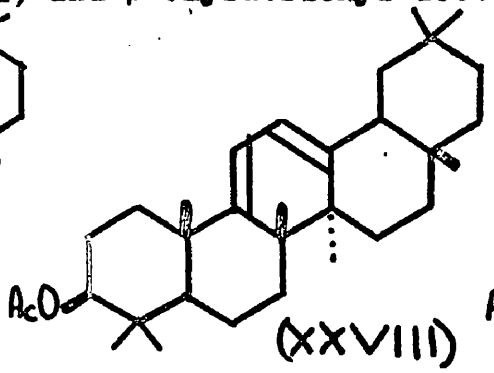

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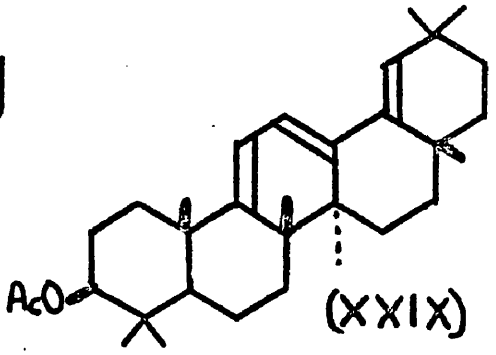
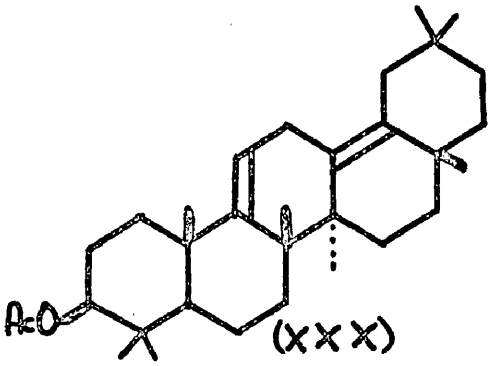
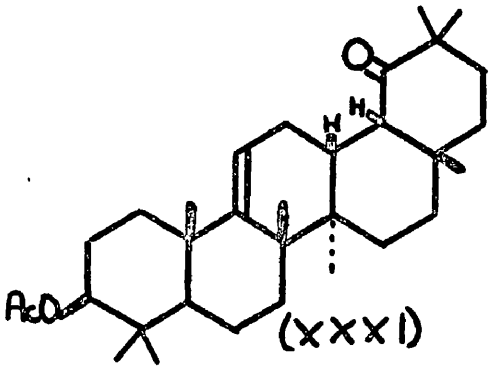


(XXIX)

Reduction of the dioxodiene (XXVI) affords a number of products, according to the nature of the reagent used.[72] Catalytic hydrogenation, for example, yields a mixture of the non-conjugated 9(11):13(18)-dienyl acetate (XXX), and the 19-oxo-9(11)-enyl acetate (XXXI).



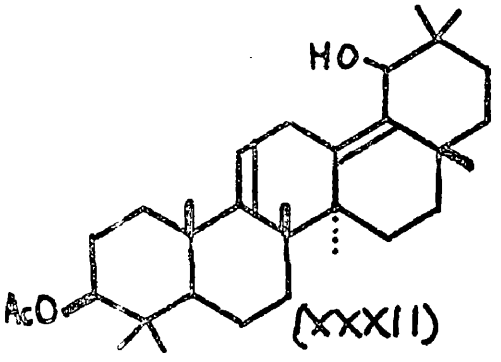
(XXX)



(XXXI)

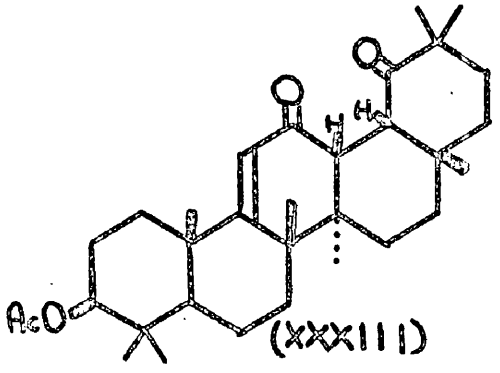
Mineral acid treatment of (XXX) isomerises the 9(11)-double bond to the stable 11:12-position, with the formation of β-amyradienyl-II-acetate in high yield.

Reduction with lithium aluminium hydride is somewhat anomalous. Of the two carbonyl groups in the dioxodiene (XXVI), the one at $C_{(12)}$ is reduced to methylene, whilst the other, at $C_{(19)}$, is converted to the expected alcohol. On subsequent acetylation, 3β-acetoxy-19-hydroxy-oleana-9(11):13(18)-diene (XXXII) is obtained.

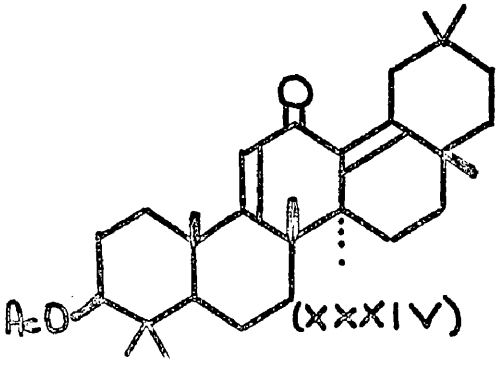


(XXXII)

Reduction of the dioxodiene with zinc and acetic acid gives a mixture of the 13β:18β-dihydro-derivative (XXXIII), and the 12-oxo-9(11):13(18)-dienyl acetate (XXXIV). The former can best be prepared from the dioxodiene by reduction with zinc and boiling ethanol.

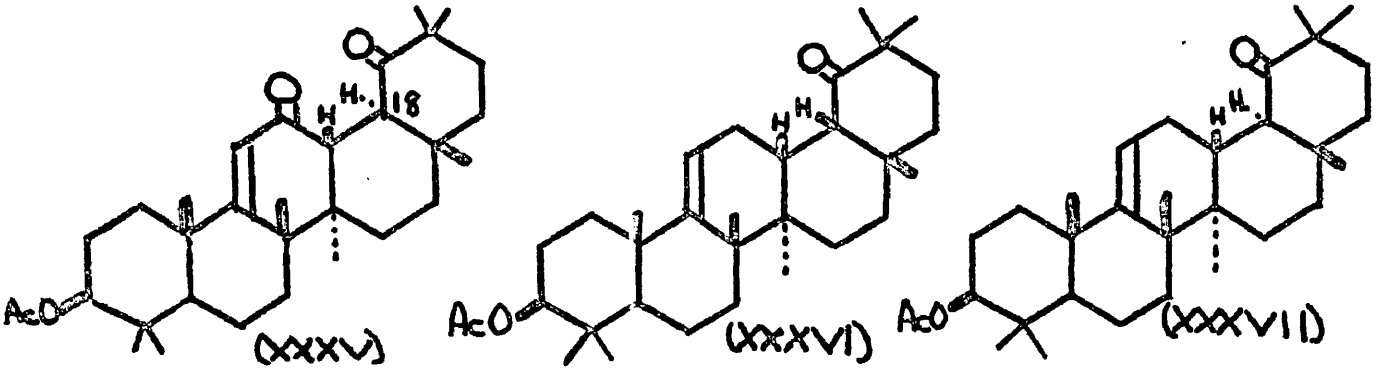


(XXXIII)

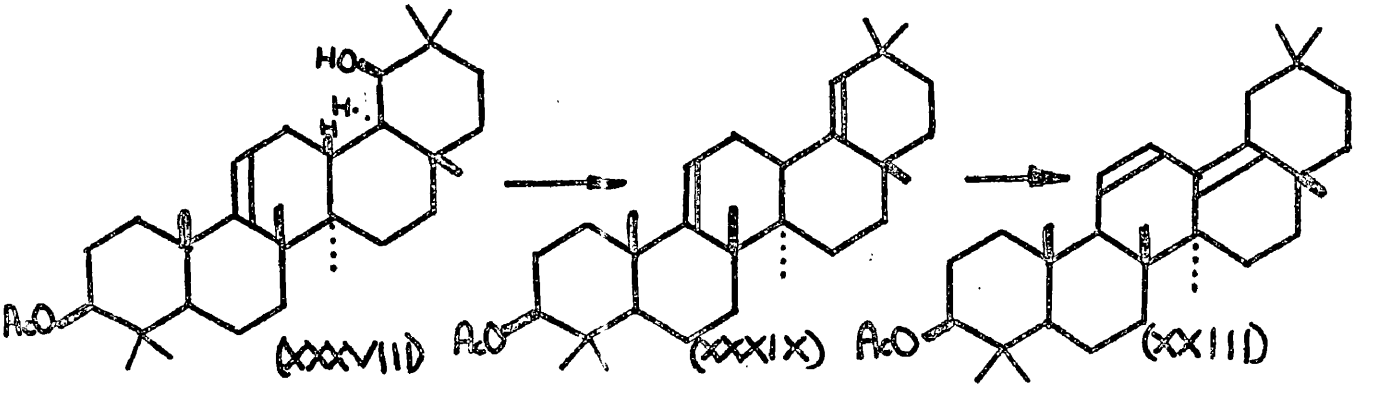


(XXXIV)

Treatment of the dihydro-derivative with alkali or acid effects epimerisation at $C_{(18)}$. After acetylation, the stable 13β:18α-dihydro-derivative (XXXV) is obtained.



Hydrogenolysis of the dihydro-dioxodienes (XXXIII) and (XXXV) attacks the activated 12-carbonyl in both compounds to give the unsaturated ketones (XXXVI) and (XXXVII) respectively. Acid or base-induced epimerisation of (XXXVI) affords (XXXVII) in high yield. Reduction of (XXXVII) with lithium aluminium hydride, followed by partial acetylation of the product, gives 3β-acetoxy--19β-hydroxy-18α-olean-9(11)-ene (XXXVIII). Dehydration of the latter compound with phosphorus oxychloride in pyridine proceeds smoothly to the non-conjugated 9(11):18-dienyl acetate (XXXIX), which isomerises with mineral acid to the heteroannular diene (XXIII).



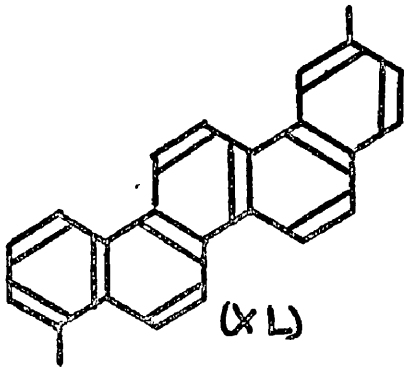
IV. HISTORICAL SECTION.

Over the past 50 years, many compounds have been isolated from soyabeans.[73] They include the enzyme urease, palmitic, stearic, oleic, linoleic, linolenic and palmitic acids,[74] flavonic glycosides such as genistin and daidzin,[75] the unsaturated hydrocarbon squalene,[76] the plant sterols stigmasterol and sitosterol,[77] and the sugars arabinose, galactose, sucrose, raffinose and stachyose.[78] In 1923, Murumatsu investigated the defatted bean meal, and isolated a substance, m.p. 224°, which he named hispidic acid.[79] Later workers showed that Murumatsu's 'acid' was in fact a crystalline saponin mixture with typical haemolytic action, giving on acid hydrolysis rhamnose, galactose, glucuronic acid, and a sapogenin mixture resolvable by crystallisation into several distinct fractions.[80] These fractions were difficult to purify,[81] and marked divergencies in melting point and specific rotation are recorded in the literature of this period. Nevertheless, in 1937 Ochiai and his Japanese co-workers succeeded in purifying the calcium salt of the saponin, hydrolysing with acid, and separating the mixture of sapogenins by chromatography.[82,83] The four products obtained in this way were termed the soyasapogenols A, B, C and D. Their characteristics are shown in Table IV.

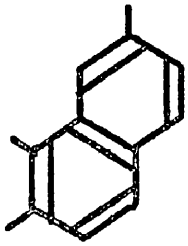
TABLE IV: The Soyasapogenols.

	Formula	m.p	$[\alpha]_D$
A	$C_{30}H_{46}(OH)_4$	321°	+102°
B	$C_{30}H_{47}(OH)_3$	260°	+ 92°
C	$C_{30}H_{46}(OH)_2$	239°	+ 71°
D	$C_{30}H_{48}(OH)_2$	299°	- 61°

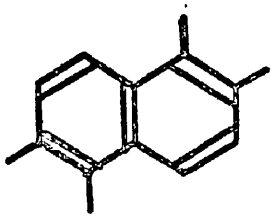
It was initially assumed by Ochiai that the four sapogenols were triterpenoid, on the basis of their molecular formulae, and the fact that each gave a purple colour with the Liebermann-Burchard reagent.[82] This view was confirmed by the selenium dehydrogenation of sapogenol B, which gave three typically triterpenoid products, viz: 1:8-dimethylpicene (XL), 1:2:7-trimethylnaphthalene (XLI), and 1:2:5:6-tetramethylnaphthalene (XLII).[84]



(XL)

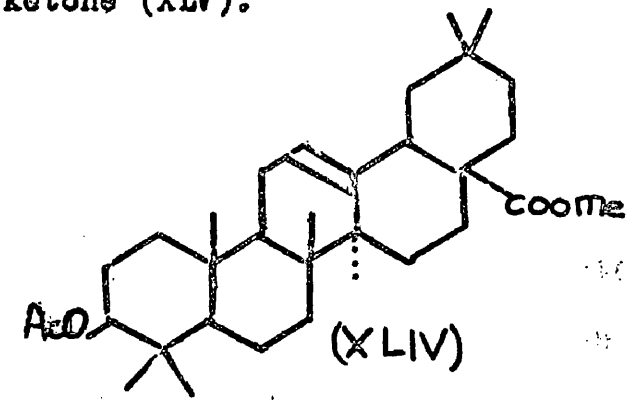


(XLI)



(XLII)

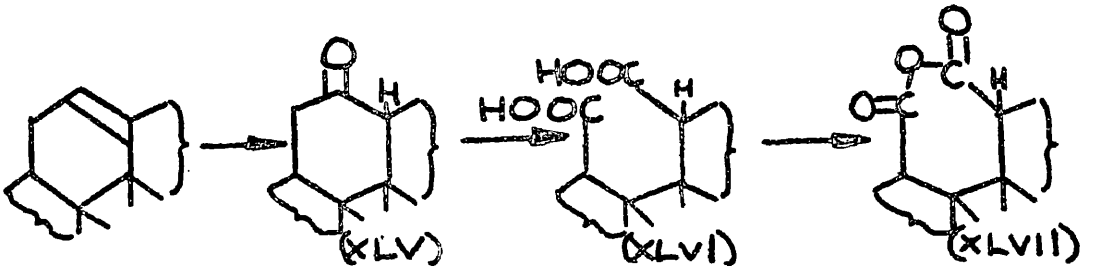
Tsuda and Kitagawa showed that catalytic reduction of sapogenol C gave a dihydro-derivative, m.p. 245°, diacetate m.p. 188°. They also examined the action of various oxidising agents on the soyasapogenols. For example, mild oxidation of sapogenol B with potassium permanganate converts it into a dihydroxy-carbonyl derivative, $C_{30}H_{48}O_5$, m.p. 218° (decomp.); oxime, m.p. 224°, diacetate m.p. 144°, presumably by preferential attack of a primary hydroxyl group. Treatment of sapogenol B triacetate with perbenzoic acid gives a saturated oxide, $C_{36}H_{56}O_7$, m.p. 213° (decomp.). Hydrogen peroxide oxidation, however, yields a different oxide, m.p. 258°, isomeric with the last compound, and giving on alkaline hydrolysis a triol, $C_{30}H_{50}O_4$, m.p. 254°. By analogy with the chemistry of methyl acetyl oleanolate (XLIV),[67] Tsuda and Kitagawa concluded that the oxide, m.p. 258°, is in fact a saturated ketone (XLV).



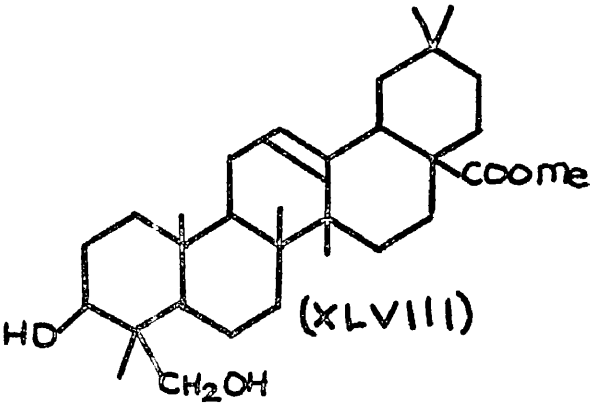
(XLIV)

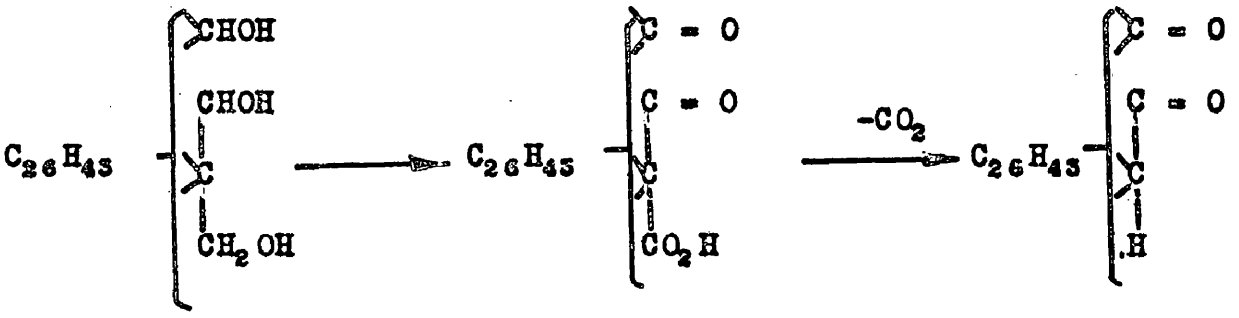
Confirmation of this view was soon obtained by treatment of the oxide, m.p. 258°, with nitric acid, which effects ring cleavage of the saturated ketone to a dicarboxylic acid,

$C_{36}H_{56}O_{10}$, m.p. 293° (decomp.) (XLVI). Heating the acid at 150° forms the anhydride, $C_{36}H_{54}O_9$, m.p. 283° (XLVII), the sequence of reactions being represented as shown.



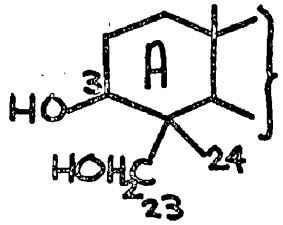
If, however, oxidation of sapogenol B is carried out with chromic acid, a product, $C_{29}H_{44}O_2$, m.p. 256° can be obtained. This product is formulated as a nordiketone, since it forms a dioxime, m.p. 266°, and a dihydrazone, m.p. 205° (decomp.) By analogy with the similar oxidation of methyl hederagenate (XLVIII),[88] it was suggested that this oxidation of sapogenol B can be represented by the partial formulae (XLIX) to (L).





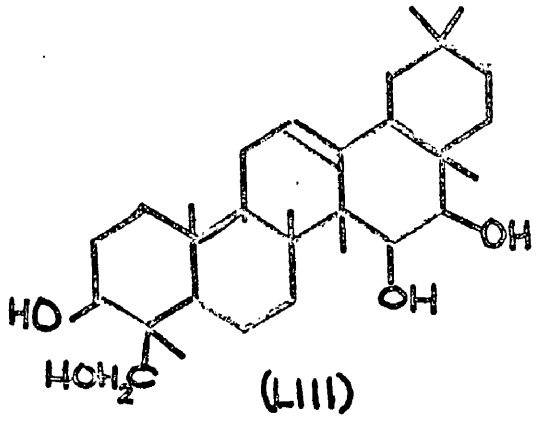
(XLIX) (L) (LI)

Confirmation of this view was obtained by the use of high temperature copper dehydrogenation, which is known to oxidise primary and secondary alcohols to the corresponding carboxylic acids and ketones.[89] Two products were isolated from the dehydrogenation of sapogenol B:formaldehyde, and a diketone, m.p. 256°, identical with the nordiketone previously obtained by chromic acid oxidation. Although data for sapogenol A is not recorded, dehydrogenation of sapogenol C, sapogenol D and dihydrosapogenol C gave in each case, formaldehyde and a corresponding nordiketone. It is thus probable that the soyasapogenols may contain a ring system similar to that in hederagenin (LII) with hydroxylation in ring A at positions 3 and 23 (or 24).[34]

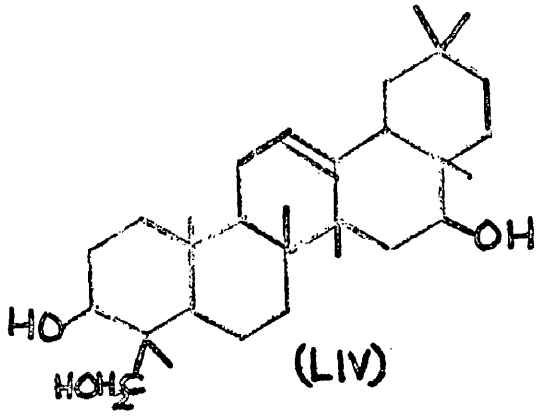


(LII)

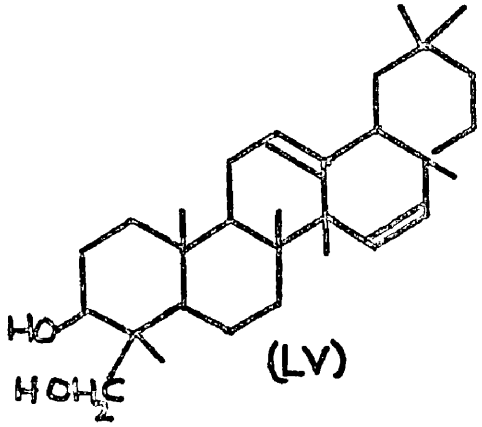
In 1950, Meyer, Jeger and Ruzicka reported on a detailed investigation of soyasapogenols A, B, C and D, which they formulated as (LIII), (LIV), (LV) and (LVI) respectively.[90,91,92]



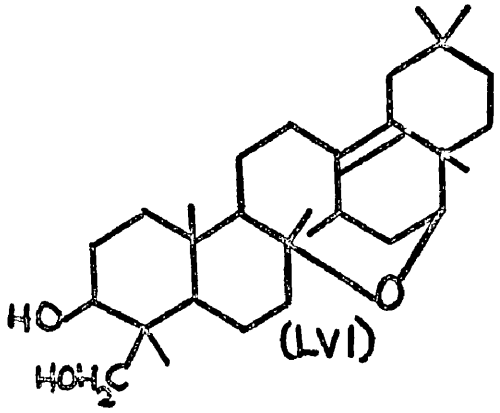
(LIII)



(LIV)



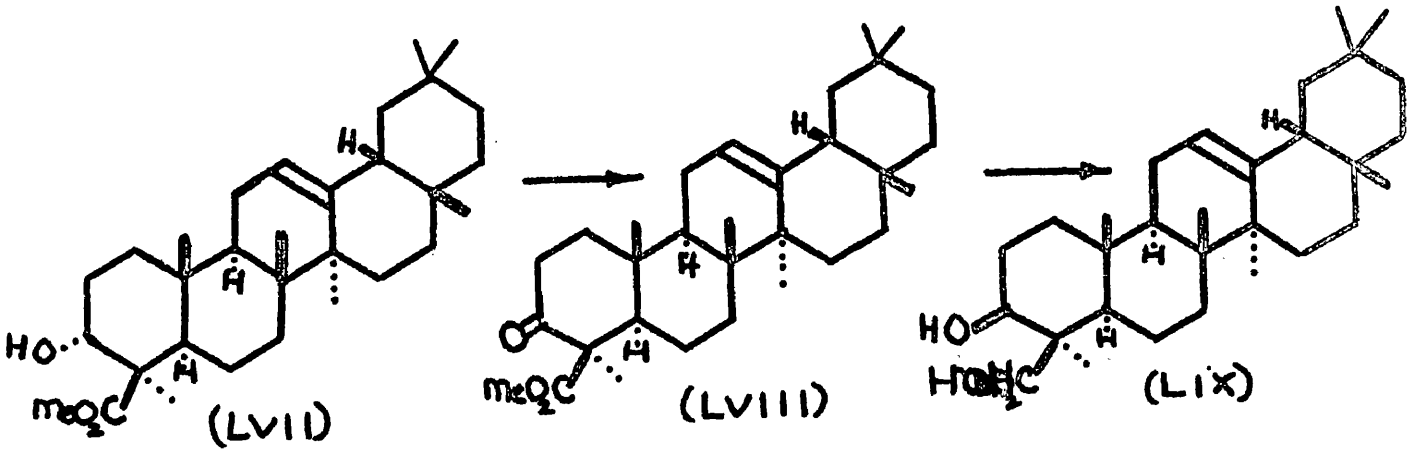
(LV)

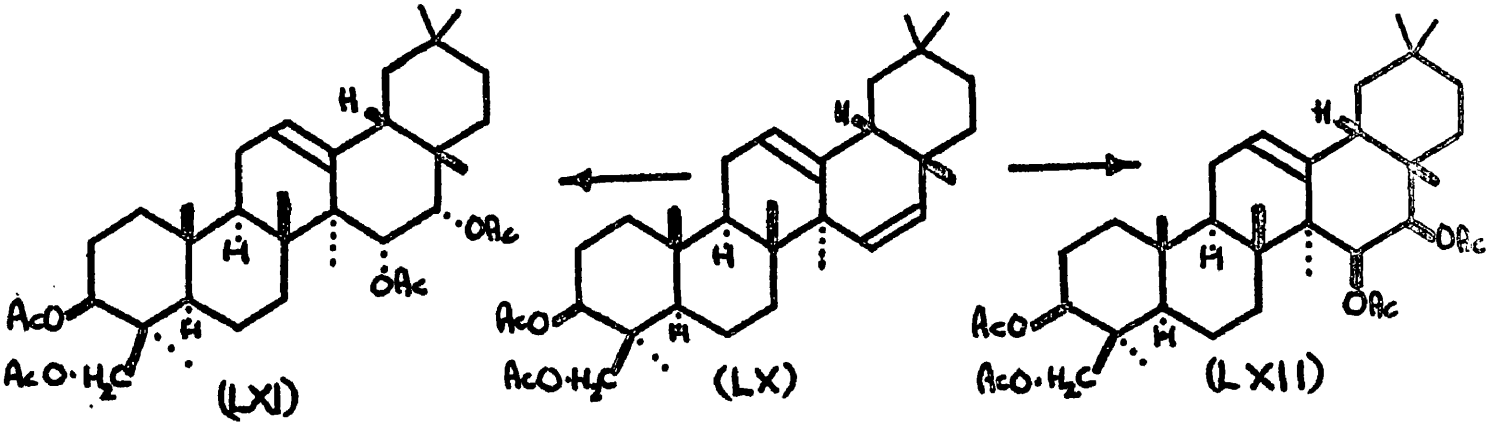


(LVI)

The sapogenols were identified as members of the oleanane group of triterpenoids. Thus, chromic acid oxidation of methyl-α-boswellate (LVII) gives a ketonic ester (LVIII) which yields epi-α-boswellene diol (LIX) on lithium aluminium hydride reduction. Direct comparison of the diol with dihydrosapogenol C, previously prepared by Ochiai,[62] showed that the two compounds were identical. On this evidence, sapogenol C can be formulated as 3β:24-dihydroxyoleana-12:x(y)-diene, where x and y designate the location of the reactive double bond. Osmium tetroxide oxidation of sapogenol C diacetate (LX) gives, after acetylation, a mixture which can be separated by

chromatography into sapogenol A tetra-acetate (LXI), and a stereoisomeric sapogenol A tetra-acetate (LXII).

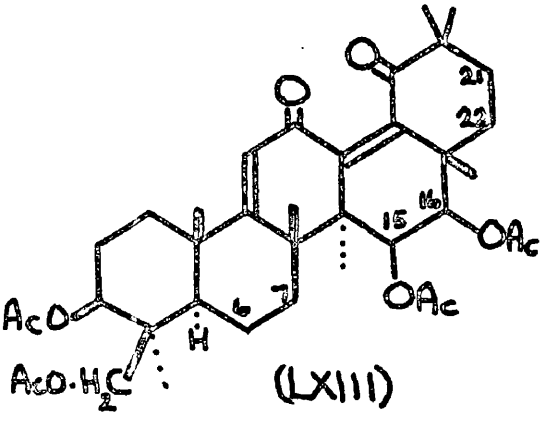




Hence, sapogenol A can be formulated as 3β:24:x:y-tetrahydroxyolean-12-ene.

The location of x and y is limited to three possible sites in the molecule. Since lead tetra-acetate oxidation of sapogenol A utilises only one mole of reagent, x and y cannot be in ring A. Moreover, vigorous selenium dioxide oxidation

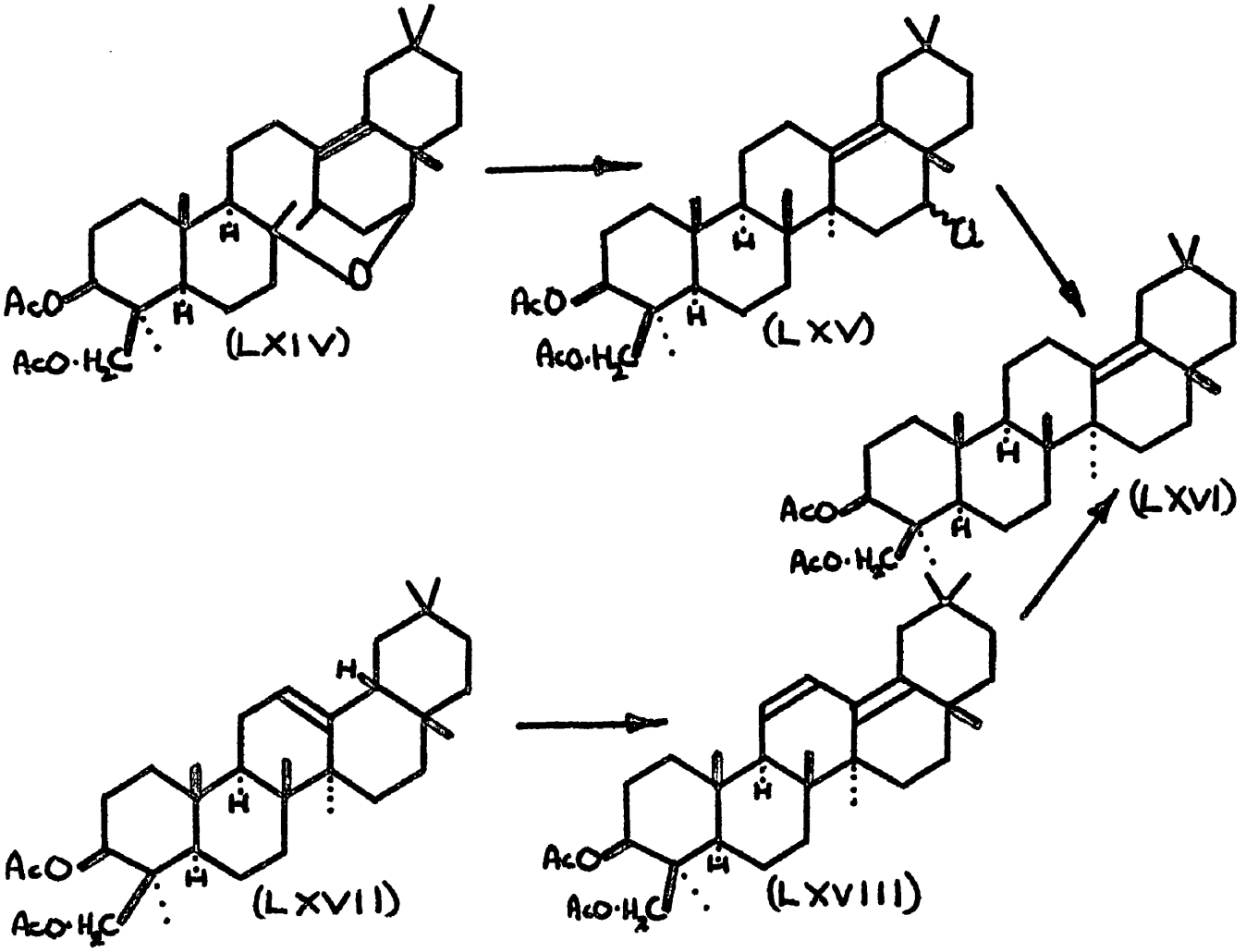
of sapogenol A tetrabenzoate, followed by alkaline hydrolysis and acetylation, gives a tetra-acetoxy-12:19-dioxo-9(11):13(18)--diene (LXIII), the formation of which excludes positions 9,11, 12,13,18, and 19 from further consideration. Accordingly, x and y can only be at positions 6:7, 21:22, or 15:16.



(LXIII)

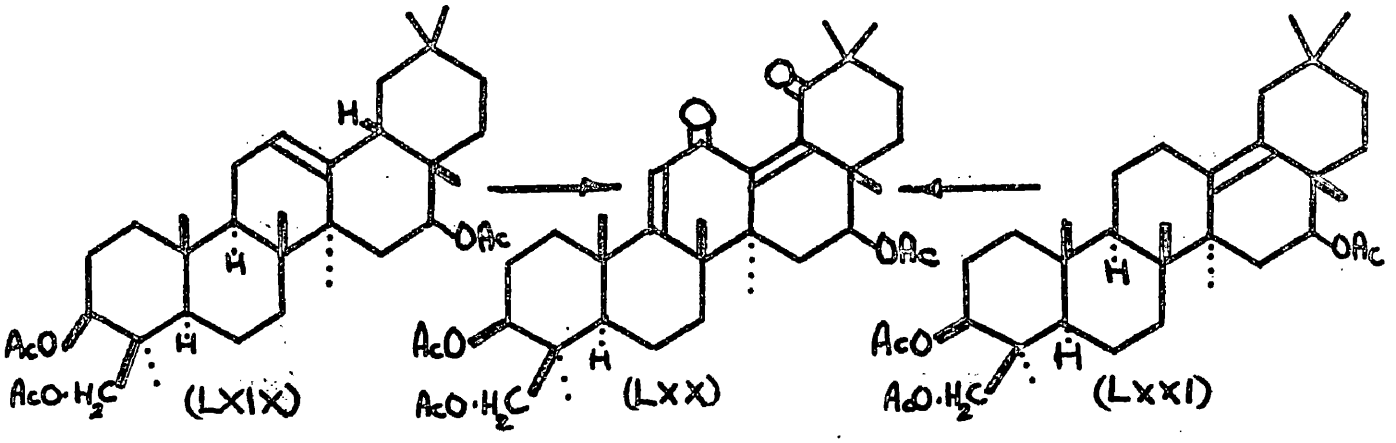
Sapogenol D was shown by Meyer et al. to be a tetracyclic oxide, $C_{30}H_{50}O_3$.[91,92] Of the three oxygen atoms in the molecule, two are present as hydroxyls, and a diacetate, m.p. 194°, can be prepared. The third oxygen, however, is present in an oxide ring, which is related to a strong absorption band at 9.1 μ in the infrared. Furthermore, a close structural relationship exists with the other three sapogenols, since fission of the oxide link with normal ether-splitting reagents gives a variety of olean-13(18)-ene derivatives. For example, mild treatment of the diacetate (LXIV) with hydrochloric and acetic acids gives 3β:24-diacetoxy-x-chloro-olean-13(18)-ene (LXV), characterised by its conversion into 3β:24-diacetoxy-olean-

-13(18)-ene (LXVI) by treatment with Raney nickel and hydrogen. The latter compound is prepared more directly from dihydrosapogenol C diacetate (LXVII) by mild oxidation with selenium dioxide to the heteroannular diene (LXVIII), followed by catalytic reduction to the required diacetoxy-oleanene (LXVI)



Treatment of sapogenol D diacetate (LXIV) with boron trifluoride and acetic anhydride affords a triacetate, $C_{36}H_{56}O_6$. This was formulated as the 13:18-double bond isomer of sapogenol

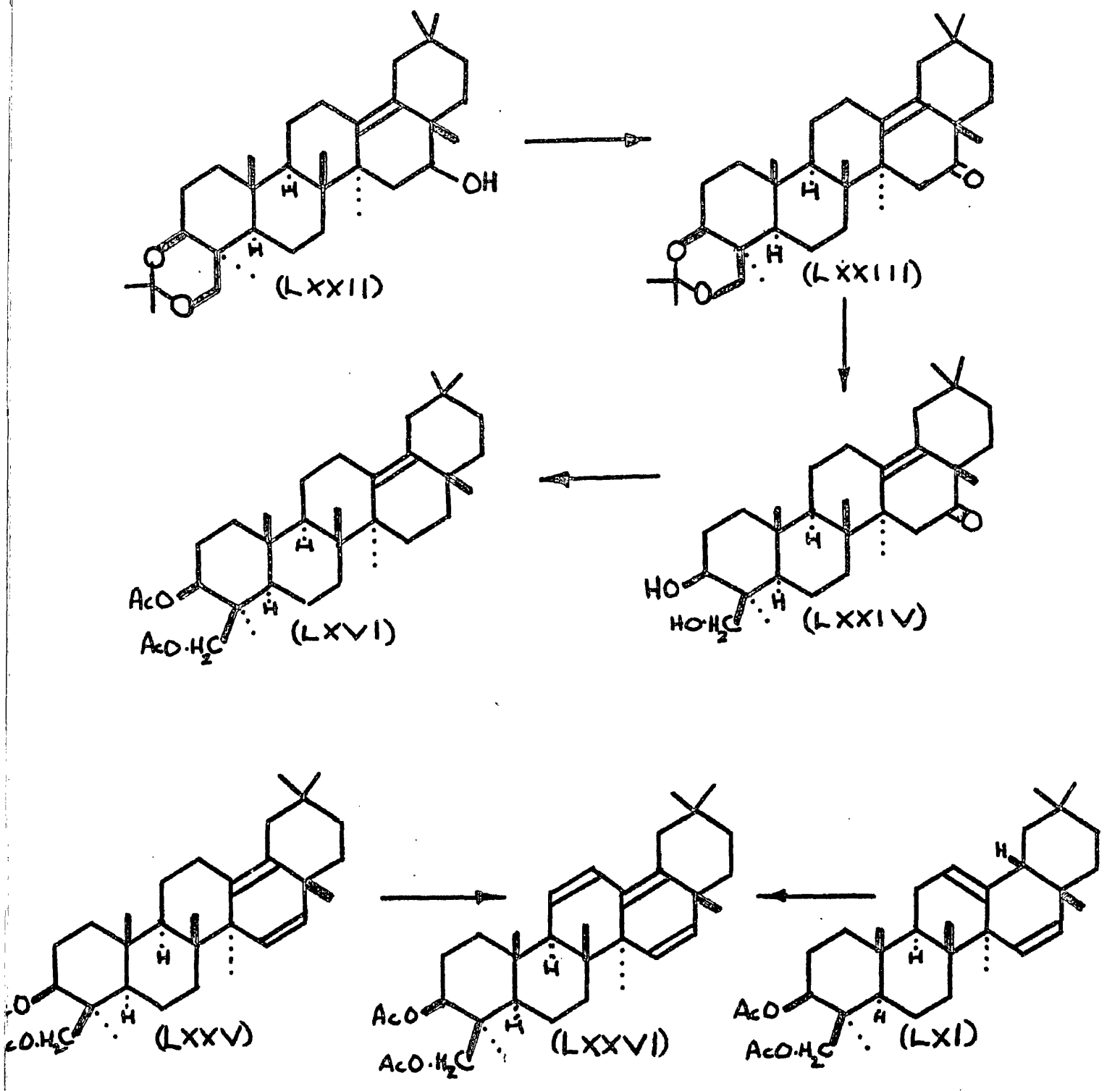
B triacetate (LXIX), since both give a common dioxodiene derivative (LXX) on vigorous selenium dioxide oxidation.



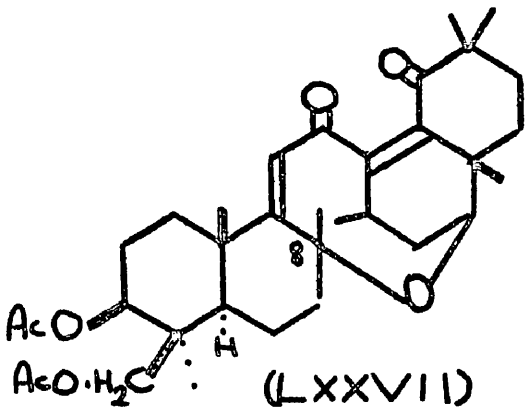
Alkaline hydrolysis of the isomeric triacetate (LXXI), followed by treatment with acetone and sulphuric acid gives a hydroxy-isopropylidene derivative (LXXII) which could be oxidised with chromic acid to the corresponding ketone (LXXIII). Brief treatment of the latter with mineral acid gave the dihydroxyketone (LXXIV) which was reduced by the Wolff-Kishner method, and acetylated to the diacetoxy-oleanene derivative (LXVI), previously obtained from both the diacetoxy chloro-compound (LXV), and from dihydrosapogenol C diacetate (LXVII).

Treatment of the chloro-compound (LXV) with sodium iodide in acetone affords a non-conjugated diene, formulated as (LXXV)[92] Oxidation of the diene with selenium dioxide in acetic acid gives a diacetoxy-triene, which is also obtained by the similar oxidation of sapogenol C diacetate. On this

evidence, the triene is formulated as 3β:24-diacetoxy-oleana--11:13(18):x(y)-triene (LXXVI).



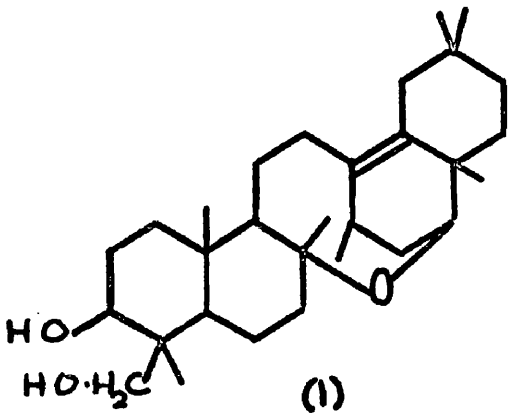
Meyer *et al.* were able to throw additional light on the location of the oxide ring in sapogenol D, by selenium dioxide oxidation of the diacetate to a dioxodiene derivative (LXXVII)[21]. Infrared and ultraviolet absorption spectra showed that the last compound is a typical 12:19-dioxo-9(11):13(18)-diene, and that the oxide ring is still intact. On the basis of this and earlier reactions, it was deduced that one end of the oxide ring was attached to position x in the molecule, with the other end located at some nearby carbon atom such as C_8.



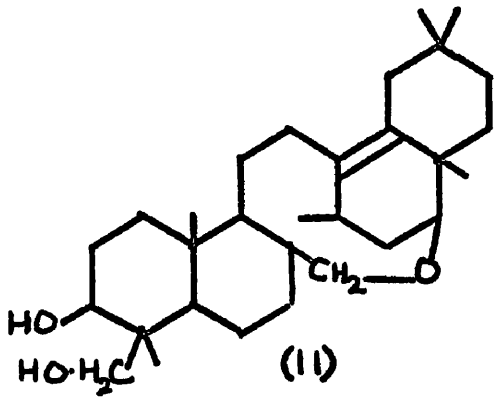
(LXXVII)

THEORETICAL

Our interest in the soyasapogenols was aroused by the remarkable constitution ascribed by Ruzicka to sapogenol D (I)[90].



(I)



(II)

Recently, in a review of the chemistry of the soyasapogenols, a modified structure (II) for sapogenol D is proposed in which the oxide ring is attached to carbon atoms 16 and 26.[93] Although this structure is perhaps more feasible than that of Ruzicka, it still retains several unattractive features. It was therefore decided to reinvestigate the chemistry of the soyasapogenols, with a view to establishing the true nature of the oxide ring in sapogenol D. An account of this work will now be given.

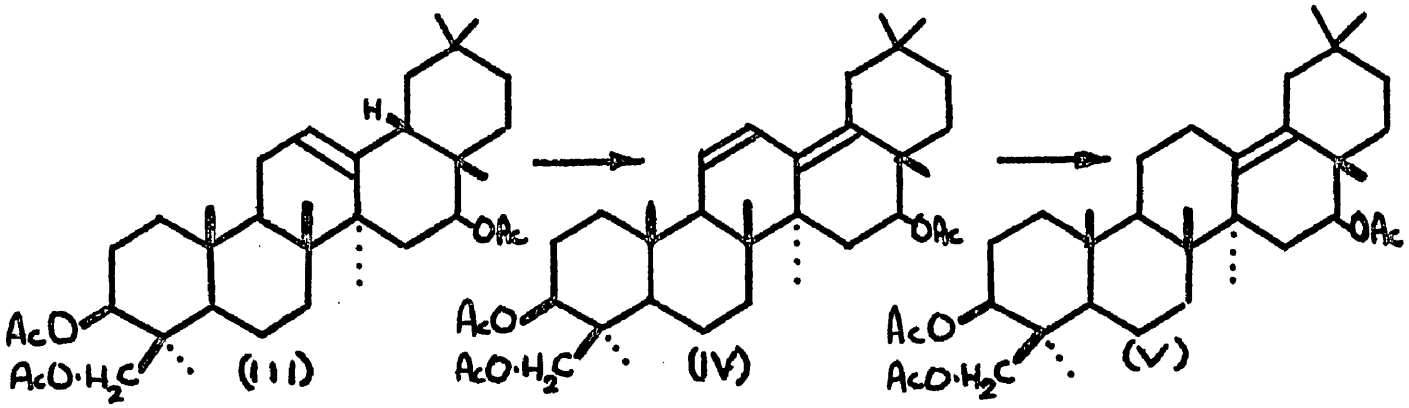
I. Isolation of the Soyasapogenols.

Two additional sources of the soyasapogenols have recently been reported. From the saponin of ladino clover (Trifolium repens), Djerassi has isolated sapogenols B, C, and possibly A,[94] while McLean and Thomson have shown sapogenol C to be a constituent of common whin (Ulex europaeus).[95] Since sapogenol D is apparently absent from both these sources, it was decided to isolate all four sapogenols from soyabean meal. Two different methods for this are available. The first method, used by Ochiai and his co-workers, involves preparation of the calcium salt of the saponin, which is then subjected to acid hydrolysis and subsequent chromatography.[82] In our hands, this method failed to gave a satisfactorily crystalline calcium salt. The second method, employed by Ruzicka, involves acid and alkaline hydrolyses of the crude saponin, followed by benzene extraction and chromatography.[90] It was decided to adopt the latter procedure with several modifications, to minimise time spent in isolating the sapogenols. Accordingly, the crude sapogenin mixture obtained was fractionally crystallised prior to acetylation and chromatography. In this way, appreciable amounts of sapogenols A, B, C, and D were obtained and purified by crystallisation. Without exception, the physical constants of all four sapogenols and their acetates were found to be in close agreement with the values recorded in the literature.

In addition to the sapogenols, a fifth compound was isolated during chromatography of the acetylated sapogenin mixture. This was identified as the acetate of the well-known phytosterol, β-sitosterol, by direct comparison with authentic specimens of the alcohol and derived acetate.

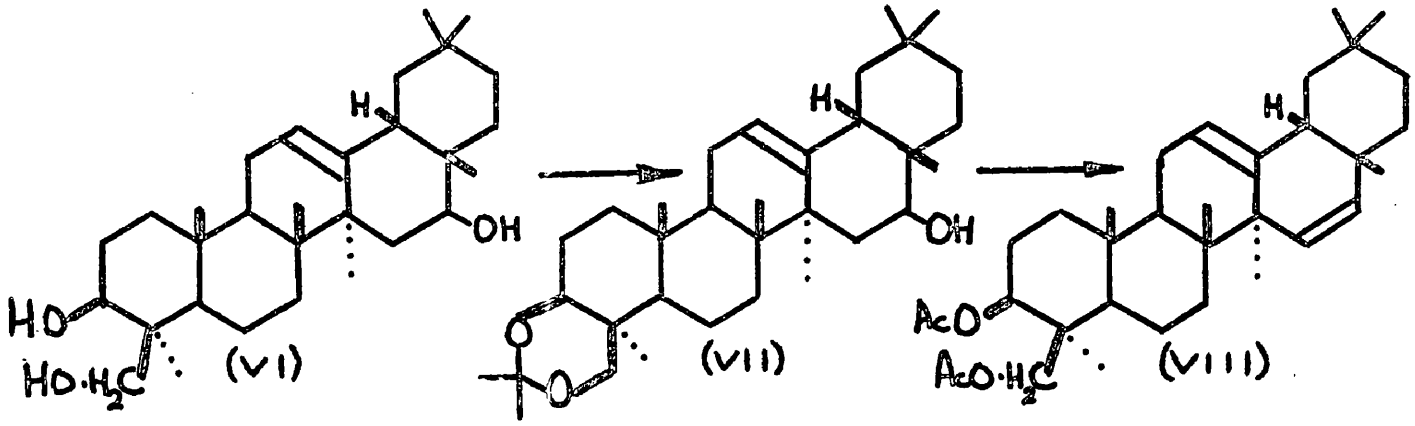
II. Structural Inter-relationships.

Ruzicka was able to inter-relate sapogenols A and C by osmium tetroxide oxidation.[90] He also obtained a common dioxodiene derivative from sapogenols B and D, and concluded that the triacetate produced by treatment of sapogenol D diacetate with boron trifluoride and acetic anhydride, is the 13(18)-double bond isomer of sapogenol B triacetate.[91] This view has been confirmed by direct conversion of sapogenol B triacetate into its 13(18)-double bond isomer. Oxidation of sapogenol B triacetate (III) with selenium dioxide in acetic acid gives the heteroannular 11:13(18)-diene (IV), which on catalytic hydrogenation affords the required isomeric sapogenol B triacetate (V). The latter is further characterised by hydrolysis to the corresponding triol.



A third inter-relationship has been achieved in the direct conversion of sapogenol B (VI) into sapogenol C. Treatment of the former with acetone and sulphuric acid yields the 3β:24--isopropylidene derivative (VII), which is dehydrated by

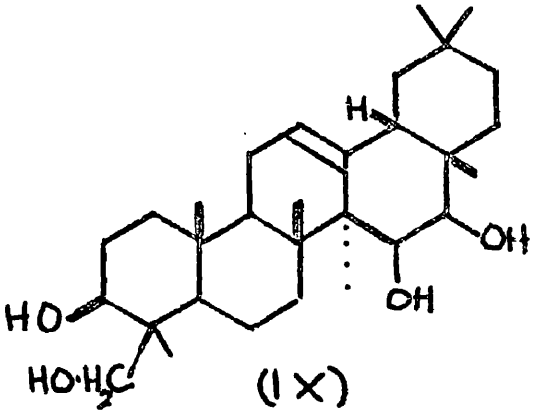
phosphorus oxychloride in pyridine. Brief mineral acid hydrolysis furnishes sapogenol C, identified as the diacetate (VIII).



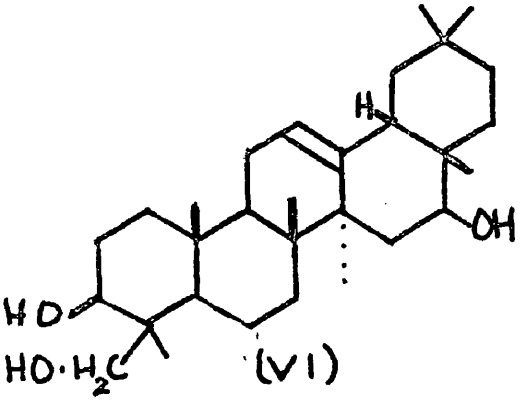
On the basis of established relationships, and the assignment of the unlocated double bond in sapogenol C to positions x and y, partial structures can be given for the other sapogenols. Thus, sapogenol A is 3β:24:x:y-tetrahydroxyolean--12-ene, sapogenol B is 3β:24:x(or y)-trihydroxyolean-12-ene, and one terminal of the oxide ring in sapogenol D is attached to position x(or y).

III. The Oleanane - Taraxerane Rearrangement.

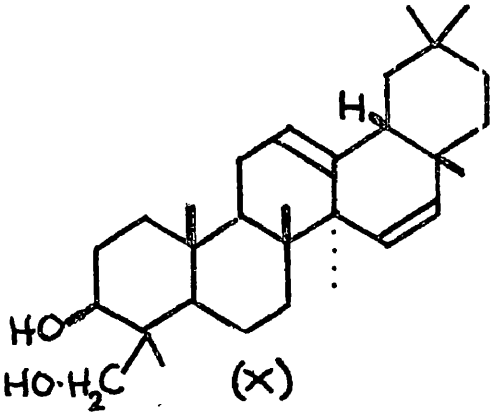
Having isolated sapogenols A, B, C and D, our immediate aim was to test the structures (IX, VI, X, and I) postulated by Ruzicka.[90]



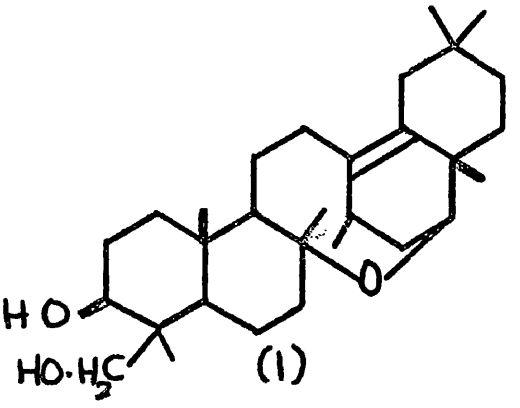
(IX)



(VI)

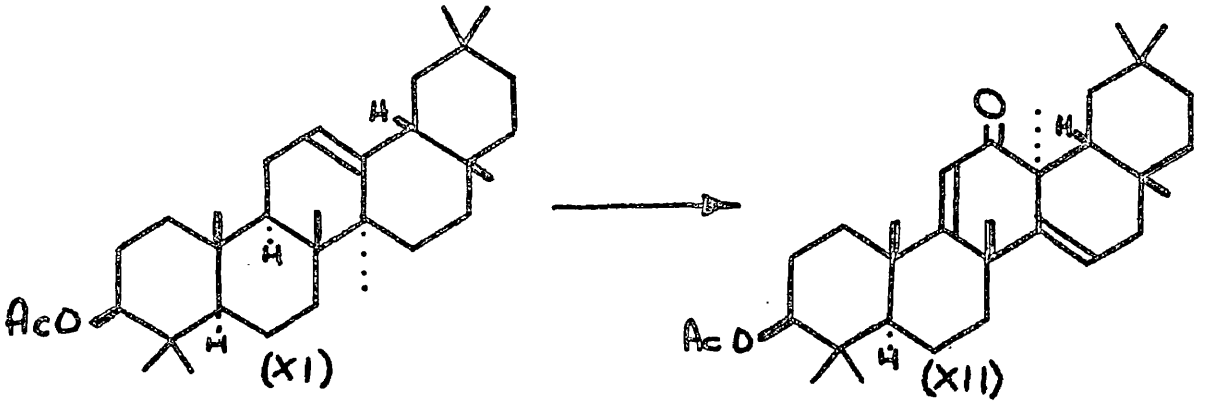


(X)

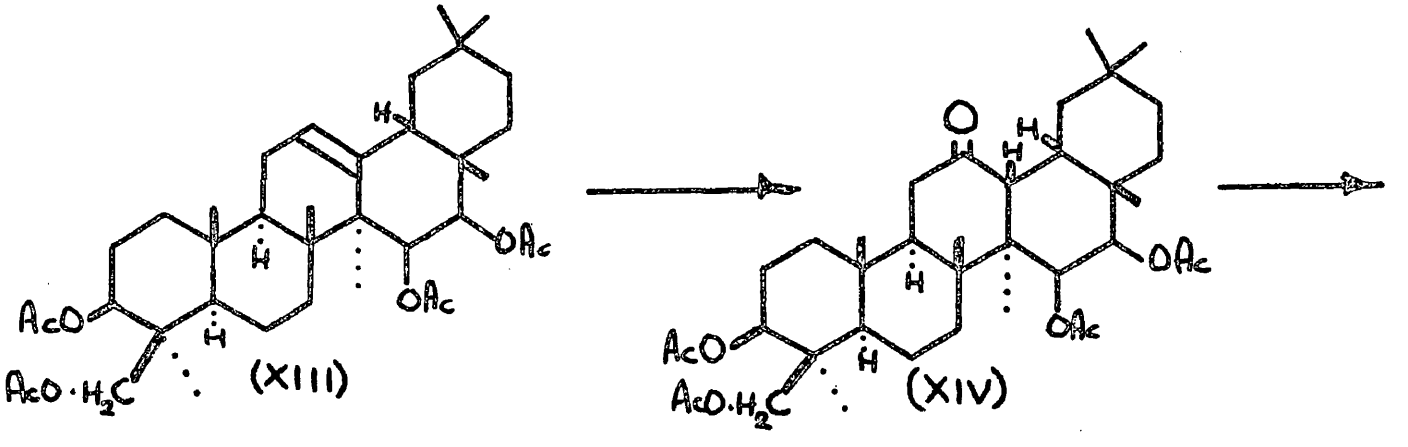


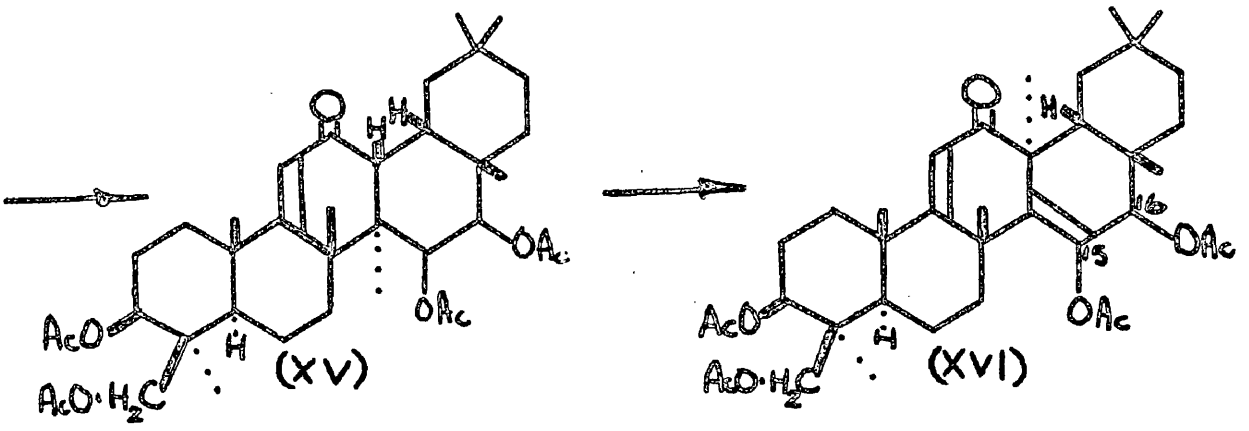
(I)

Accordingly, it was decided to apply to sapogenol A tetra-acetate a series of reactions first carried out on β-amyrin acetate (XI), involving the preparation of the taraxerane derivative, iso-β--amyradienonyl acetate (3β-acetoxy-12-oxotaraxera-9(11):14-diene, XII)[65,66]



Oxidation of sapogenol A tetra-acetate (XIII) with performic acid gave the saturated 12-ketone, 3β:24:x:y-tetra-acetoxy-12--oxo-oleanane (XIV). Treatment of the latter with bromine furnished the αβ-unsaturated ketone, 3β:24:x:y-tetra-acetoxy--12-oxo-olean-9(11)-ene (XV), which rearranged as anticipated during selenium dioxide oxidation to yield the isodienonyl-derivative, 3β:24:x:y-tetra-acetoxy-12-oxo-taraxera-9(11):14-diene (XVI).





Proof that the two reaction series are structurally analogous is best demonstrated by the method of molecular rotation differences,[63] and by comparison of the corresponding ultraviolet absorption spectra, as shown in Tables I and II.

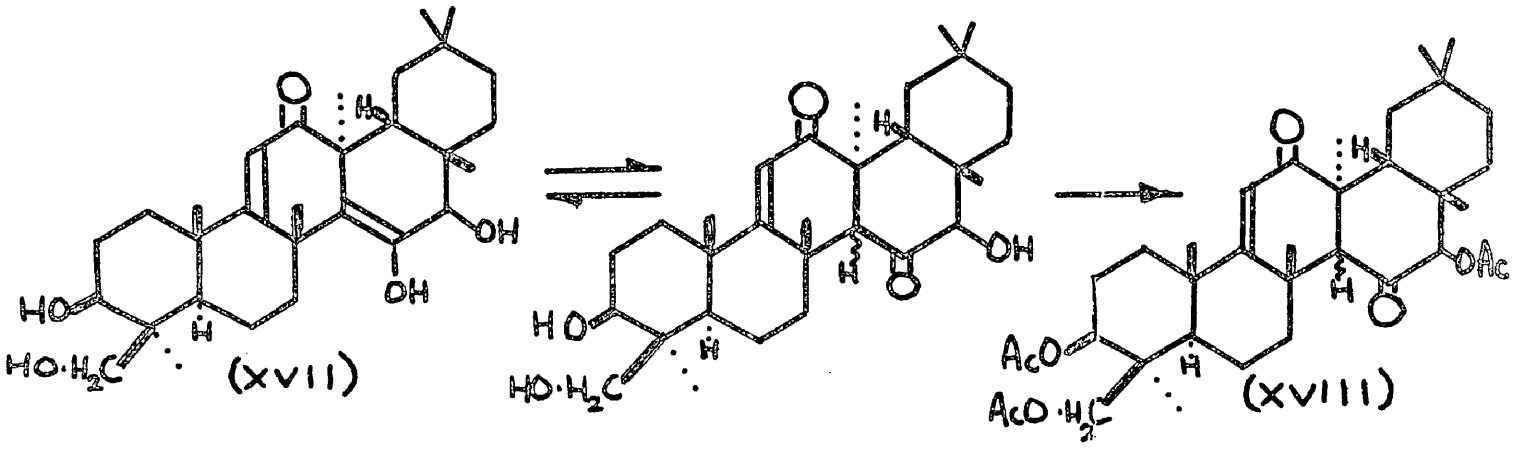
Table I: β-Amyrin Series.

Compound	λ max.	ε	M_D	Δ
3β-acetoxyolean-12-ene (XI)	End absorption		+374°	-447°
3β-acetoxy-12-oxo-oleanane	-	-	- 73°	+367°
3β-acetoxy-12-oxo-olean-9(11)-ene.	2470 Å.	12,000	+294°	-486°
3β-acetoxy-12-oxotaraxera-9(11):14-diene (XII)	2080 Å. 2450 Å.	8,400 11,200	-192°	

Table II : Sapogenol A Series.

Compound	λ Max.	ε	M_D	Δ
3β:24:x:y-tetra-acetoxyolean--12-ene (XIII)	End absorption		+546°	
3β:24:x:y-tetra-acetoxy-12--oxo-oleanane (XIV)	- -	- -	+129°	-417°
3β:24:x:y-tetra-acetoxy-12--oxo-olean-9(11)-ene (XV)	2470 Å.	11,000	+476°	+347°
3β:24:x:y-tetra-acetoxy-12--oxotaraxera-9(11):14--diene (XVI)	2080 Å. 2440 Å	8,000 11,200	-11°	-487°

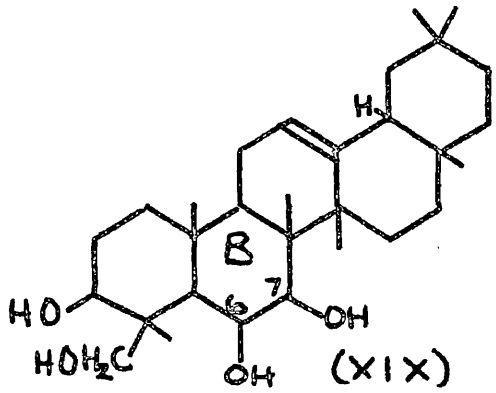
If Ruzicka's structure for sapogenol A (IX) is assumed, with the x:y-glycol group located at positions 15 and 16, the structure assigned to the isodienonyl derivative (XVI) must include an enol-acetate grouping at the former position. The presence of such a group can be readily established. Hydrolysis with alkali furnishes the tetrol (XVII) which would reacetylate under standard conditions to the triacetoxy-ketone (XVIII).



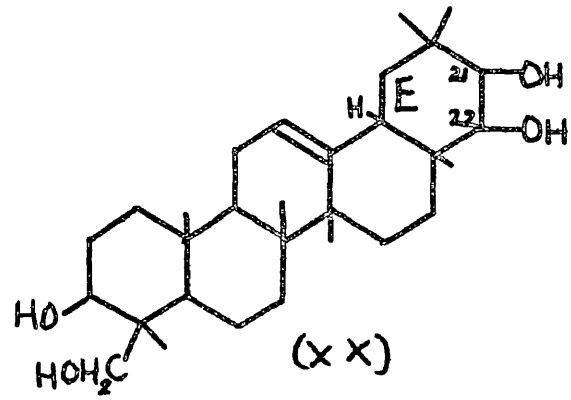
However, when these reactions were carried out on the isodienonyl--tetra-acetate, a crystalline tetrol, $C_{30}H_{48}O_5$ was obtained which did not give a colour with ferric chloride solution, and on cold acetylation regenerated the parent tetra-acetate in excellent yield. This shows conclusively that the x and y hydroxyl groups in sapogenol A cannot be at positions 15 and 16, and that the four structures (IX, IV, X and I) assigned to the sapogenols by Ruzicka,[20] are erroneous.

IV. Preliminary Studies in Structural Elucidation.

With the rejection of the provisional structures for the soyasapogenols, attention could now be turned to the more exacting task of locating the x:y-glycol grouping in sapogenol A. Possible sites are in ring B, at positions 6 and 7 (XIX), and in ring E, at positions 21 and 22 (XX).

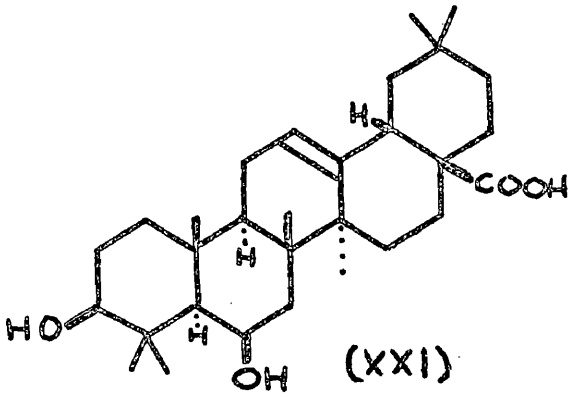


(XIX)

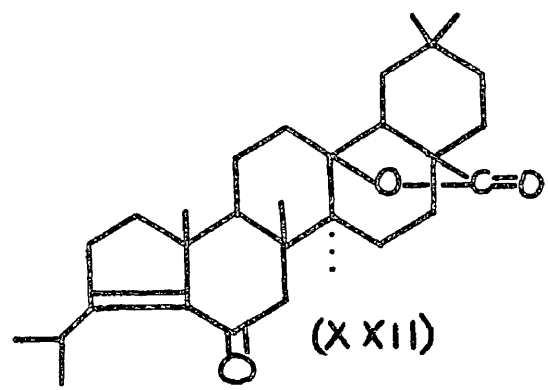


(XX)

Sumaresinolic acid (XXI), the 6β-hydroxy-derivative of oleanolic acid, is the only known oleanane triterpenoid oxygenated in ring B. The 6β-hydroxyl substituent is unaffected by standard acetylation procedures. Its location was established by dehydration of the derived 6-ketone, which yielded the αβ-unsaturated ketone (XXII).[96]



(XXI)

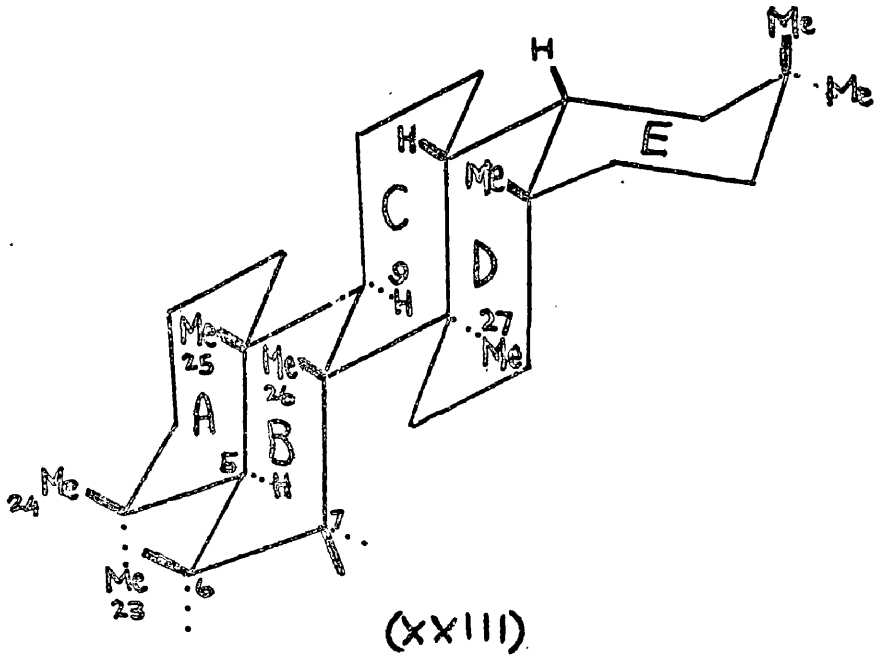


(XXII)

Such a method would have obvious complications if applied to the sapogenols, because of the additional hydroxyl group at position 24. Consequently, attention was turned to the possibility of partially acetylating sapogenols A and B. Since Ruzicka reports that all four hydroxyl groups in the former are acylable at room temperature,[90] it was decided to investigate the acetylation of the two sapogenols under milder conditions. Parallel reactions were carried out using pyridine and acetic anhydride at 5°. In the case of sapogenol B, it was found that all three hydroxyl groups had esterified, whereas chromatography of the product from sapogenol A gave a triacetate, m.p. 261°, and the expected tetra-acetate, m.p. 228°. Hot acetylation of the triacetate furnished the tetra-acetate in high yield. Furthermore the latter was found to be stable to the conditions employed for the chromatographic separation, thus showing that the triacetate had not originated by partial hydrolysis of the tetra-acetate.

A consideration of the non-bonded interactions prevailing in ring B of the oleanane molecule (XXIII) reveals that steric hindrance of substituents at positions 6 and 7 diminishes in the order $6\beta > 7\alpha > 6\alpha > 7\beta$, as shown in Table III.

Assuming x and y to be in ring B, the most attractive of the possible structures for sapogenol B is therefore 3β:7β:24--trihydroxyolean-12-ene (XXIV). Similarly sapogenol A can be tentatively formulated as 3β:6α:7α:24-tetrahydroxyolean-12-ene



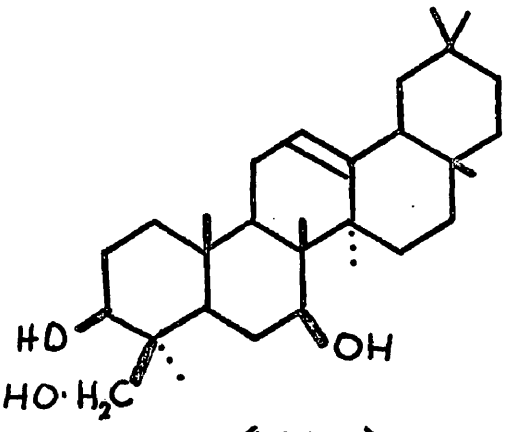
(XXIII)

Table III

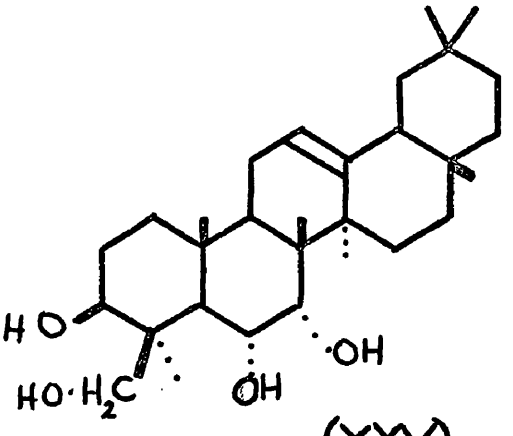
Substituent	Neighbouring Groups for 1:3-Interference
6β	24, 25, 26
7α	27, 9α, 5α
6α	23
7β	-

(XXV). This latter structure is however, open to criticism, since the possibility cannot be excluded of ester interchange from the 7β- to the hindered 6β- position during acetylation.

In an attempt to best the structure (XXIV) for sapogenol B, it was decided to carry out an oxidative procedure successfully

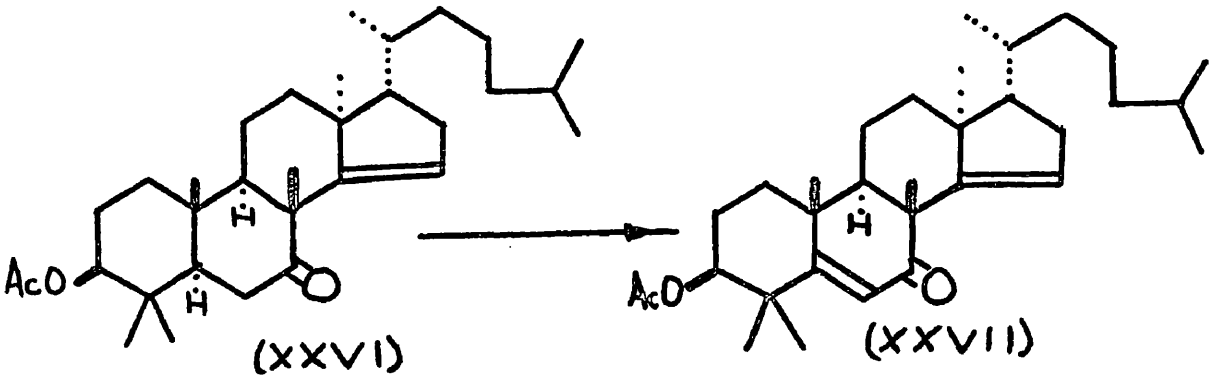


(XXIV)



(XXV)

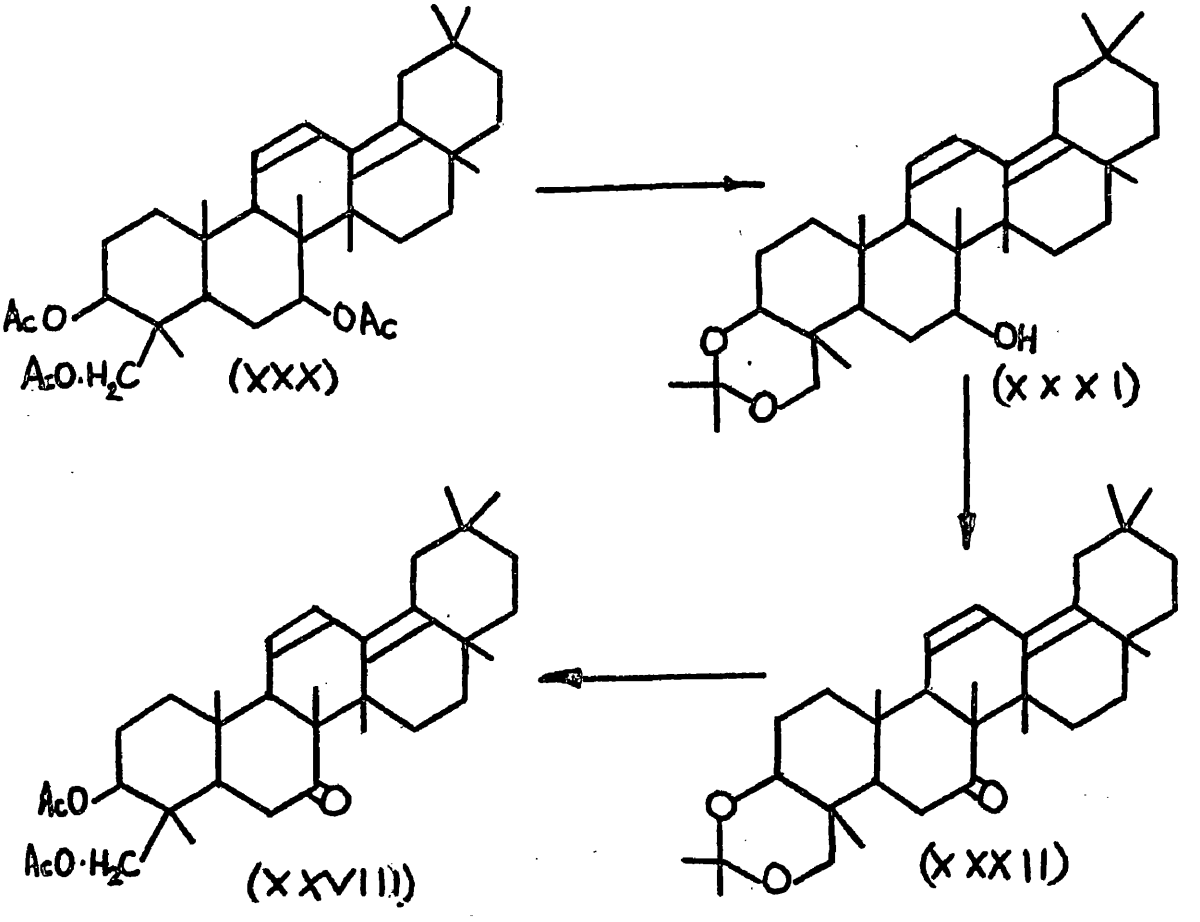
employed in the tetracyclic triterpenoid series.[97] Oxidation of 7-oxoapoeuphenyl acetate (XXVI) with selenium dioxide in acetic acid, gives in high yield, the αβ-unsaturated ketone, 7-oxoapo-eupha-5:14-dienyl acetate (XXVII).



(XXVI) (XXVII)

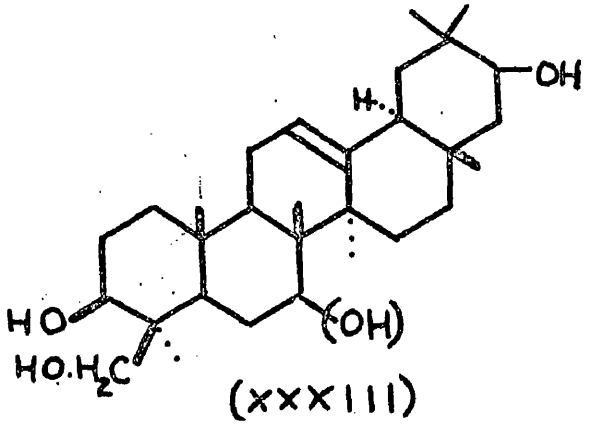
It was anticipated that similar treatment of the diacetoxy-ketone derived from sapogenol B (XXVIII) would introduce a 5:6-double bond into the molecule, and yield the αβ-unsaturated ketone (XXIX). Since the 12:13-oleanene double bond is itself readily oxidised, it was decided to prepare the heteroannular 11:13(18)--diene prior to oxidation, as this system is stable to mild

treatment with selenium dioxide. Accordingly, the x-oxo-dienyl diacetate (XXVIII) was prepared in the following way. Oxidation of sapogenol B triacetate with selenium dioxide in acetic acid gave the 11:13(18)-dienyl-derivative (XXX) in high yield. This was hydrolysed with lithium aluminium hydride and treated with acetone and sulphuric acid to produce the 3β:24-isopropylidene derivative (XXXI), which on oxidation with the chromium trioxide--pyridine complex gave the x-oxodienyl acetonide (XXXII). Brief acid hydrolysis of the latter and subsequent acetylation yielded the required diacetoxy-oxodiene (XXVIII).

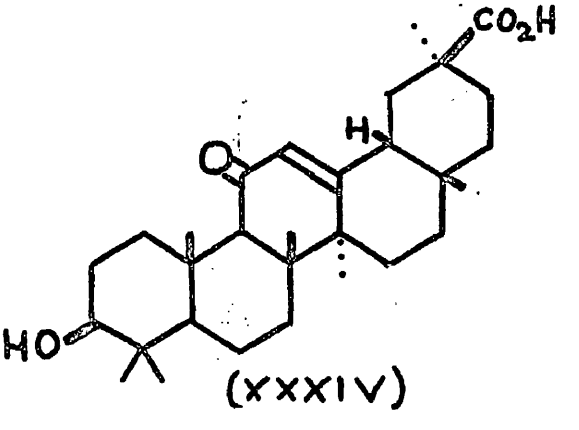


When the latter was oxidised with selenium dioxide in acetic acid, the product was obtained as an intractable gum. The ultraviolet absorption spectrum indicated the presence of unchanged starting material, but careful chromatography did not give a homogeneous product.

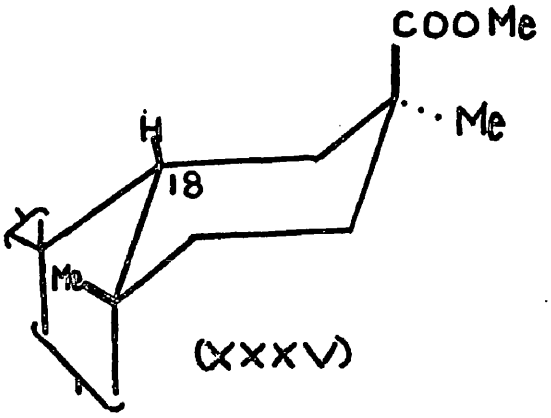
At this stage it was decided to prepare the 18α-isomer of sapogenol B (XXXIII). Since equatorially bonded substituents in ring E in an oleanane derivative become axially bonded in the 18α-oleanane isomer, and similarly ring E substituents axially bonded in an oleanane derivative become equatorially bonded in the derived 18α-oleanane isomer. This effect has been used to establish the axial conformation of the $C_{(30)}$-carboxyl group in glycyrrhetic acid (XXXIV), since methyl glycyrrhetate (XXXV) is much more resistant towards hydrolysis than methyl-18α-glycyrrhetate (XXXVI).[98]



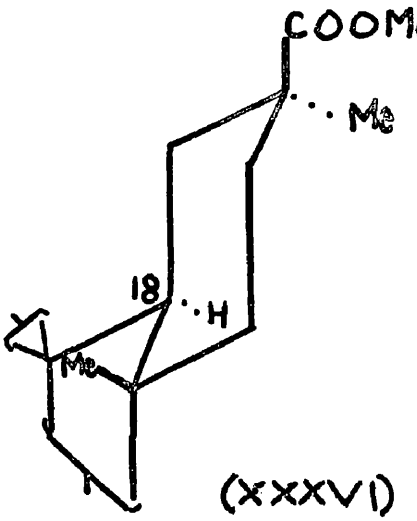
(XXXIII)



(XXXIV)

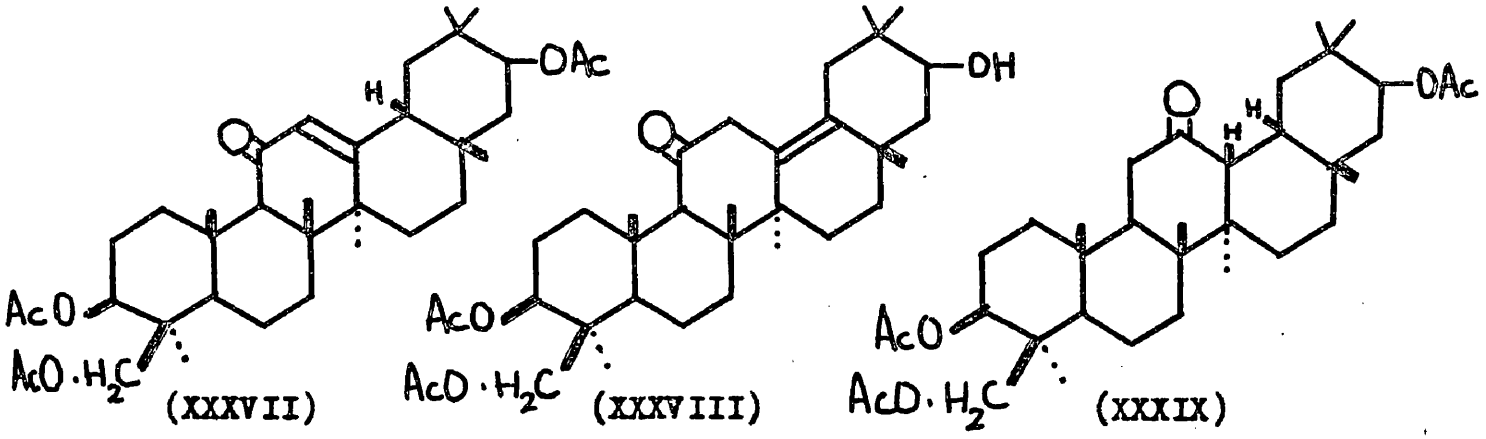


(XXXV)



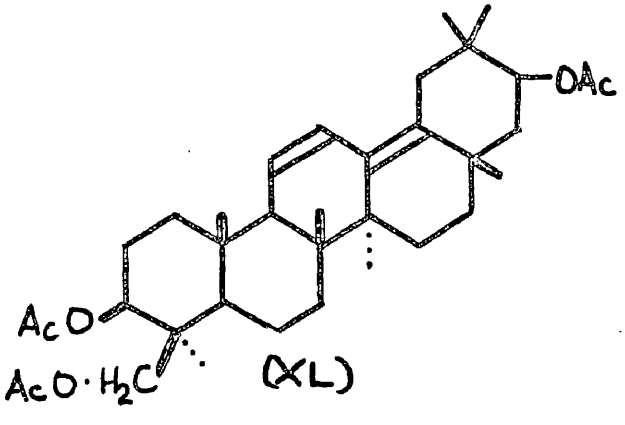
(XXXVI)

It was anticipated that acetylation of 18α-sapogenol B might reveal a change in its ease of esterification attributable to a hydroxyl group present at positions 21 or 22. Using the procedure for the conversion of β-amyrin acetate into its 18α-isomer,[99] sapogenol B triacetate was oxidised with chromium trioxide in acetic acid. The product was a mixture, m.p.242-248°, from which pure 3β:24:x-triacetoxy-11-oxo-olean-12-ene (XXXVII) could not be separated. Its ultraviolet absorption spectrum, $\lambda_{max.}$ 2480 A., ε = 6000, showed that the mixture contained an appreciable amount of the required αβ-unsaturated ketone, but chromatography and fractional crystallisation failed to purify this component. The mixture was treated with strong alkali in the hope that the αβ-unsaturated ketone would be inverted at $C_{(18)}$. Surprisingly, the product did not show any appreciable absorption in the neighbourhood of 2480 Å., but it did show strong absorption in the ethylenic region of the spectrum, from which it is concluded that the αβ-unsaturated ketone component (XXXVII) had been converted into the non-conjugated unsaturated ketone (XXXVIII).

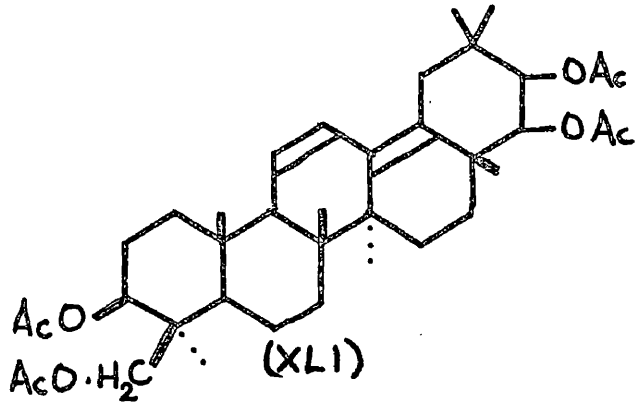


Acetylation of this product and chromatography of the acetate mixture yielded a saturated ketone as the only crystalline fraction. This ketone is 3β:24:x-triacetoxy-12-oxo-oleanane (XXXIX), the reference specimen of which was prepared by oxidation of sapogenol B triacetate with performic acid; this saturated ketone has been obtained by Tsuda and Kitagawa[88] by oxidation of sapogenol B triacetate with either chromium trioxide or hydrogen peroxide in acetic acid. Finally, the mixture, m.p. 242-248°, was treated with hydrochloric and acetic acids. The product, m.p. 285-300°, showed an absorption maximum at 2420 Å. The shift in the position of the absorption maximum indicated that the αβ-unsaturated ketone component had been converted into its 18α-isomer. The latter compound, however, could not be separated from the mixture either by fractional crystallisation or by chromatography.

In view of these difficulties, it was decided to suspend work on the preparation of 18α-sapogenol B, in favour of the more accessible 11:13(18)-diene, to determine whether the consequential change in the conformation of ring E is reflected in a change in the ease of esterification of the x and y hydroxyl groups. The triacetoxy-11:13(18)-diene (XL) was prepared as before, and hydrolysed with lithium aluminium hydride to the corresponding triol. Acetylation of the latter at 5° overnight regenerated the parent triacetate in high yield. Similar treatment, however, of the tetrol prepared from the analogous sapogenol A tetra-acetoxy-diene (XLI) gave a mixture of a triacetate, m.p. 256° and a tetra-acetate, m.p. 241°. The tetra-acetate was also obtained by hot acetylation of the triacetate, this behaviour corresponding to that of sapogenol A triacetate.



(XL)

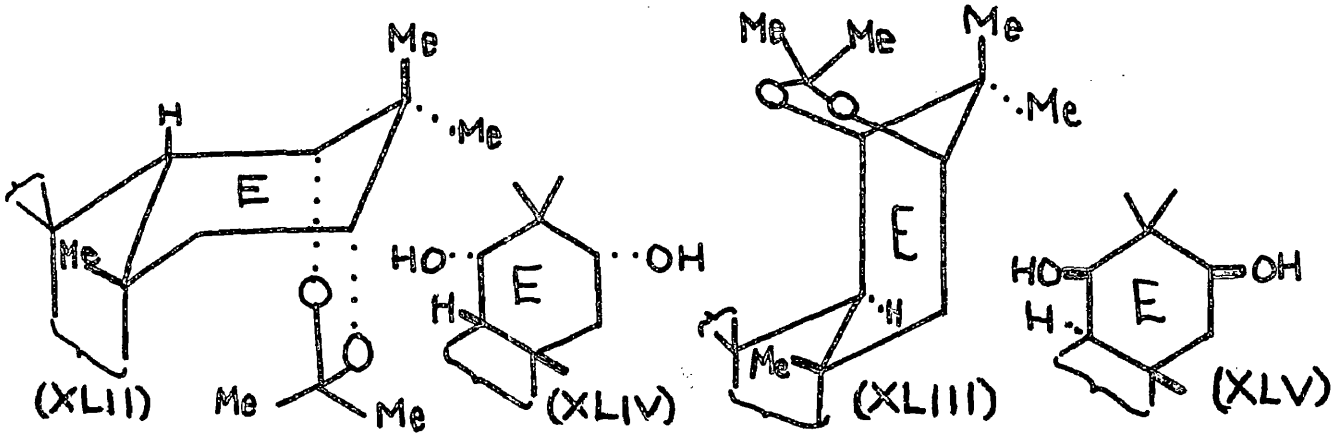


(XLI)

Proof that the same hydroxyl group in both triacetates is resistant to acetylation at 5°, was established by the conversion of sapogenol A triacetate into the diene triacetate by mild

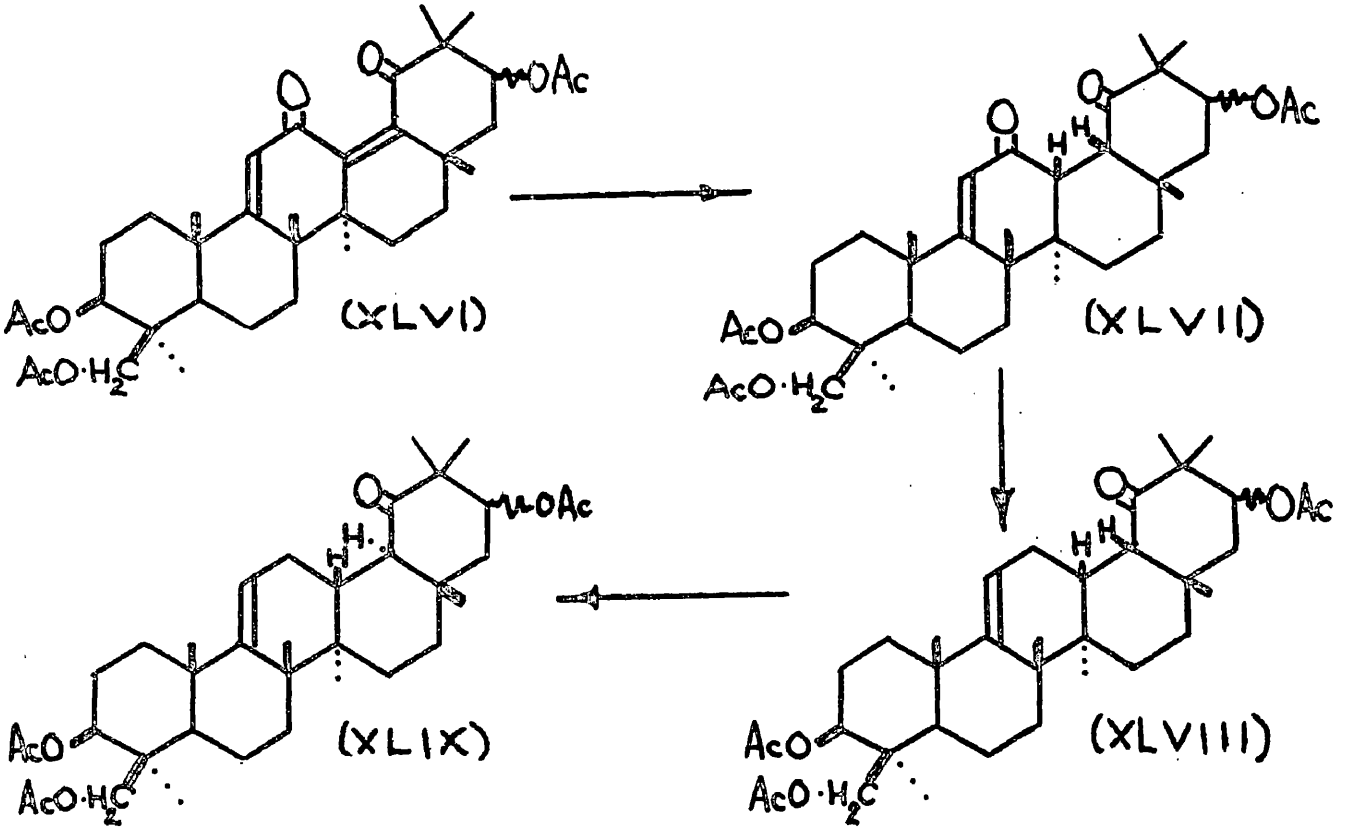
selenium dioxide oxidation. Consequently, if the x and y hydroxyl groups of sapogenol A are located at $C_{(21)}$ and $C_{(22)}$, it is evident that the above method is not sufficiently delicate to detect the conformational change in ring E associated with the conversion of an olean-12-ene derivative into the related 11:13(18)-diene.

The last series of reactions discussed in this section were attempts to establish that the third hydroxyl group in sapogenol B is at position 21. Inspection of molecular models of the sapogenol suggested that, if a fourth hydroxyl group were introduced at $C_{(19)}$, a 19:21-isopropylidene derivative could be prepared in both the oleanane (XLII) and 18α-oleanane series (XLIII).



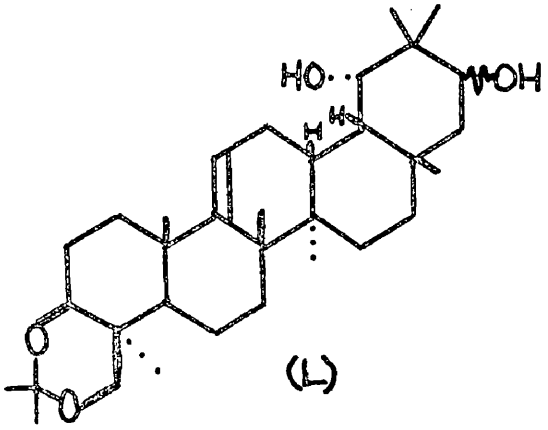
A necessary condition for the formation of this isopropylidene derivative is that the participating hydroxyl groups at $C_{(19)}$ and $C_{(21)}$ must be axially bonded, as in structures (XLIV) and (XLV). To test this postulate, a number of reactions which had previously been performed on β-amyrin acetate were carried out.[72] Sapogenol B triacetate was oxidised with selenium dioxide in benzyl acetate

to give the 12:19-dioxo-9(11):13(18)-diene (XLVI). Reduction of the latter with zinc in boiling ethanol gave the 13β:18β--dihydro-derivative (XLVII) which was hydrogenolysed over platinum in acetic acid to yield the 19-oxo-9(11)-enyl-derivative (XLVIII). Treatment of this product with mineral acid afforded the 18α-isomer, 3β:24:x-triacetoxy-19-oxo-18α-olean-9(11)-ene (XLIX).

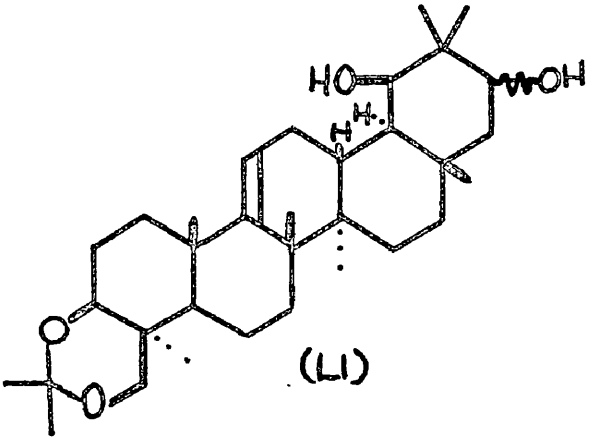


Lithium aluminium hydride reduction of the isomeric 19-oxo-9(11)--enyl-triacetates (XLVIII and XLIX) gave the corresponding 3β:19:24:x-tetrols. If x is at $C_{(21)}$, one of these tetrols must possess

the required 19:21-diaxial glycol structure, as in (XLIV) or (XLV). However, treatment of both compounds with acetone and sulphuric acid gave only the 3β:24-isopropylidene derivatives (L) and (LI) respectively.

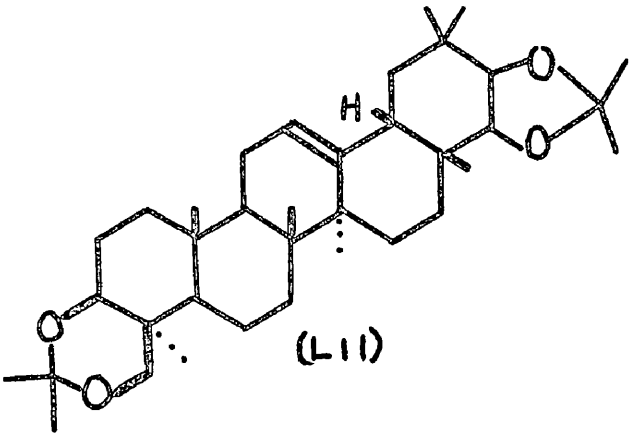


(L)



(LI)

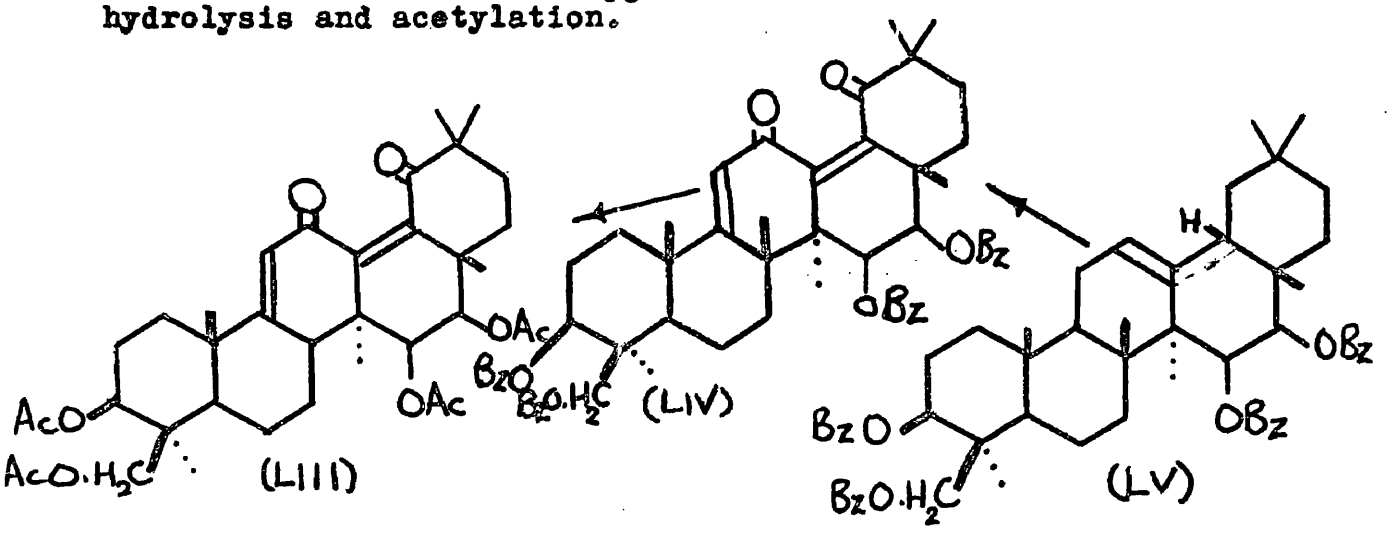
It is the author's opinion that this lack of reactivity of the ring E hydroxyl groups is the result of steric blocking by the carbon atoms in ring D, and by the gem.-dimethyl group at $C_{(20)}$, since sapogenol A forms a bis-isopropylidene derivative (LII) under analogous conditions.



(LII)

V. The Structures of Sapogenols A and C.

In an attempt to locate the x:y-glycol group in sapogenol A, attention was turned to the preparation of the tetra-acetoxy--12:19-dioxo-oleana-9(11):13(18)-diene (LIII). This compound, according to Ruzicka, has m.p. 323-324°, $[\alpha]_D$ - 48°, $\lambda_{max.}$ 2780 Å., and is prepared from sapogenol A tetrabenzoate (LIV) by vigorous selenium dioxide oxidation, followed by alkaline hydrolysis and acetylation.[80]



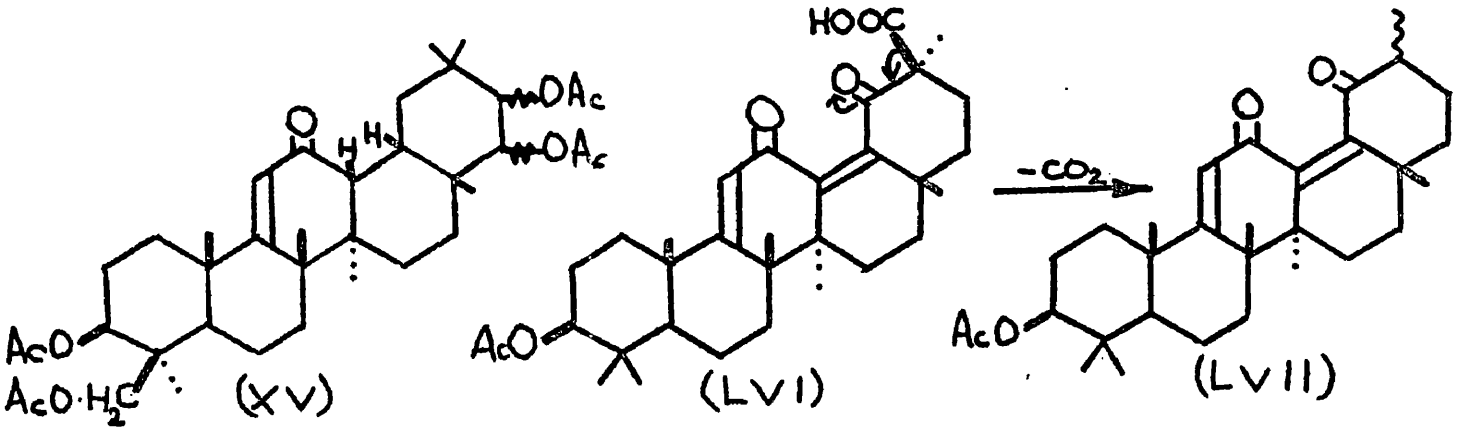
Since the dioxodiene tetrabenzoate (LIV) obtained by Ruzicka was stated to be amorphous, it was decided to repeat the oxidation of sapogenol A in a more direct manner, using the tetra-acetate. Surprisingly, when the latter was oxidised with selenium dioxide in benzyl acetate, a tetra-acetoxydioxodiene was obtained, the constants of which did not agree with the product obtained by Ruzicka. It appeared to be an isomer, and had m.p. 265-266°, $[\alpha]_D$ - 42°, $\lambda_{max.}$ 2780 Å. Furthermore, when hydrolysed with alkali and reacetylated, the previously expected tetra-acetoxydioxodiene (m.p. 330-332°, $[\alpha]_D$

-48°, $\lambda_{max.}$ 2780 Å.) was obtained. A mixture of the two compounds had m.p. 254°, thus confirming their non-identity.

From these results it would appear that the two dioxodienes are stereoisomers, and that their interconversion involves an intramolecular rearrangement. The infrared and ultraviolet absorption spectra of the compounds indicate that both are true 12:19-dioxo-oleana-9(11):13(18)-dienes. Rearrangement can therefore only have affected the x:y-glycol diacetate group and must have included the formation of an equatorial from an axial bond. Also, epimerisation must have occurred during the original preparation of the higher melting dioxodiene, when the dioxodiene tetrabenzoate (LIV) was treated with alkali before acetylation. A third, isomeric tetra-acetoxydioxodiene was now prepared. Sapogenol C diacetate was oxidised with oxmium tetroxide according to the method of Ruzicka, and gave, after acetylation and chromatography, sapogenol A tetra-acetate and a stereoisomeric sapogenol A tetra-acetate. The latter, on vigorous selenium dioxide oxidation, afforded a third isomeric tetra-acetoxydioxodiene, m.p. 212-214°, $[\alpha]_D$ - 95°, $\lambda_{max.}$ 2780 Å. As anticipated, treatment of this dioxodiene with alkali and subsequent acetylation gave the stable higher melting isomer, m.p. 330-332°.

Since sapogenol A tetra-acetate is recovered unchanged after alkaline hydrolysis and acetylation of the product, it is

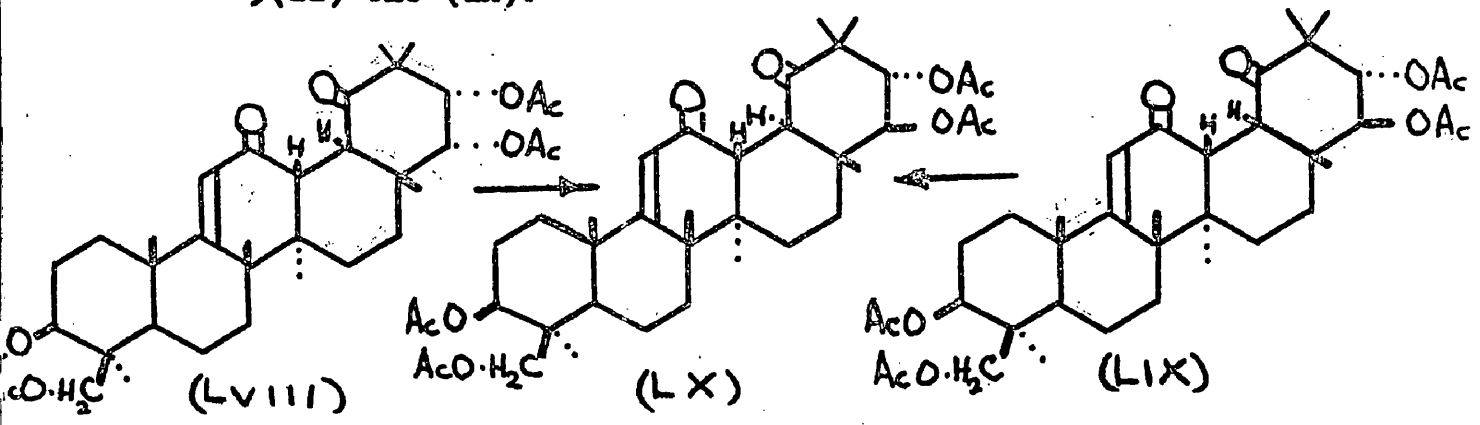
evident that the interconversion of the three tetra-acetoxy-dioxodienes is supported by the 12:19-dioxo-9(11):13(18)-diene system, or by part of this system, and is to be represented as a base-induced epimerisation of the groups attached to $C_{(x)}$ and/or $C_{(y)}$. Although the 12:19-dioxo-9(11):13(18)-diene system is normally regarded as a stable grouping, it can exhibit electrophilic character, as in the decarboxylation of the dioxodiene from glycyrrhetic acid acetate (LVI) to the nordioxodienyl-derivative (LVII).[100]



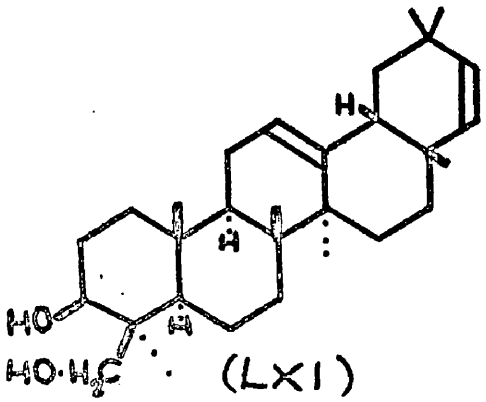
Now, 3β:24:x:y-tetra-acetoxy-12-oxo-olean-9(11)-ene (XV) is recovered unchanged after hydrolysis with alkali and acetylation of the product, thus showing that the 12-oxo-9(11)--ene part of the dioxodiene system is not in itself sufficient to support the base-induced epimerisation at $C_{(x)}$ and/or $C_{(y)}$. That the 19-carbonyl is the activating group was shown as follows.

Reduction of the isomeric dioxodienes, m.p. 265-266° and

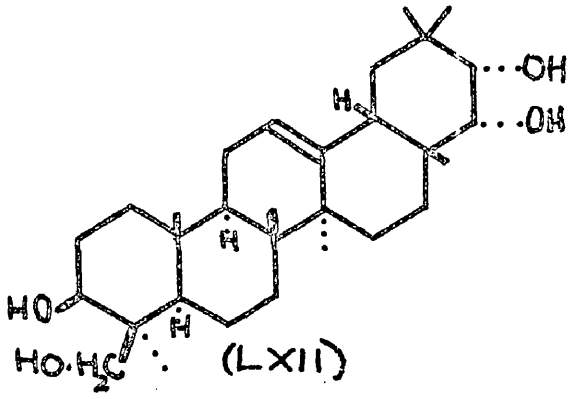
m.p. 330-332°, with zinc in ethanol gave the corresponding 13β:18β-dihydro-derivatives (LVIII, m.p. 274-275°, $[\alpha]_D$ + 103°) and (LIX, m.p. 239-241°, $[\alpha]_D$ + 153°). Subsequent alkaline hydrolysis and reacetylation of (LVIII) and (LIX) gave the same product, 3β:24: x: y-tetra-acetoxy-12:19-dioxo-18α-olean-9(11)-ene (LX).

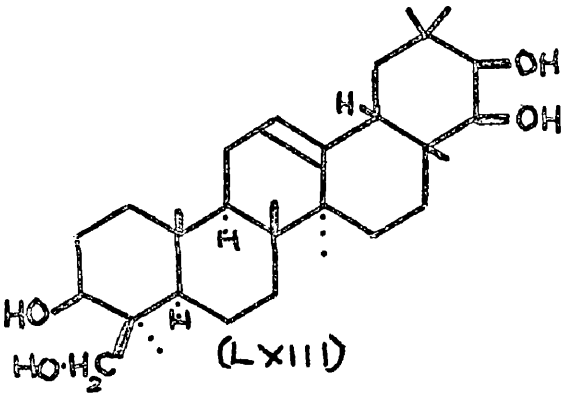


Thus, the 19-carbonyl group, and not the complete dioxodiene system, is responsible for the base-induced epimerisations at x and y, which must therefore be situated at $C_{(21)}$ and $C_{(22)}$. It also follows that the structure of sapogenol C must be 3β:24-dihydroxyoleana-12:21-diene (LXI).

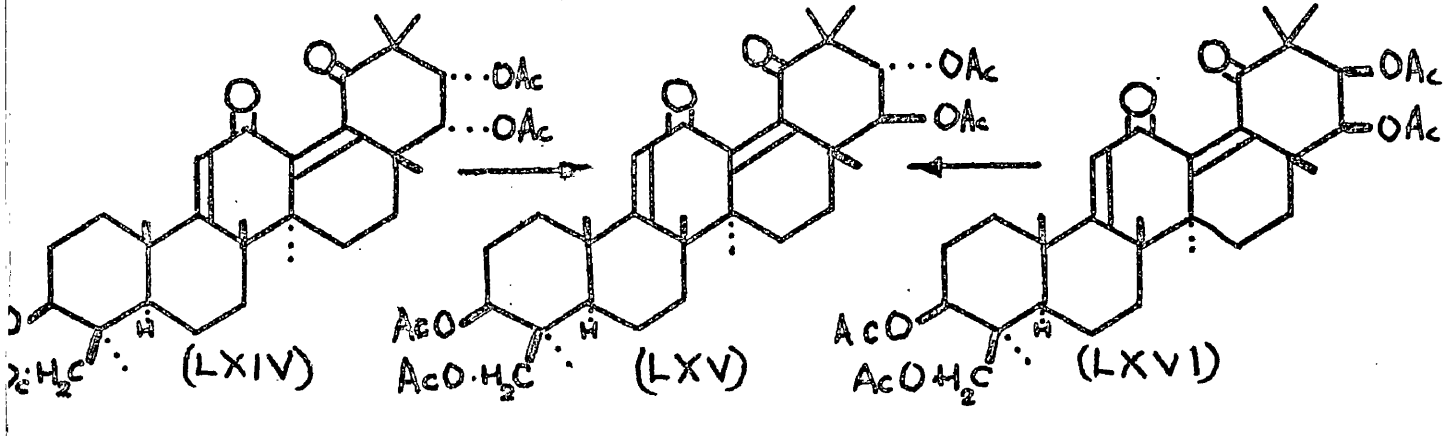


Since sapogenol A is formed as minor product in the osmium tetroxide oxidation of sapogenol C, and the stereoisomeric tetrol is the major product, the latter is probably formed by attack at the less hindered β-face, and the former by attack at the more hindered α-face of ring E. Consequently, sapogenol A is provisionally formulated as 3β:21α:22α:24-tetrahydroxyolean--12-ene (LXII), and the stereoisomeric sapogenol A as 3β:21β:22β :24-tetrahydroxyolean-12-ene (LXIII).



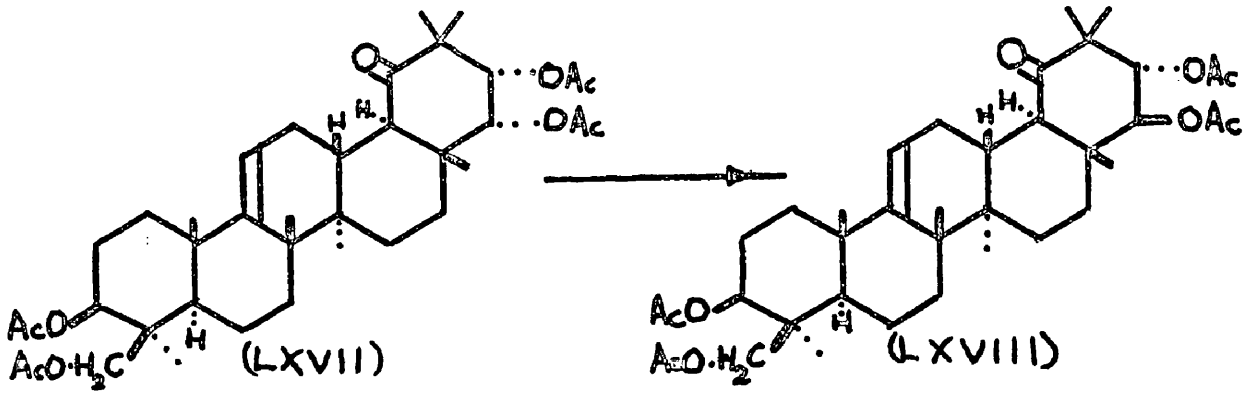


Accordingly, the three isomeric dioxodienes, m.p. 265-266°, m.p. 330-332°, and m.p. 212-214°, are represented as (LXIV), (LXV) and (LXVI) respectively.



An attempt was now made to prepare and interconvert the

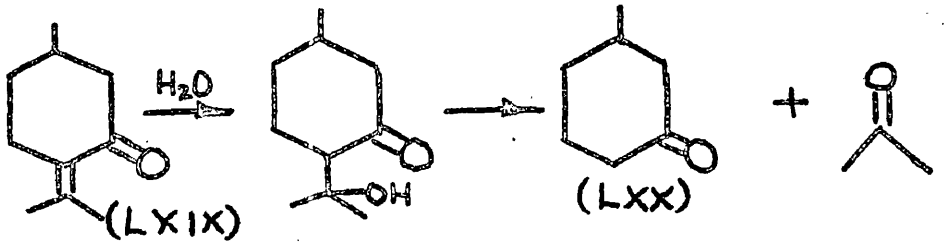
19-oxo-9(11)-enyl-derivatives (LXVII) and (LXVIII) by hydrogenolysis of the dihydro-dioxodienes (LVIII) and (LIX), followed by acid-induced equilibration of the $C_{(18)}$-hydrogen atom. Subsequent treatment of (LXVII) with alkali, followed by reacetylation, would then limit conformational inversion to



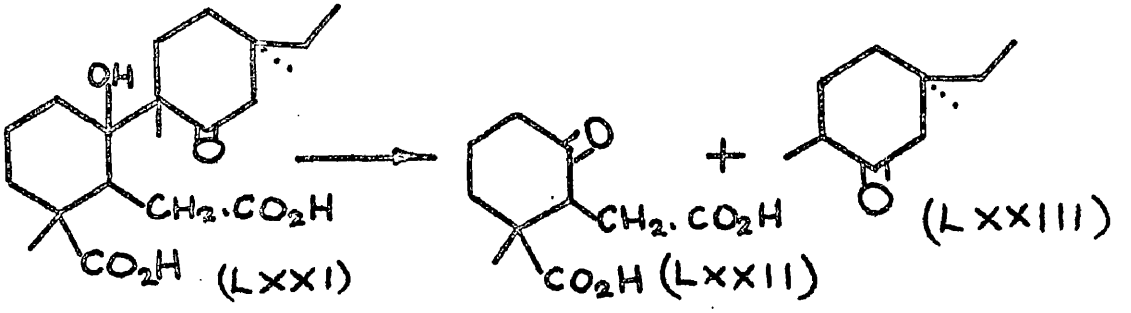
the $C_{(22)}$ hydroxyl group, and give (LXVIII). Surprisingly, when these reactions were attempted, it was found that, in contrast to the behaviour of the analogous β-amyrin[56] and sapogenol B derivatives, hydrogenolysis of the dihydro--dioxodienes (LVIII) and (LIX) was slow, and incomplete even after several weeks. The resulting mixture could not be separated by crystallisation or by chromatography, and the project was accordingly abandoned.

VI. The Reverse-Aldol Condensation Reaction.

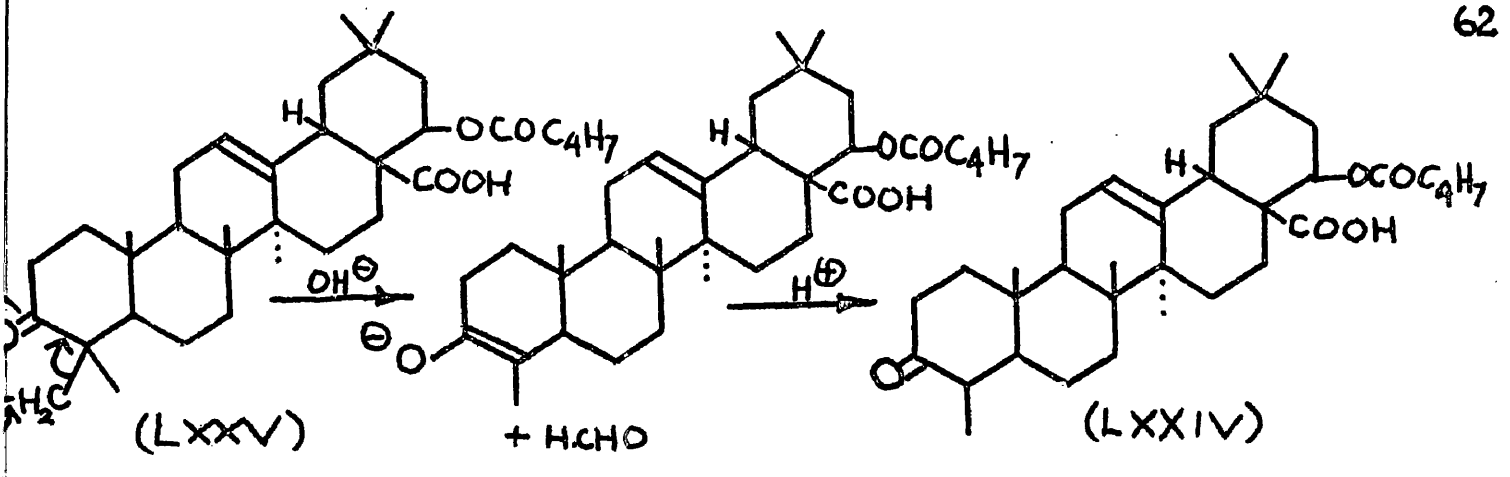
At this juncture, mention must be made of the well-known Reverse- or Retro-Aldol condensation reaction, involving the rupture of a saturated carbon-carbon bond. Many examples of the reaction are known in terpenoid chemistry, e.g. the acid or base-induced decomposition of pulegone (LXIX) into acetone and 3-methylcyclohexanone (LXX).[101]



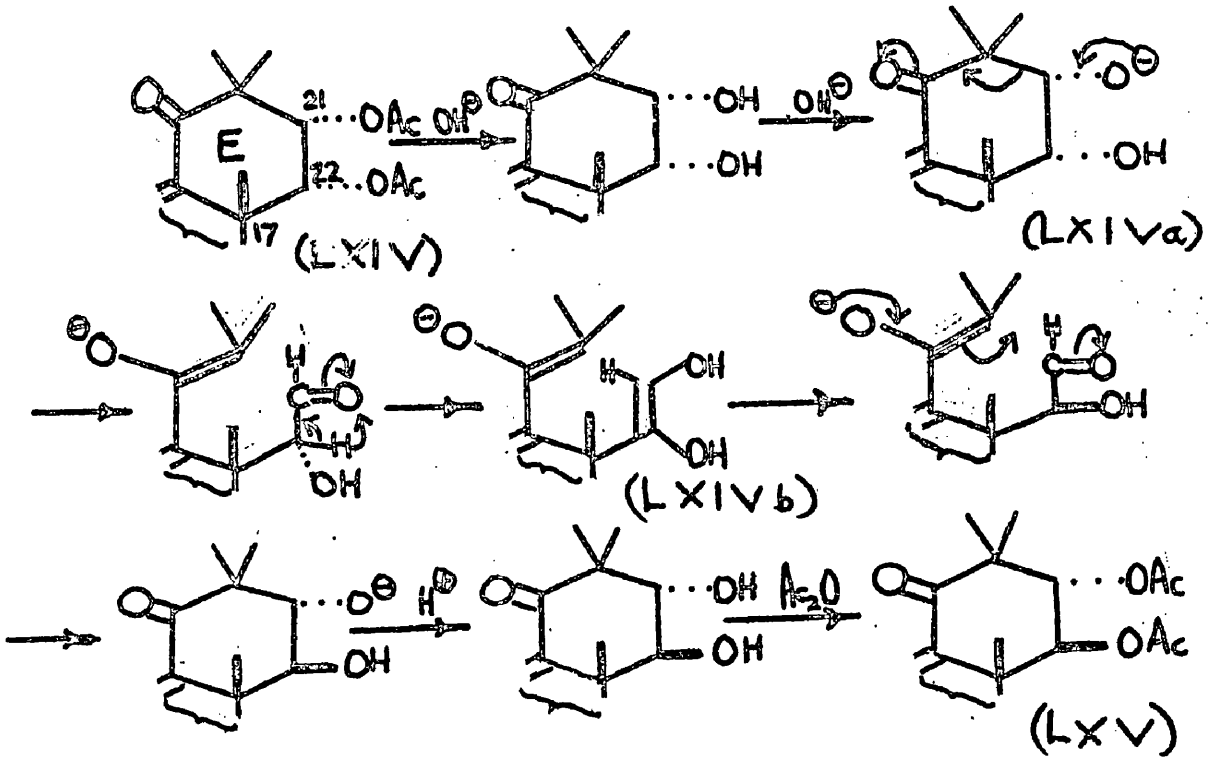
Similarly, treatment of the keto-acid from rosenonolactone (LXXI) with alkali, effects fission of the molecule into the components (LXXII) and (LXXIII).[102]



The mechanism of the Reverse-Aldol reaction is well-defined. The process is essentially a reversal of the Aldol Condensation, a typical example in the triterpenoid series being the formation of the norketone (LXXIV) from icterogenin (LXXV).[103]



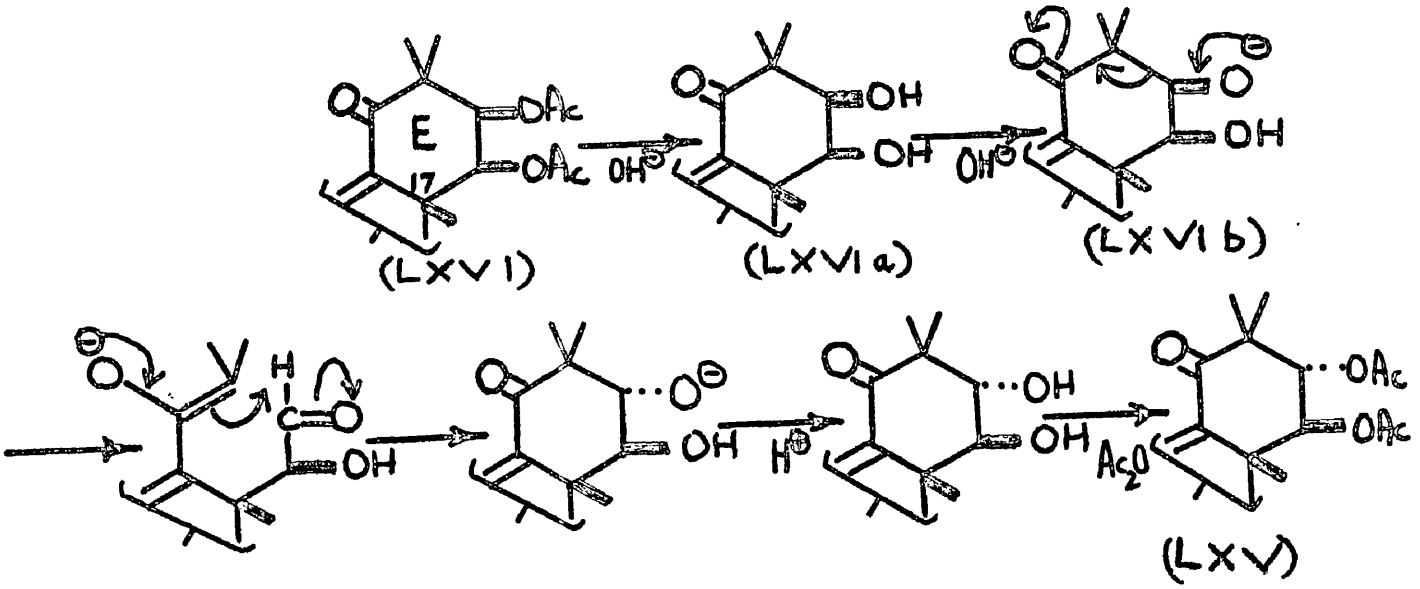
In common with other reported instances from terpenoid chemistry, these three examples of the Reverse Aldol reaction are bimolecular processes, involving the formation of distinct products, which do not recondense. Nevertheless, a consideration of the implications of this reaction led us to the view that it alone could provide a rational explanation for the base-induced dioxodiene epimerisations mentioned above. The epimerisations are viewed as proceeding by an intramolecular Reverse Aldol reaction, followed by subsequent aldol condensation to give the stable diequatorial isomer. Conversion of the dioxodiene, m.p. 265-266° (LXIV) into the higher melting isomer (LXV) is represented by the following reaction sequence, which includes the formation of an anionic intermediate (LXIVa), fission of ring E by reverse aldolisation, equilibration at $C_{(22)}$ via the enolised aldol (LXIVb), and direct aldol condensation to reform ring E:



In this sequence, the rate-determining step is the formation of the anion (LXIVa), without which reverse aldolisation cannot take place. Since the $C_{(21)}$ hydroxyl group is already in the stable equatorial conformation, the anion should be formed readily, and in consequence, the overall yield for the reaction is high (ca. 75%).

Epimerisation of the dioxodiene, m.p. 212-214° (LXVI) is represented similarly, with inversion of configuration confined to the $C_{(21)}$-hydroxyl group.

The rate-determining step in the reaction sequence is again the formation of an anionic intermediate (LXVIb). This time, however, its precursor (LXVIa) contains an axial hydroxyl group at $C_{(21)}$, and in consequence the overall yield for the reaction is only moderate (40%).



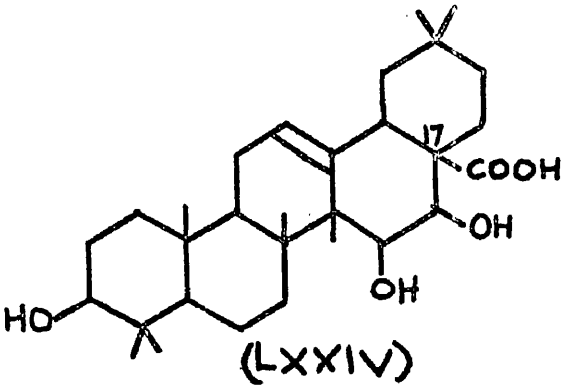
In both these sequences, the reformation of the bond between $C_{(20)}$ and $C_{(21)}$ is due to the effective "locking" of the aldolic fragment by its attachment to the remainder of the molecule at $C_{(17)}$. This locking ensures that the reactive centres are maintained in close proximity throughout the reaction sequence, and permits the necessary direct aldol condensation to take place.

In view of the importance of the conclusions drawn from these epimerisations, it was decided to carry out parallel studies with other pentacyclic triterpenoids. Preliminary results appear to indicate that epimerisations of the above type may have wider application in the rearrangement of suitably "locked" cyclic α:α-disubstituted β-aldols and ketols, of the type:

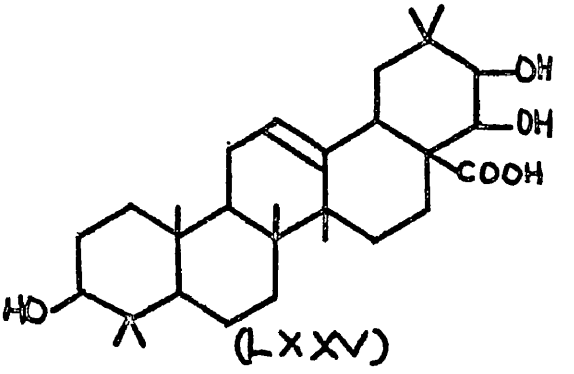
$$R - CO - \overset{|}{\underset{|}{C}} - CH(OH) -$$

where the hydroxyl group is in an unstable configuration. One possible application of the reaction is now discussed.

Entagenic acid is a triterpenoid derived from the giant Sword Bean (Entada phaseoloides). Two possible structures (LXXIV) and (LXXV) have been proposed by Barua.[104] In the author's opinion, it may be possible to distinguish between these two structures in the following manner:

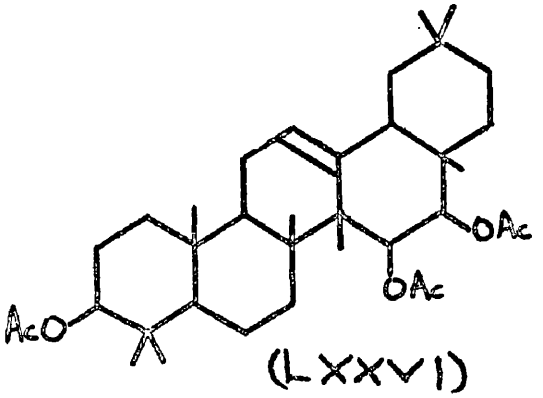


(LXXIV)

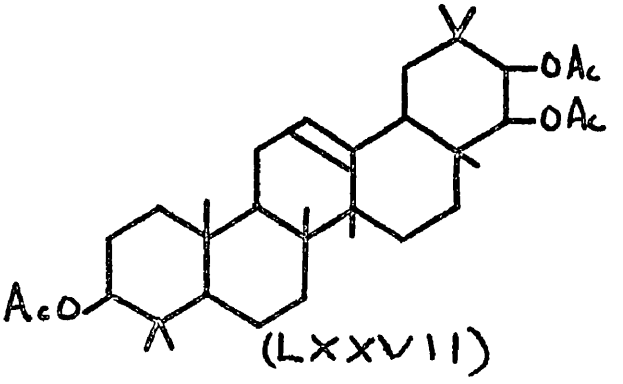


(LXXV)

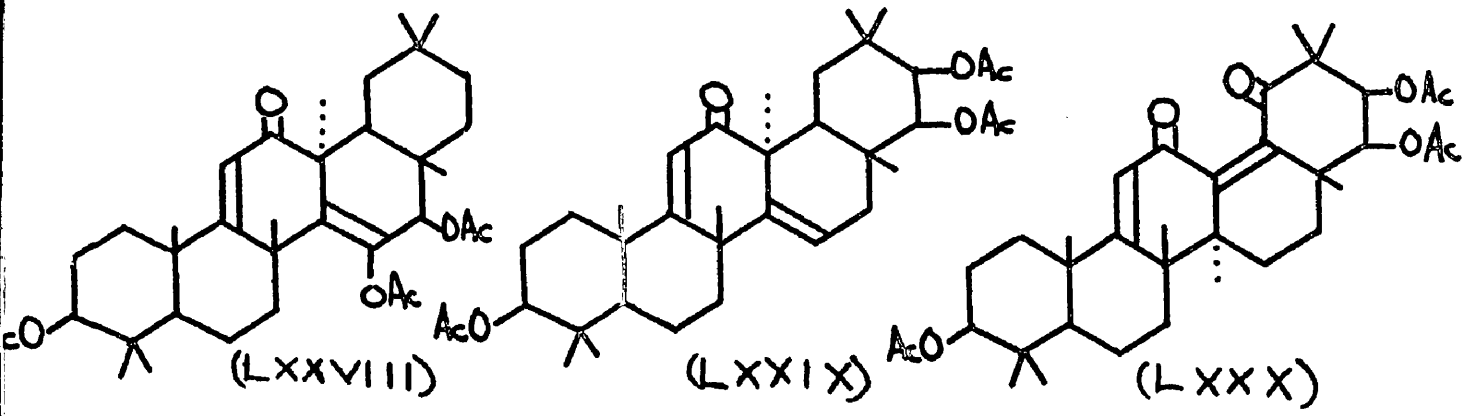
First, the $C_{(17)}$-carboxyl group must be reduced by standard procedures involving Rosenmund reduction of the triacetoxy-acid chloride, followed by Wolff-Kishner reduction of the resultant aldehyde. In this way, either 3β:15:16-triacetoxyolean-12-ene (LXXVI) or 3β:21:22-triacetoxyolean-12-ene (LXXVII) will be formed.



(LXXVI)

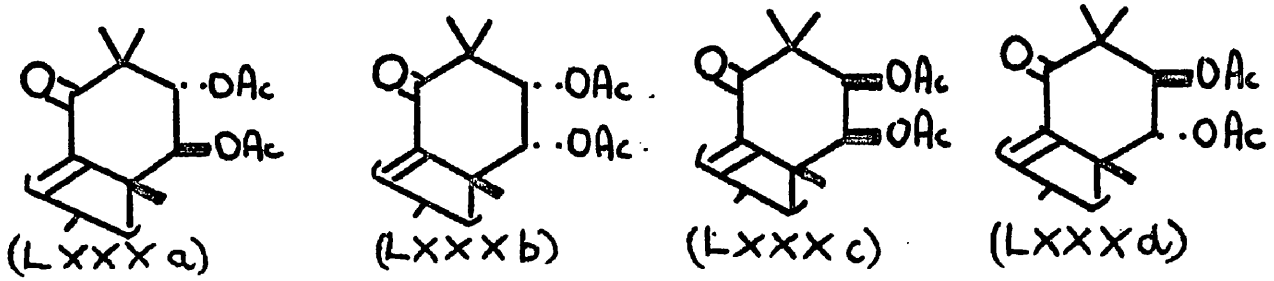


(LXXVII)



(LXXVIII) (LXXIX) (LXXX)

Secondly, by carrying out the oleanane→taraxerane rearrangement outlined in a previous section of this thesis, the triacetate can be converted into its isodienonyl derivative (LXXVIII) or (LXXIX).[33,66] If the product is stable towards alkali, and subsequent acetylation, the glycol grouping cannot be in ring D, and must therefore be at $C_{(21)}$ and $C_{(22)}$, as in (LXXIX). Assuming the latter possibility to be the case, assignment of configuration at $C_{(21)}$ and $C_{(22)}$ can be made as follows. Oxidation of the triacetate (LXXVII) with selenium dioxide in benzyl acetate will yield the dioxodiene (LXXX), which can be subjected to treatment with alkali and reacetylation, under the same conditions as were used for the epimerisation of the sapogenol A dioxodienes.



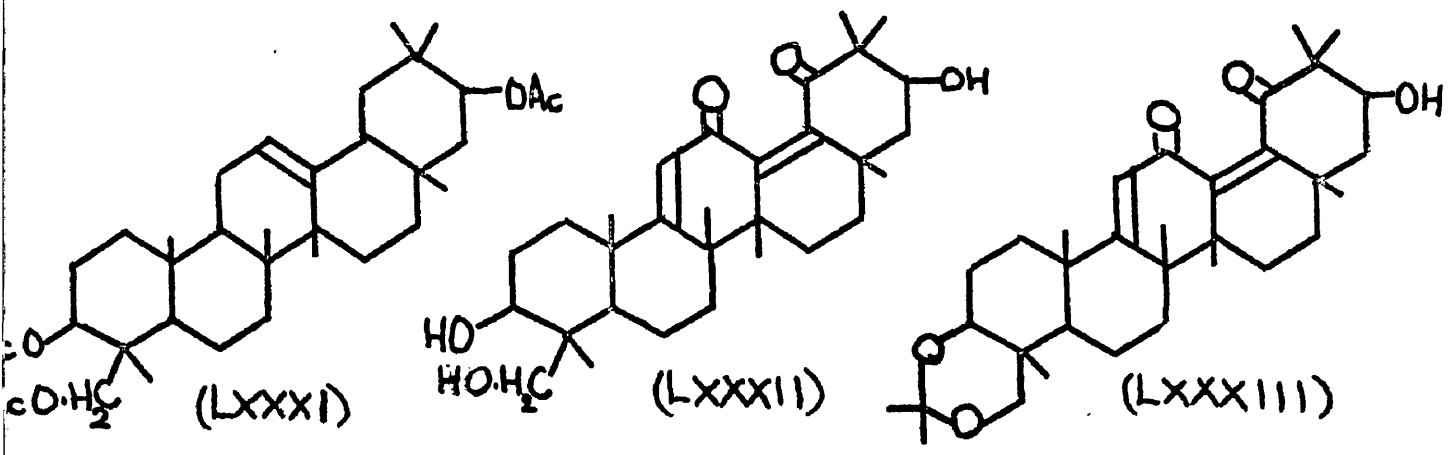
(LXXXa) (LXXXb) (LXXXc) (LXXXd)

Quantitative recovery of unchanged dioxodiene is consistent only with the stable, diequatorial 21α:22β-conformation (LXXXa).

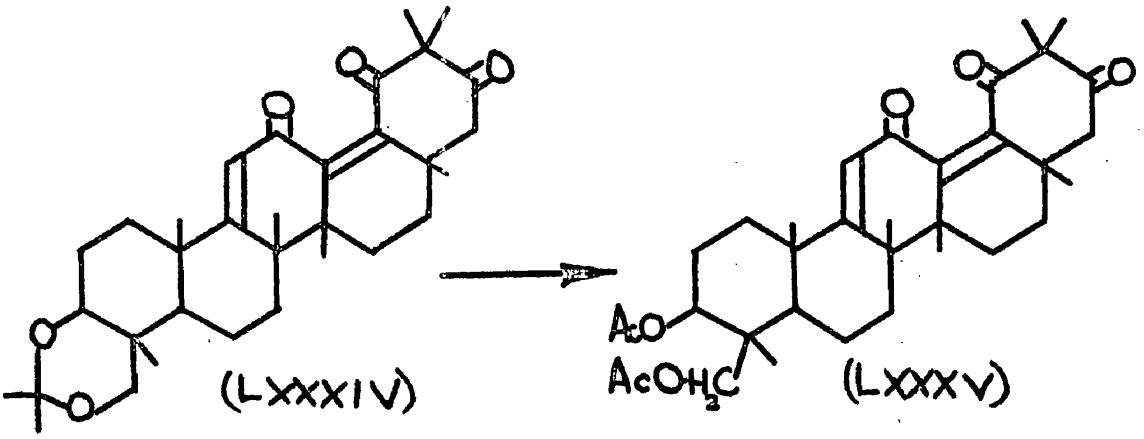
If the epimerisation is successful, the latter possibility can be ruled out, and the configurations of the $C_{(21)}$ and $C_{(22)}$-hydroxyl groups established by an evaluation of the overall yield in the reaction. If the yield is high (>60%), the dioxodiene must have the 21α:22α configuration, as in (LXXXb). If the yield is moderate (30-50%), the dioxodiene has the di-β-configuration (LXXXc). Finally, if the yield is less than 30%, the dioxodiene probably has the 21β:22α-structure (LXXXd), which has not so far been encountered. It is expected, however, that such a structure would be sterically hindered, and slow to epimerise.

VII. The Structure of Sapogenol B.

With the elucidation of the structures of sapogenols A and C, it follows that sapogenol B must be 3β:21 or 22:24--trihydroxyolean-12-ene. The presence of a hydroxyl group at $C_{(21)}$ was first postulated after the preparation of an unstable diacetoxytrioxodiene from sapogenol B triacetate (LXXXI).

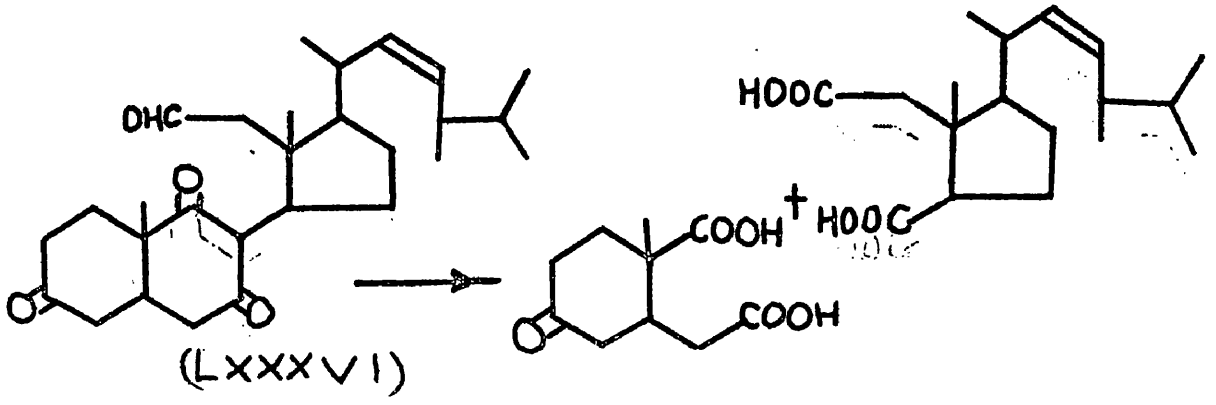


Oxidation of the triacetate with selenium dioxide in benzyl acetate gave the dioxodiene derivative, which was hydrolysed with alkali to yield 3β:21 or 22:24-trihydroxy-12:19-dioxo--oleana-9(11):13(18)-diene (LXXXII). Treatment of the last product with acetone and sulphuric acid furnished the 3β:24-isopropylidene-derivative (LXXXIII) which was oxidised with the chromium trioxide-pyridine complex to the corresponding ketone (LXXXIV). Brief mineral acid treatment of the ketone furnished the 3β:24-diol, which was acetylated to give 3β:24--diacetoxy-12:19:21-trioxo-oleana-9(11):13(18)-diene (LXXXV).

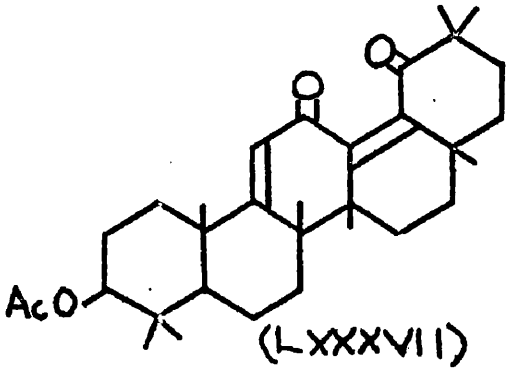


Hydrolysis of the latter was attempted with alkali, but no crystalline product could be isolated, even after acetylation and esterification. However, an unstable acid product, which showed maximal absorption at 2040 and 2740 Å., was extracted from the reaction mixture. This reactivity of the trioxodiene towards alkali is indicative of an α:γ-diketone system in the molecule. It follows therefore that there is a carbonyl group at position 21.

The diacetoxytrioxodiene (LXXXV) was treated with hydrogen peroxide in methanolic potassium hydroxide solution according to the method used for the degradation of the analogous ergosterol derivative (LXXXVI).[108]



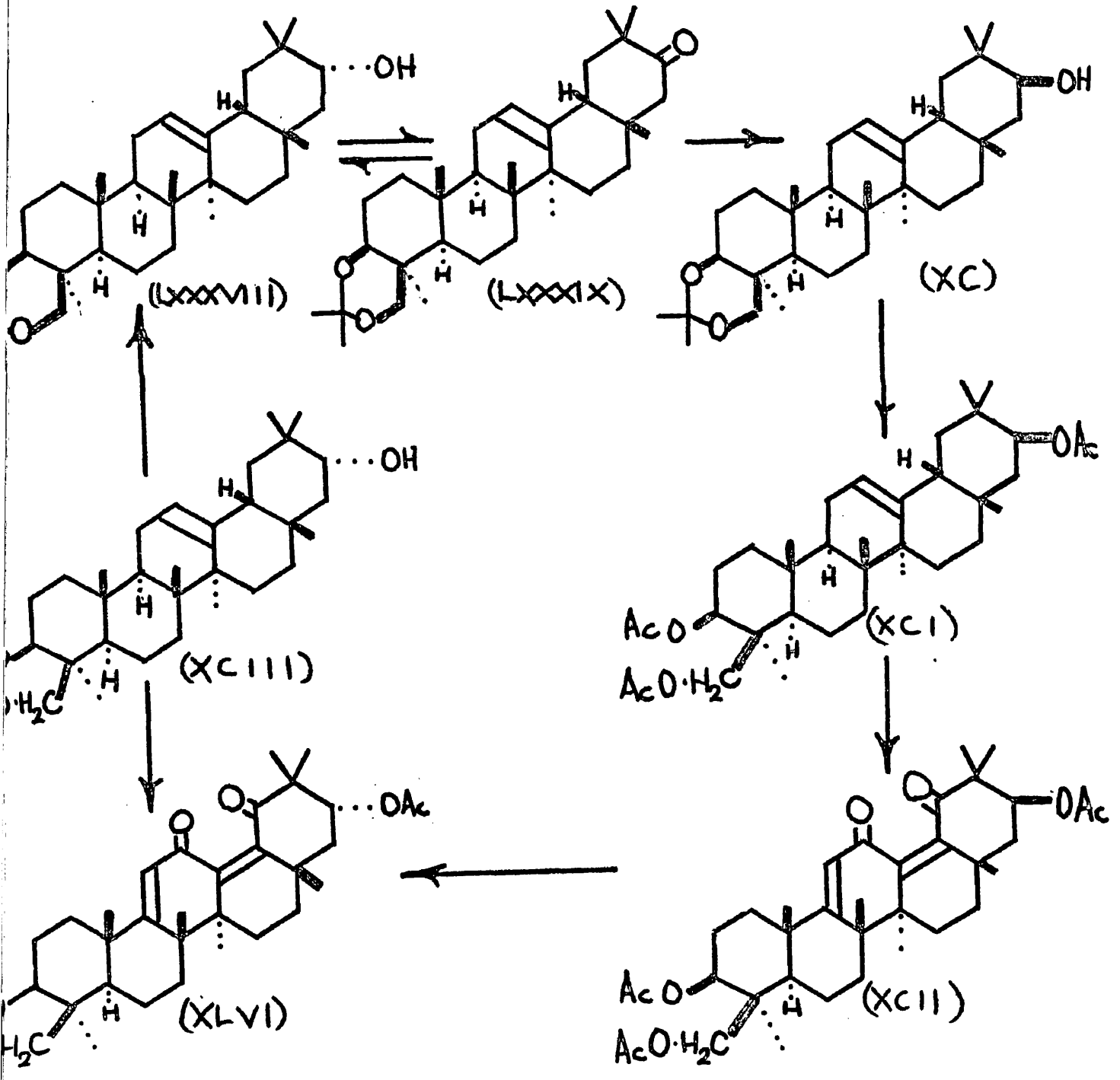
An unstable acid product, which could not be purified, and which rapidly decomposed, was again obtained. A parallel reaction on 3β-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (LXXXVII) recovered only starting material. This is further evidence for the presence of a $C_{(21)}$-carbonyl group in the diacetoxytrioxodiene.



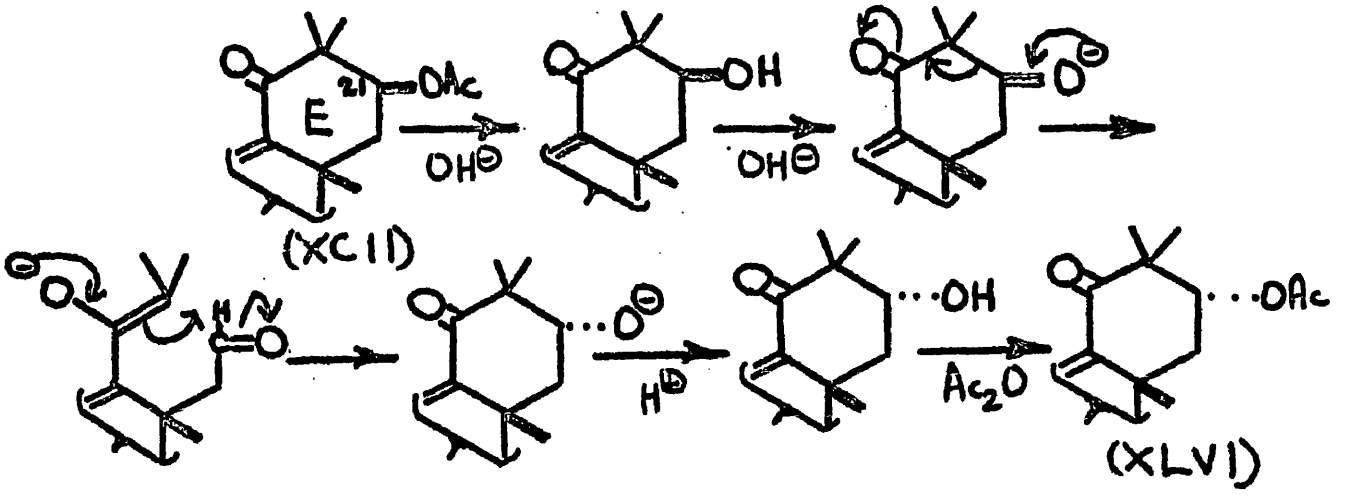
(LXXXVII)

The structure of sapogenol B was rigidly established by the preparation of a second triacetoxydioxodiene. Oxidation of the 3β:24-isopropylidene derivative (LXXXVIII) with the chromium trioxide-pyridine complex gave the 21-ketone (LXXXIX). Lithium aluminium hydride reduction of the ketone furnished a mixture, from which 21α-hydroxy-3β:24-isopropylidenedioxyolean-12-ene (LXXXVIII) and its 21β-hydroxy epimer (XC) were obtained by chromatography. Acid hydrolysis and acetylation of the 21β-epimer gave a stereoisomeric sapogenol B triacetate (XCI, m.p. 214-215°, $[\alpha]_D$ + 65°). Vigorous selenium dioxide oxidation of the latter furnished the dioxodiene derivative, 3β:21β:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (XCII, m.p.

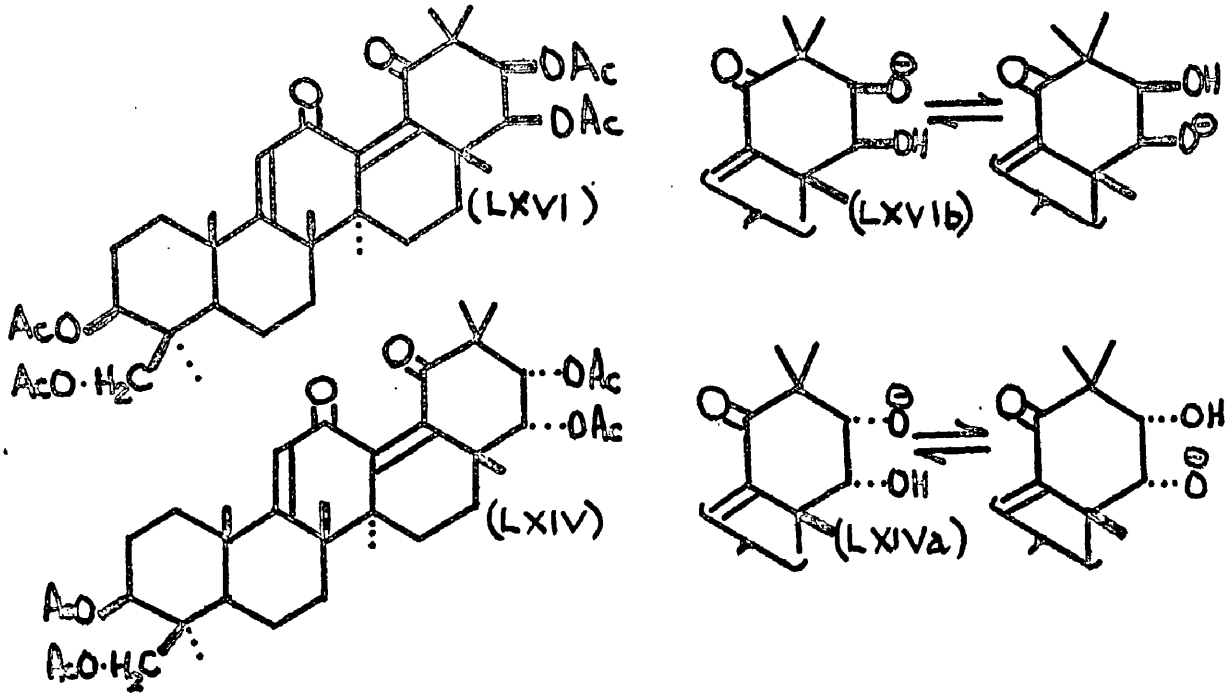
241-242°, $[\alpha]_D$ - 57°, $\lambda_{max.}$ 2800 Å.). A mixture of the isomeric sapogenol B dioxodienes (XCII) and (XLVI) had m.p. 231°, confirming their non-identity.



Treatment of the triacetoxydioxodiene, m.p. 241-242° (XCII), with alkali, followed by reacetylation, gave the higher-melting isomer, (XCIII; m.p. 274-275°, $[\alpha]_D$ - 52°). Hence, the conformation of the hydroxyl group attached to $C_{(21)}$ must have been altered in (XCII) from the axial to the more stable equatorial form during treatment of this compound with alkali. The process is again considered to proceed via a reverse aldol-direct aldol condensation sequence, which involves the opening and closing of ring E, and the formation of the stable equatorial epimer, as shown below:



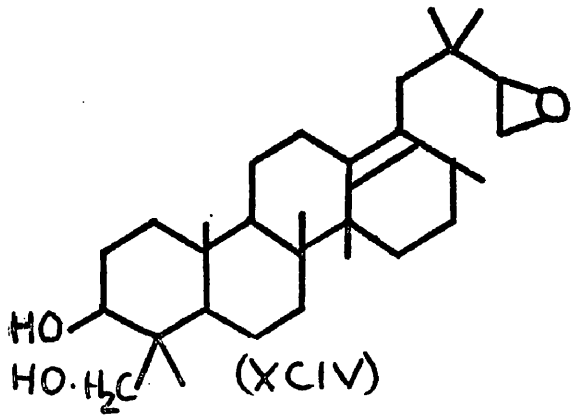
Sapogenol B must therefore have the structure 3β:21α:24--trihydroxyolean-12-ene (XCIII). Again, the rate-determining step in the above reaction sequence is the formation of the anionic intermediate (XCIIa) from the 21β-epimer. In the epimerisation, the overall yield was found to be high (ca. 70%), as opposed to the moderate (40%) yield for the epimerisation of the corresponding 21β:22β-dioxodiene-derivative of sapogenol A (LXVI):



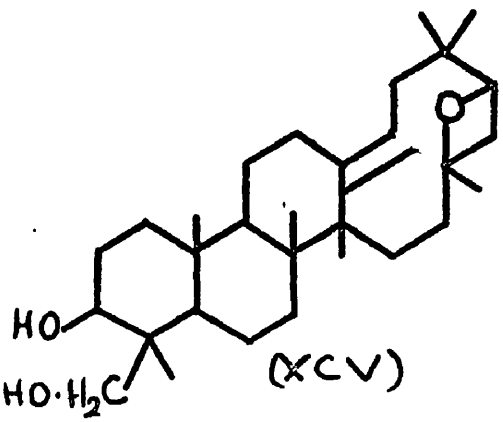
It is possible to explain this anomaly, if a secondary effect operates during the epimerisation of the sapogenol A dioxodienes (LXIV) and (LXVI), which hinders formation of the required anionic intermediates (LXIVa) and (LXVIb), but is not present in the epimerisation of the sapogenol B dioxodiene (XCII). This effect may well be of the type shown above, and is bound to retard the effective opening of ring E by reverse aldolisation in the sapogenol A series. Since, however, sapogenol B does not contain a $C_{(22)}$-hydroxyl group, no hindering effect is observed in the epimerisation of (XCII).

VIII. The Structure of Sapogenol D.

It was concluded by Meyer, Jeger and Ruzicka that sapogenol D is a tetracarbocyclic oxide, isomeric with sapogenol B, containing a 13(18)-double bond and a cyclic ether system.[90,91] From the work described in the earlier sections of this thesis it is evident that one end of the oxide ring must be attached to $C_{(21)}$, with the other end at some suitably adjacent carbon atom to facilitate acid rearrangement to pentacyclic oleanene derivatives. A number of reactions were carried out on the diacetate. These reactions served to demonstrate the stability of the oxide ring to reduction. Thus, prolonged hydrogenolysis, and reduction with zinc in ethanol or with zinc in acetic acid has no effect, while treatment of the diacetate with lithium aluminium hydride affords the diol. Examination of the infrared spectra of sapogenol D and its derivatives reveals a well-defined absorption band at 1100 $cm.^{-1}$, indicative of a five or higher-membered oxide ring.[106] Structures such as (XCIV) and (XCV), which contain a 3- or a 4-membered oxide ring are therefore improbable.

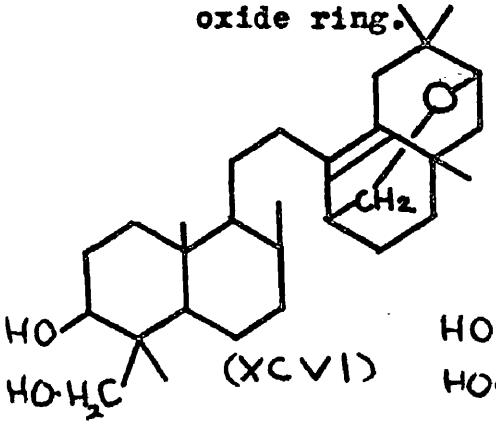


(XCIV)

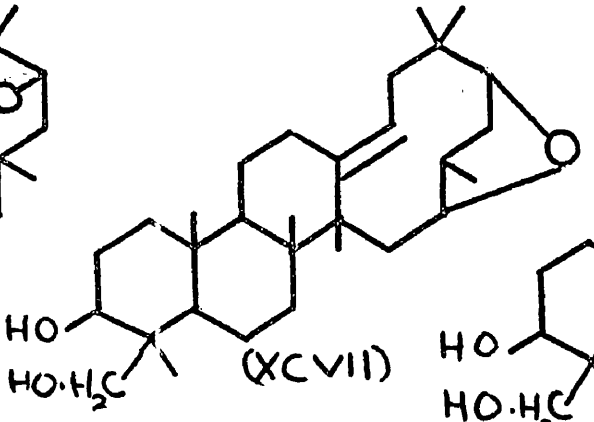


(XCV)

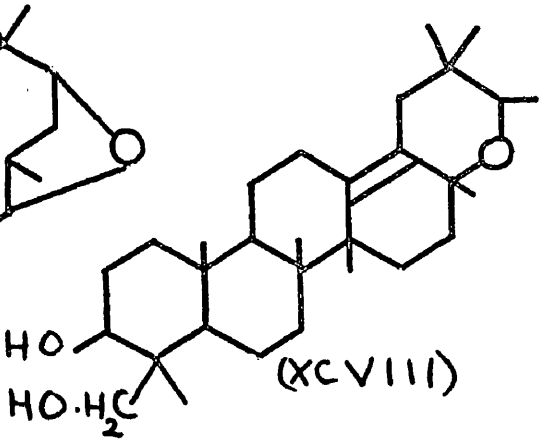
Other possible structures are limited by the observation that a 12:19-dioxo-9(11):13(18)-diene, which still contains an oxide ring, is formed on vigorous selenium dioxide oxidation of the diacetate.[24] Formulae such as (XCVI) and (XCVII) cannot therefore be considered, since it is impossible sterically for these to form a dioxodiene derivative without fission of the oxide ring.



(XCVI)



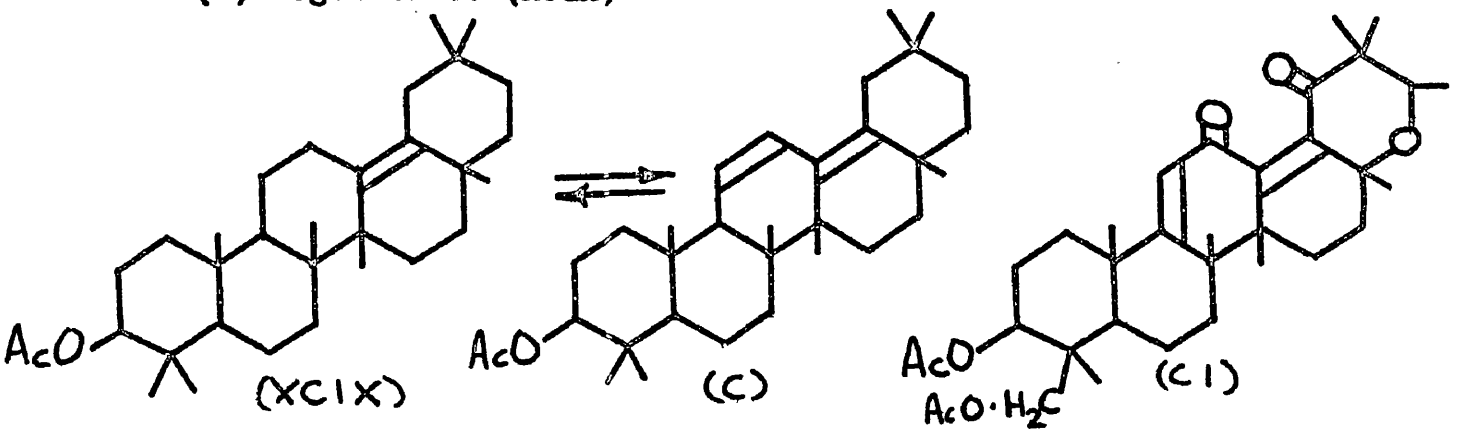
(XCVII)



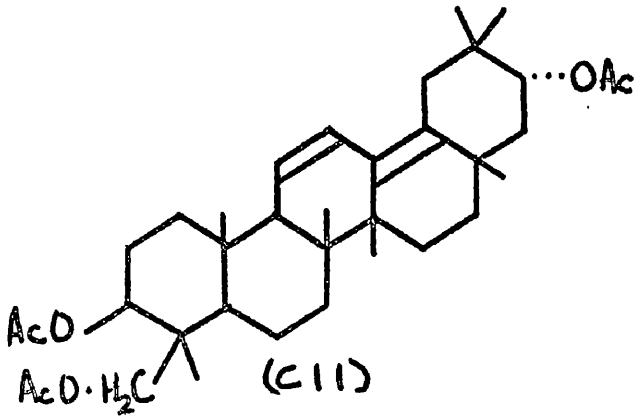
(XCVIII)

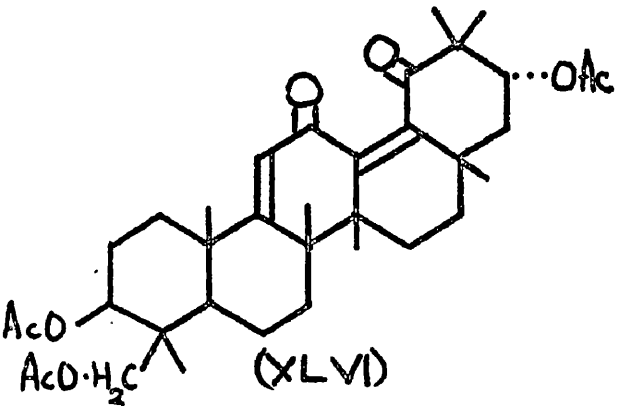
At this stage it was decided to adopt the provisional structure (XCVIII) for sapogenol D, and to test it by rigid chemical means. Attention was first turned to the location of the double bond, previously assigned to position 13(18) by Meyer et al., on the basis of ultraviolet and infrared absorption characteristics, and by the ready rearrangement of soyasapogenol D to olean-13(18)-ene derivatives. A series of reactions first performed on δ-amyrin acetate (3β-acetoxy-olean-13(18)-ene, XCIX) were therefore carried out. These reactions involve mild selenium dioxide oxidation to the heteroannular-11:13(18)-diene (C). Subsequent hydrogenation of

(C) regenerates (XCIX).[68,69]

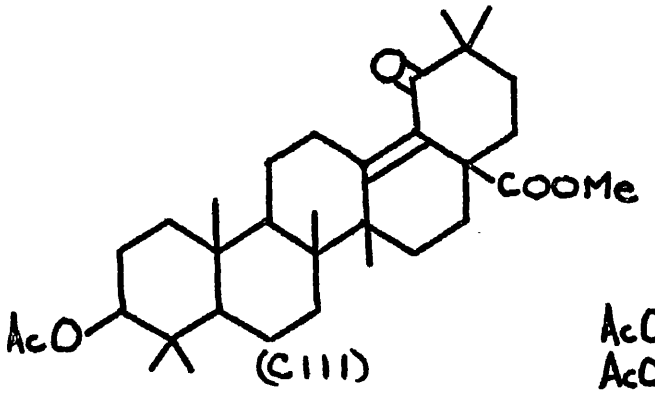


Sapogenol D diacetate was oxidised with selenium dioxide in benzyl acetate solution. Unexpectedly, the sole product obtained was the 12:19-dioxo-9(11):13(18)-diene (CI). Milder oxidation with selenium dioxide in acetic acid gave a mixture of the required 11:13(18)-diene, dioxodiene, and starting material; and this mixture could not be purified by chromatography or by crystallisation. Furthermore, prolonged oxidation with selenium dioxide in aqueous acetic acid yielded a mixture of 3β:21α:24-triacetoxyoleana-11:13(18)-diene (CII) and 3β:21α:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (XLVI). Fission of the oxide ring has thus taken place during the oxidation.

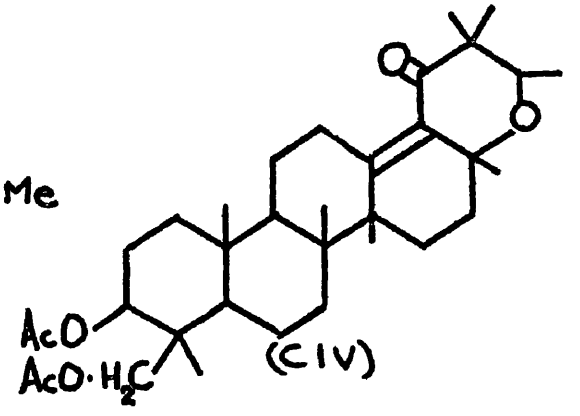




The location of the double bond between $C_{(13)}$ and $C_{(18)}$ in sapogenol D was finally established by means of two further oxidation reactions. In the first, mild chromic oxidation of sapogenol D diacetate gave an αβ-unsaturated ketone, $C_{34}H_{52}O_6$, $\lambda_{max.}$ 2540 Å. (ε = 7000). This compound, which shows the same ultraviolet absorption characteristics as methyl-19-oxo-olean--13(18)-enolate acetate (CIII), is formulated as the 19-oxo--13(18)-ene (CIV).[107]



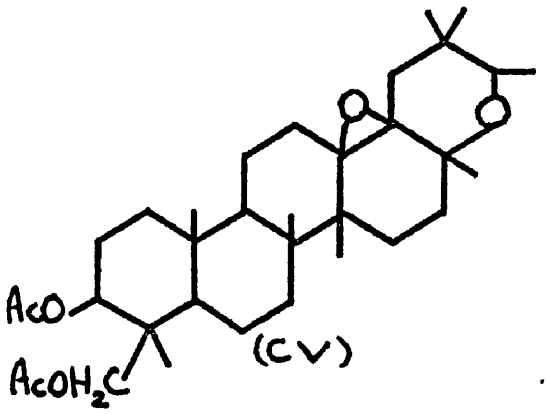
(CIII)



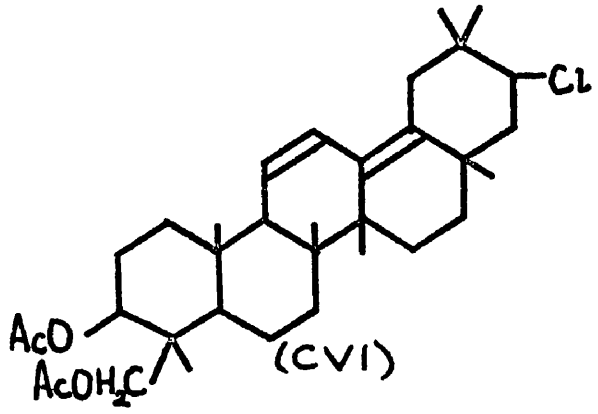
(CIV)

Performic acid oxidation of sapogenol D diacetate affords a second product, $C_{34}H_{54}O_6$, which gives no colour with tetranitromethane, and does not contain an isolated carbonyl or hydroxyl group. By analogy with the similar oxidation of δ-amyrin acetate (XCIX),[108] the product is formulated as the 13(18)-epoxide (CV). In support of this view, there is good agreement between the molecular rotation differences accompanying the formation of the 13(18)-epoxides from δ-amyrin acetate (+ 200) and sapogenol D diacetate (+ 237).[63] Moreover, on treatment of sapogenol Ddiacetate epoxide with mineral acid,

both oxide rings are opened, and a chlorine-containing 11:13(18)-diene is obtained. This product is considered to have the structure (CVI).

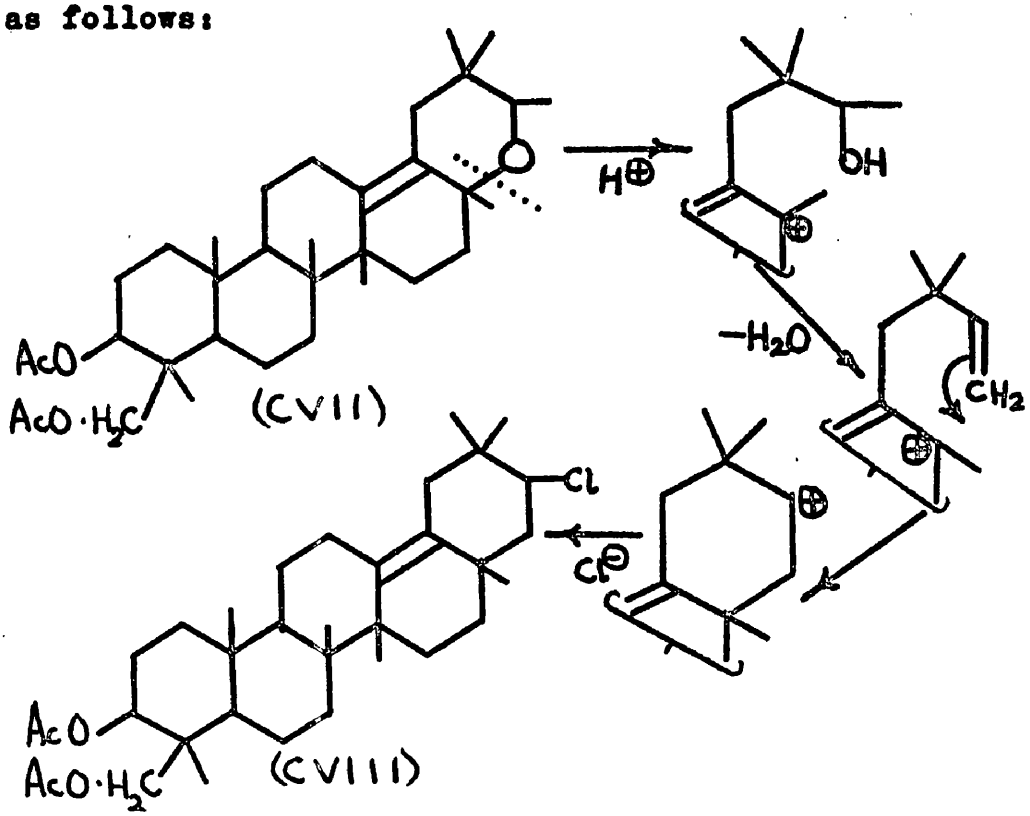


(CV)



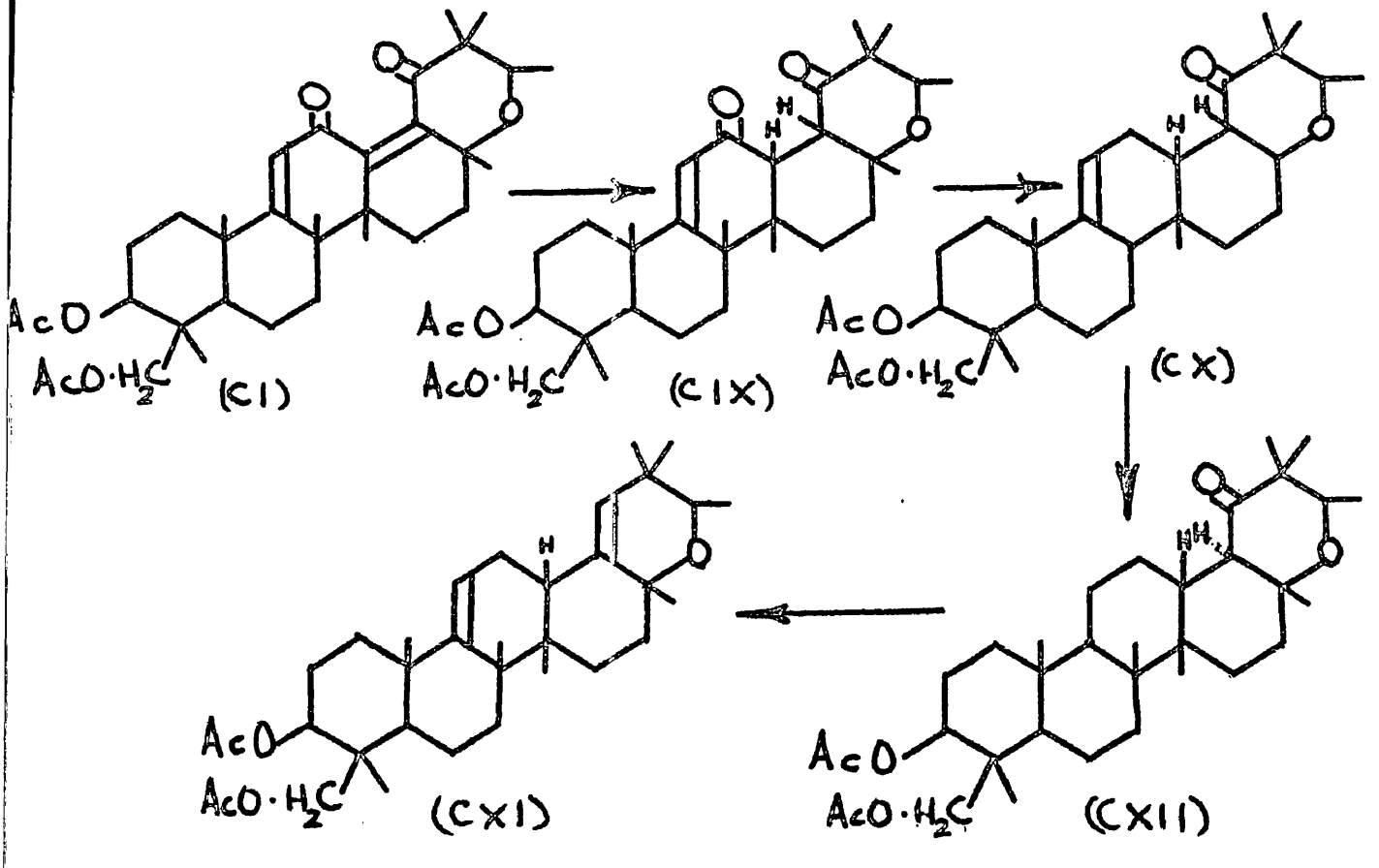
(CVI)

It has now been established that the double bond in sapogenol D is situated between $C_{(13)}$ and $C_{(18)}$. A Study of the effect of mineral acid on the oxide ring was undertaken. If sapogenol D diacetate is assumed to have the structure (CVII), the formation of 21-chloro-3β:24-diacetoxyolean-13(18)-ene (CVIII) may be formulated as follows:



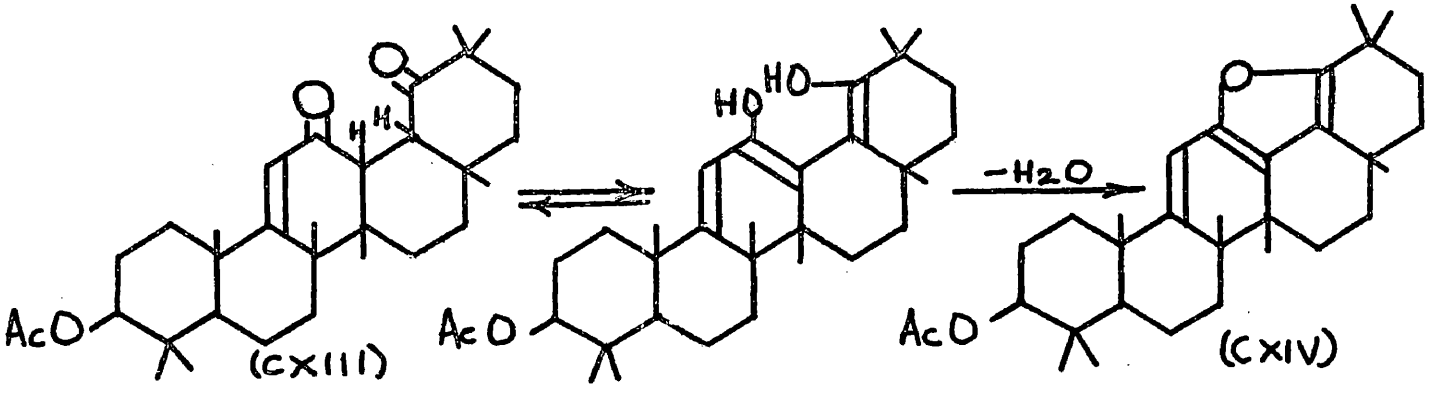
Since the cisoid αβ-unsaturated ketone (CIV) is recovered unchanged after treatment with mineral acid, it is concluded that introduction of a 19-carbonyl function deactivates the molecule to acid rearrangement, by minimising the electron-donating effect of the 13(18)-double bond towards the oxide ring.

The stability of the dioxodiene and its derivatives towards acid was also examined. Vigorous selenium dioxide oxidation of sapogenol D diacetate gave the expected 12:19-dioxo-9(11):13(18)--diene ($C_{34}H_{50}O_7$, $\lambda_{max.}$ 2800 Å., CI) which was reduced with zinc in ethanol to the 13β:18β-dihydro-derivative (CIX).[72] Hydrogenolysis of the latter gave the 19-oxo-9(11)-ene (CX), and on subsequent acid equilibration, the 18α-isomer (CXI) was obtained. Lithium aluminium hydride reduction of (CXI), followed by partial acetylation afforded the non-conjugated diene, $C_{34}H_{54}O_5$, (CXII), which still contains the oxide ring. Mild acid treatment of the diene (CXII), in common with the other derivatives, has no effect on the oxide ring. However, when stronger acid conditions were tried, in an attempt to conjugate the isolated double bonds at positions 9(11) and 18, the oxide ring was broken, and a chlorodiene again obtained. It would thus appear that an essential requirement for the opening of the oxide ring in the sapogenol molecule is the presence of a tetrasubstituted double bond allylic to the oxide ring. This is in agreement with the known reactivity of allylic ethers towards mineral acid.

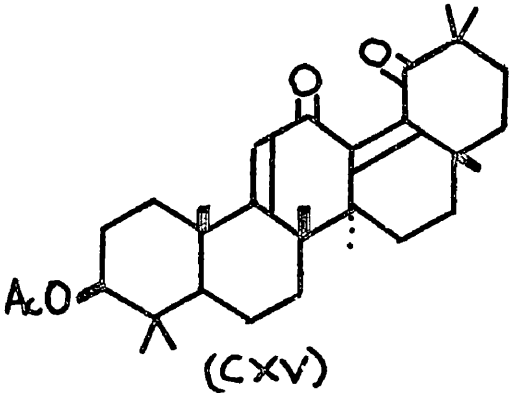


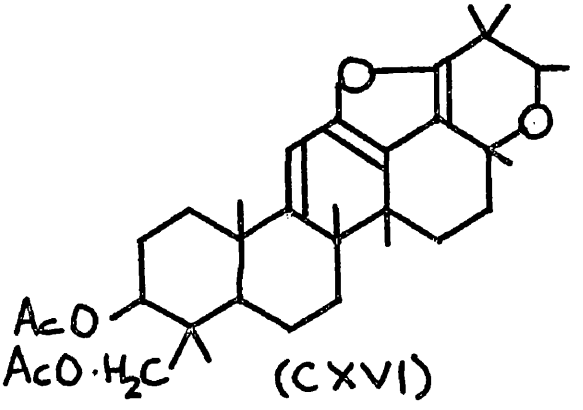
As previously mentioned, mild acid treatment of the dioxodiene and its derivatives had no effect upon the oxide ring. However, brief treatment of the 13β:18β-dihydro-derivative (CIX) with hydrochloric and acetic acids, gave a product which still contained the oxide ring, but decomposed on crystallisation, and could not be purified. Initially, the product showed maximal absorption at 2200 and 3200 Å. (ϵ = 5000 and 12,400). This, however, was found to change on treatment with acetic anhydride and pyridine, the product showing absorption bands at 2030, 2280 and 2760 Å. (ϵ = 9000, 6000 and 10,600). Investigation of the nature of this acid rearrangement was carried out with the analogous dihydro-derivative of dioxo-β-amyradienyl acetate

(CXIII), similar treatment of which gave, in good yield, 3β-acetoxy-12:19-epoxyoleana-9(11)-12:18-triene (CXIV; $\lambda_{max.}$ 2190, 3220 Å., ε = 5,500 and 13,400).

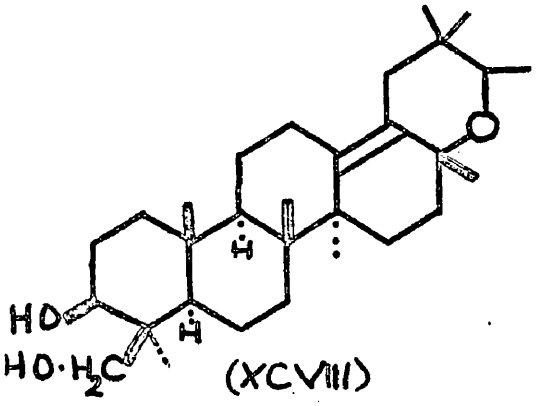


The last compound (CXIV) was originally prepared in low yield from the dihydrodioxodiene (CXIII) by Dr. L. C. McKean of this Laboratory, by the action of isopropenyl acetate and sulphuric acid.[109] Furthermore, it was noted that when traces of mineral acid are present during crystallisation of the triene-ether (CXIV), decomposition takes place to give the dioxodiene (CXV), thus accounting for the instability of the sapogenol D analogue, which is formulated as (CXVI).

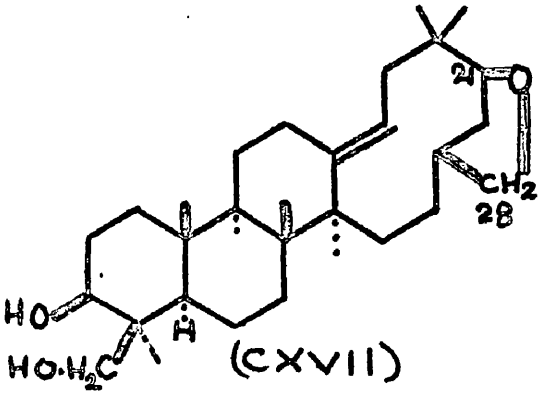




To accommodate the known facts, two main structures have been considered for sapogenol D. The first (XCVIII) has been used throughout this section, and is preferred by the author. A second structure (CXVII) is however also possible. In this structure, the bond between $C_{(17)}$ and $C_{(18)}$ is absent, and a β-ether link is attached from $C_{(21)}$ to $C_{(28)}$. A summary of the evidence in favour of structure (XCVIII) will now be given.



(XCVIII)



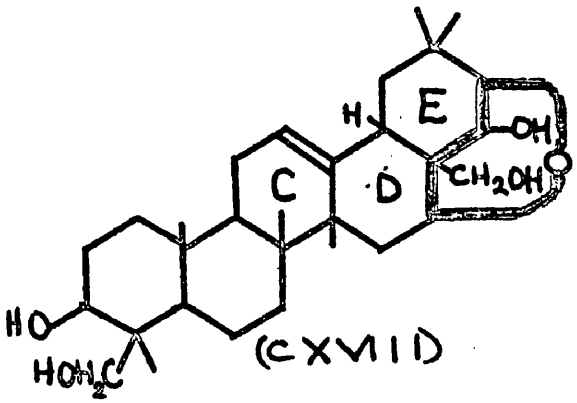
(CXVII)

1. Spectroscopic evidence: The high intensity ultraviolet end absorption of sapogenol D ($\lambda_{max.}$ 2080 Å.; ε = 10,000) is indicative of a typical tetrasubstituted 13(18)-double bond.[110] Although little information is available concerning the infrared absorption of cyclic ethers, it is known that 1:2-epoxides exhibit an ether-stretching band in the region of 920 cm.$^{-1}$, and that as the ring size grows, a corresponding increase can be discerned in the ether-stretching frequency.[108] The effect is illustrated by the data in the accompanying Table.

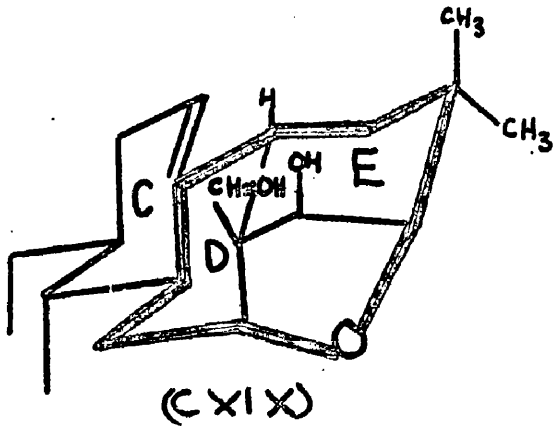
Table IV: Infrared Absorption of Cyclic Ethers.

Compound	No. of Atoms in Ring	Ether-stretching Band
Tetrahydrofuran	5	1060-1090 cm.$^{-1}$
Pentamethylene oxide	6	1090-1100 cm.$^{-1}$
Hexamethylene oxide	7	1105-1110 cm.$^{-1}$
Aliphatic ethers	∞	1080-1150 cm.$^{-1}$

In accordance with the 6-membered oxide ring required by structure (XCVIII), sapogenol D and its derivatives show a strong absorption band in the region 1090-1100 cm.$^{-1}$ It is of interest, however, to note the anomaly of aescigenin (CXVIII) which contains a five-membered oxide ring, but does not absorb between 1060 and 1090 cm.$^{-1}$ Instead a well-defined absorption band is exhibited at 1112 cm.$^{-1}$



(CXVIII)



(CXIX)

Accordingly, it appears that aescigenin behaves spectroscopically as a nine-membered cyclic ether (CXIX). Structures (CXVII) and (XCVIII) for sapogenol D are true five and six-membered cyclic oxides, and cannot therefore be expected to show this anomalous effect.

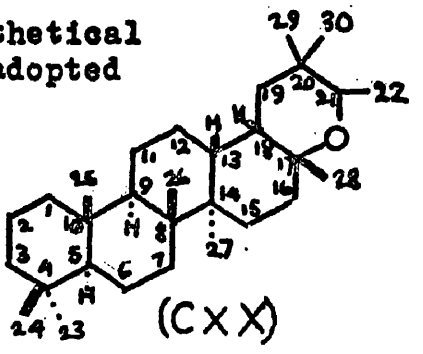
2. The Similarity of Sapogenol D and δ-Amyrin: This is shown by comparison of the molecular rotation differences, listed in Table V.*

Table V.

Compound	M_D	ΔM_D	ΔM_D	M_D	Compound
3β-Acetoxyolean-13(18)-ene	-154°			-239°	3β:24-Diacetoxy-oleanoxer-13(18)-ene
3β-Acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene	-445	-291	-204	-443	3β:24-Diacetoxy-12:19-dioxo-oleanoxera-9(11):13(18)-diene
3β-Acetoxy-12:19-dioxo-olean-9(11)-ene	+670	+1115	+1298	+855	3β:24-Diacetoxy-12:19-dioxo-oleanoxer-9(11)-ene
3β-Acetoxy-19-oxo-olean-9(11)-ene	+564	-106	-249	+606	3β:24-Diacetoxy-19-oxo-oleanoxer-9(11)-ene
3β-Acetoxy-19-oxo-18α-olean-9(11)-ene	+670	+106	- 86	+520	3β:24-Diacetoxy-19-oxo-18α-oleanoxer-9(11)-ene
3β-Acetoxy-19β-hydroxy-18α-olean-9(11)-ene	+542	-128	- 74	+446	3β:24-Diacetoxy-19β-hydroxy-18α-oleanoxer-9(11)-ene.
3β-Acetoxy-oleana-9(11):18-diene	+471	- 71	- 73	+373	3β:24-Diacetoxy-oleanoxera-9(11):18-diene

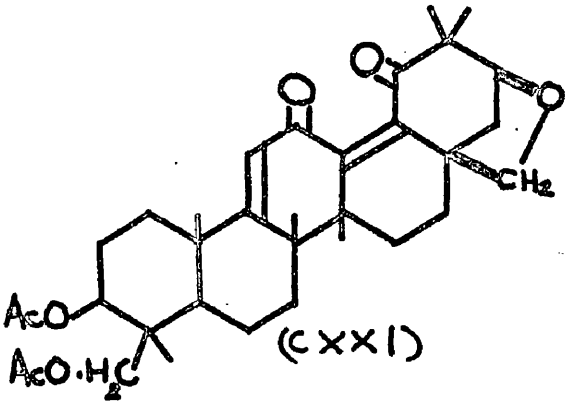
This evidence tends to favour the acceptance of formula (XCVIII) for sapogenol D. The second structure (CXVII) can only be correct if closure of the bond between $C_{(17)}$ and $C_{(18)}$ has taken

*For the purposes of this thesis, the hypothetical triterpenoid oleanoxerane (CXX) has been adopted as a basis for the naming of sapogenol D derivatives. Thus, the diacetate is 3β:24-diacetoxyoleanoxer-13(18)-ene.



(CXX)

place during oxidation to the dioxodiene (CXXI).



(CXXI)

That the dioxodiene has in fact a bond between $C_{(17)}$ and $C_{(18)}$ is evidenced by the great similarity between its infrared absorption spectrum and that of 3β-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene. (Both these dioxodienes exhibit characteristic bands at 1690, 1660, 1613 and 1597 $cm.^{-1}$).

Although analyses of the dioxodiene and its derivatives do not exclude a pentacarbocyclic structure such as (CXXI), the tetracarbocyclic structure is preferred throughout, as shown in Table VI.

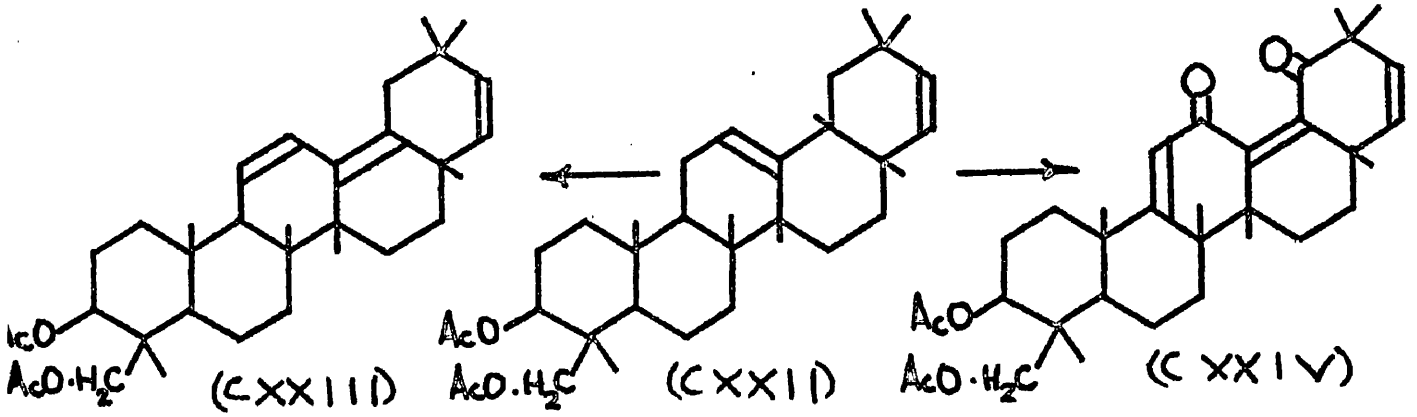
Table VI: Analytical Data for Sapogenol D Dioxodiene Diacetate and Derivatives.

	Found		Tetracyclic Structure		Pentacyclic Structure.	
Compound	% C	% H	% C	% H	% C	% H
Dioxodiene	71.7	8.7	71.8	8.5	72.05	8.2
Dihydro-derivative	71.6	8.8	71.55	8.8	71.8	8.5
19-Oxo-9(11)-ene	73.45	9.35	73.3	9.4	73.6	9.1
19-Oxo-18α-9(11)-ene	73.4	9.3	73.3	9.4	73.6	9.1
19β-Hydroxy-10α-9(11)-ene	72.9	9.5	73.1	9.7	73.3	9.4
9(11):18-diene	75.6	9.9	75.5	9.7	75.8	9.4

Until such time as evidence to the contrary is forthcoming, the first structure (XCVIII) is preferred for sapogenol D. The author considers that this sapogenol is derived in the plant from sapogenol B. In agreement with the biosynthetic concepts outlined in an earlier section, the formation of the soyasapogenols is viewed as proceeding by enzymatic hydroxylation of β-amyrin to give A and B. Subsequent dehydration of B affords C, while reaction of the 21-hydroxyl group with either $C_{(17)}$ or $C_{(22)}$, and subsequent bond fission at either 17:22 or 17:18, gives sapogenol D.

IX. The Vigorous Oxidation of Sapogenol C Diacetate

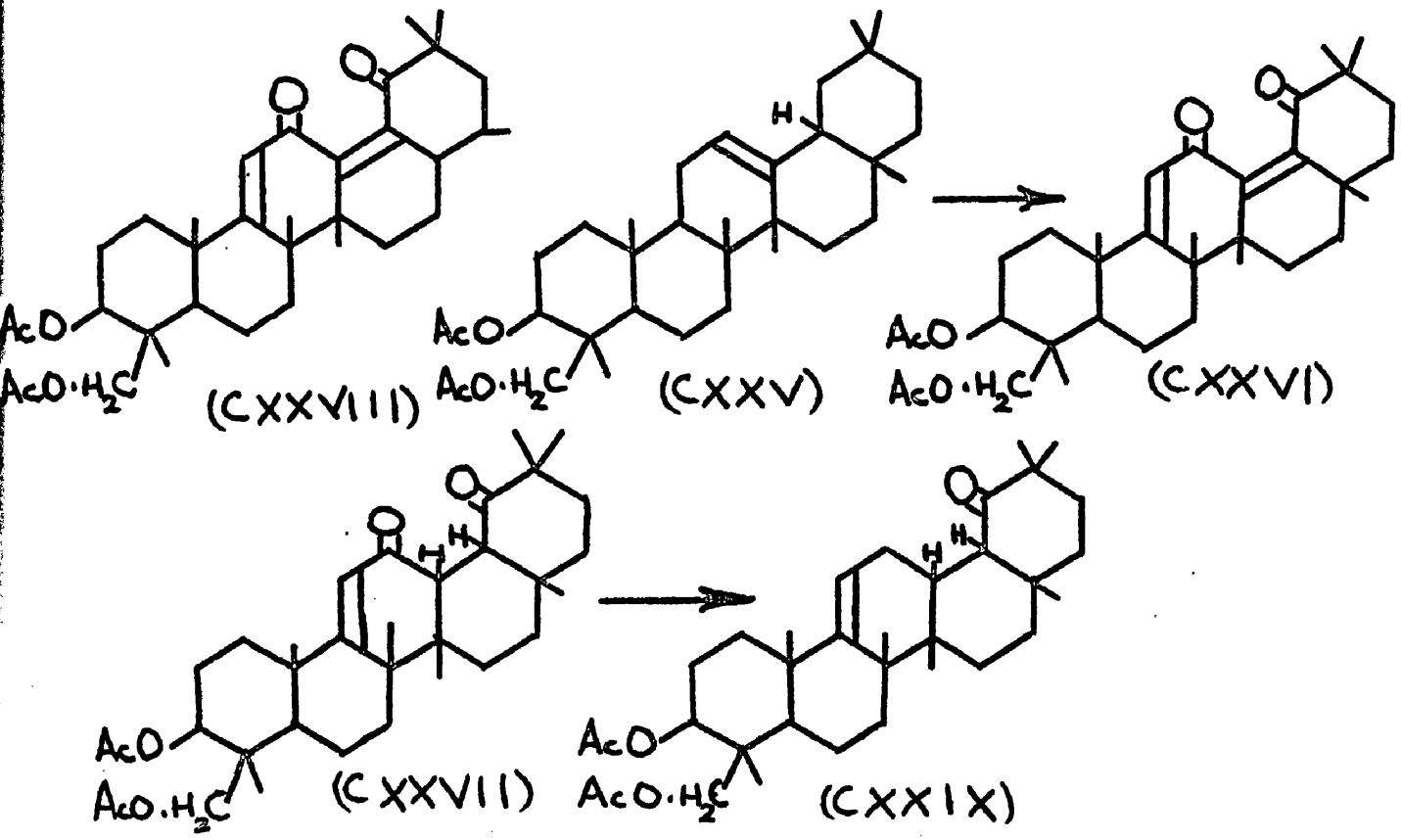
Although Meyer, Jeger and Ruzicka describe the oxidation of sapogenol C diacetate (CXXII) with selenium dioxide in acetic acid to 3β:24-diacetoxyoleana-11:13(18):x(y)-triene (CXXIII),[92] they did not apparently attempt the oxidation under more drastic conditions. It was therefore decided to prepare the dioxodiene derivative (CXXIV), and make a study of its properties.



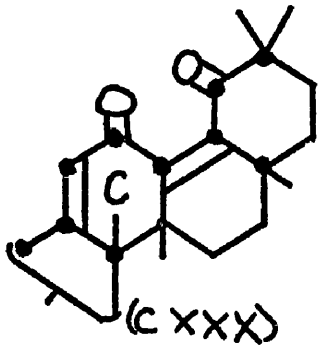
Accordingly, sapogenol C diacetate was refluxed for 18 hr. with selenium dioxide in benzyl acetate. The 12:19-dioxo-9(11):13(18)-diene obtained, m.p. 217-218°, $[\alpha]_D$ - 78.5°, gave on reduction with zinc in ethanol the 13β:18β-dihydro-derivative, m.p.266-268°, $[\alpha]_D$ + 106°. Surprisingly, both compounds failed to give the expected yellow colour with tetranitromethane in chloroform, attributable to the cis-disubstituted double bond[111] between C_{21} and C_{22}. Furthermore, ethylenic absorption was absent from their ultraviolet spectra.

One explanation of these anomalies is that saturation of the 21:22-double bond has taken place during the formation of the dioxodiene from sapogenol C diacetate. This possibility was

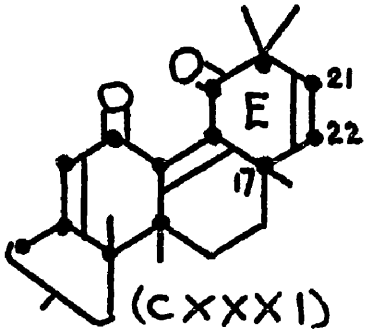
eliminated by means of a parallel series of reactions carried out on dihydrosapogenol C diacetate (CXXV), which was oxidised with selenium dioxide in benzyl acetate to give 3β:24-diacetoxy-12:19--dioxo-oleana-9(11):13(18)-diene (CXXVI, m.p. 247-248°, $[\alpha]_D$ - 77°). Reduction of (CXXVI) with zinc in ethanol gave 3β:24-diacetoxy--12:19-dioxo-olean-9(11)-ene (CXXVII), m.p. 249-251°, $[\alpha]_D$ + 120°). A second possibility, that methyl group migration had occurred during saturation of the 21:22-double bond in the formation of the dioxodiene, m.p. 217-218°, as in (CXXVIII), was eliminated when hydrogenolysis of the dihydro-derivatives, m.p. 266-268°, and m.p. 249-251°, gave the same product, 3β:24-diacetoxy-19-oxo-olean--9(11)-ene (CXXIX).



On examination of the dioxodiene structure (CXXVI), it can be seen that the carbon atoms in ring C of the molecule must be coplanar with $C_{(16)}$, $C_{(17)}$, $C_{(18)}$, $C_{(19)}$, and $C_{(20)}$, as in (CXXX). Furthermore, in structure (CXXIV), all six carbon atoms in ring E must be coplanar with the carbon atoms in ring C, as shown in (CXXXI).



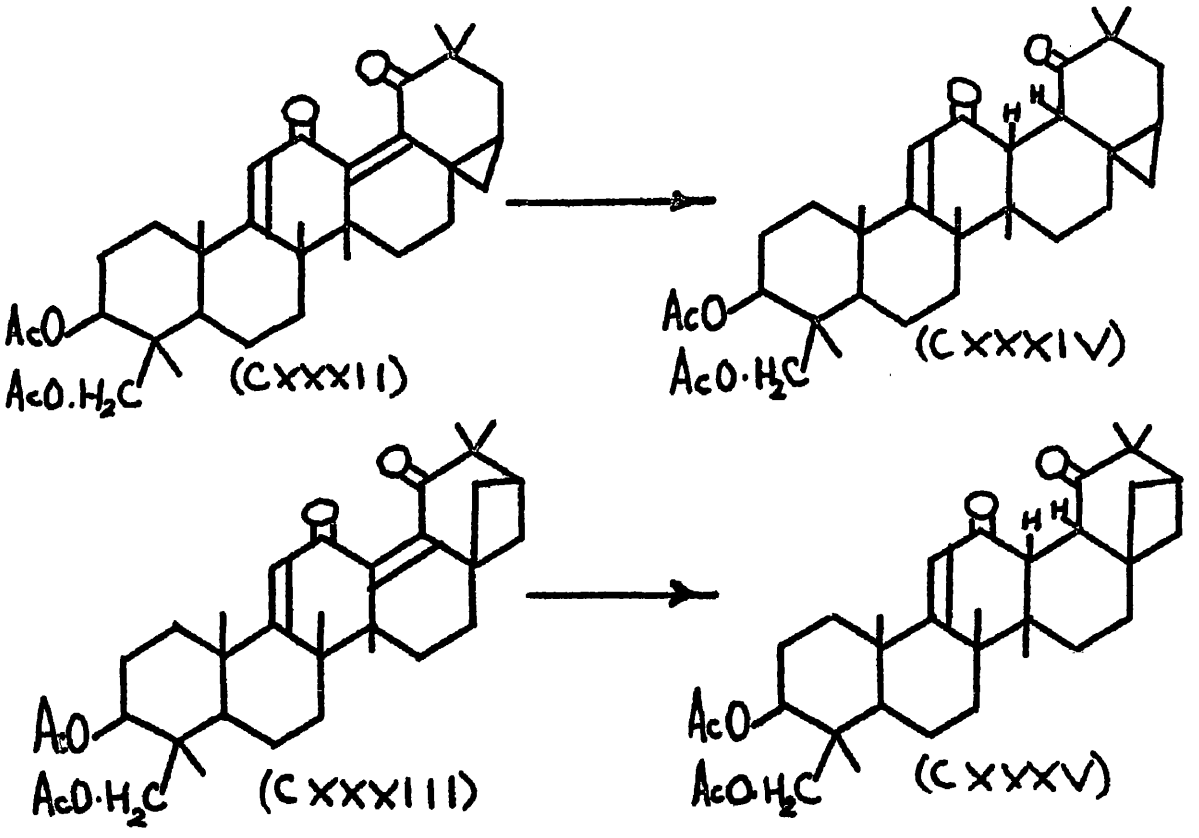
(CXXX)



(CXXXI)

Since four substituent groups are attached to ring E in (CXXXI), the author considers an extended planar structure of this type to be unlikely. Instead, it is postulated that during the oxidation of sapogenol C diacetate an additional cycloalkane ring system has been formed by attachment of the $C_{(17)}$-methyl group to either $C_{(21)}$ or $C_{(22)}$. The dioxodiene, m.p. 217-218° is accordingly formulated as (CXXXII) or (CXXXIII), and the dihydro-derivative, m.p. 266-268°, as (CXXXIV) or (CXXXV).

In support of this argument, it was observed that maximal ultraviolet light absorption of the dioxodiene, m.p. 217-218°, occurs at 2740 Å., whereas typical 12:19-dioxo-9(11):13(18)-dienes



absorb in the 2780-2800 Å. region. The hypsochromic effect can be explained by cross conjugation of the dioxodiene chromophore with a cyclopropane or cyclobutane ring system. On reduction of the dioxodiene with zinc in ethanol, this cross-conjugation is removed, and the 13β:18β-dihydro-derivative, m.p. 266-268°, absorbs at 2450 Å., as expected.

X. The Infrared Absorption Characteristics of 12:19-Dioxo--9(11):13(18)-Dienes and their Derivatives.

An examination of the infrared absorption spectra of 12:19-dioxo-9(11):13(18)-dienes reveals a well-defined band in the 1613-1626 $cm.^{-1}$ region which is characteristic of the dioxodiene system. A consideration of the light absorption properties of related compounds has enabled an assignment to be made of the band frequencies for the constituent groups in the 12:19-dioxo--9(11):13(18)-diene chromophore.

Table VII. Infrared Absorption Data (in Nujol Mull).

Band Frequency ($cm.^{-1}$)	Group	Examples
1695-1710 [112]	Isolated 12- or 19-Ketone.	3β-acetoxy-12-oxo-oleanane, 3β-acetoxy-12:19-dioxo-olean-9(11)-ene, and analogous sapogenol derivatives.
1664-1667	αβ-Unsaturated Ketone.	3β:24-diacetoxy-19-oxo-oleanoxer-13(18)-ene, 3β-acetoxy-12-oxo-olean--9(11)-ene, etc.
1613-1626	Conjugated dioxodiene	3β-acetoxy-12:19-dioxo-oleana-9(11):13(18)--diene, etc.
1595	Conjugated dienone	3β-acetoxy-12-oxo-oleana--9(11):13(18)-diene.

The absorption frequencies of acetate bands in the 5μ region are also recorded for a number of the acetoxy-dioxodienes (Table VIII). Whereas 3β-acetoxy-12:19-dioxo-oleana-9(11):13(18)--diene, the diacetoxy-sapogenol D dioxodiene, and the epimeric

triacetoxy-sapogenol B dioxodienes exhibit typical carbonyl stretching absorption at 1730-1736 $cm.^{-1}$,[112] all three tetra--acetoxy-sapogenol A dioxodienes show a pronounced bathochromic displacement of absorption to the 1745-1760 $cm.^{-1}$ region. The increase in stretching frequency is attributed to interaction between the carbonyls in the neighbouring $C_{(21)}$- and $C_{(22)}$- acetate groups. Moreover, since sapogenol A tetra-acetate does not show this effect, the interaction must be associated with the rigidity in the sapogenol molecule introduced by the extended dioxodiene system.

Table VIII. Infrared Absorption Data (continued)

Acetate Stretching Frequency ($cm.^{-1}$)	Compound.
1730	3β-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene.
1730	3β:24-diacetoxy-sapogenol D dioxodiene.
1736	3β:21α:24-triacetoxy-sapogenol B dioxodiene
1736	3β:21β:24-triacetoxy-sapogenol B dioxodiene
1745	3β:21α:24-tetra-acetoxy-sapogenol A dioxodiene
1754	3β:21α:22β:24-tetra-acetoxy-sapogenol A dioxodiene.
1759	3β:21β:22β:24-tetra-acetoxy-sapogenol A dioxodiene.

EXPERIMENTAL

All melting points are uncorrected. Specific rotations were measured at room temperature in a 1 dcm. tube with chloroform as solvent unless otherwise specified. Ultraviolet absorption were determined in absolute ethanol solution using a Unicam S.P. 500 and a Hilger H.700. 307 spectrophotometer. Infrared absorption spectra were determined in Nujol mull by Dr. G. T. Newbold and Miss N. Caramando. Microanalyses are by Dr. A. C. Syme and Mr. Wm. McCorkindale. Grade II alumina, and light petroleum, b.p. 60-80°, were used for chromatography.

I. Extraction of Defatted Soya Bean Flakes[90]

Soya bean flakes (3 Kg.), which had been exhaustively extracted with light petroleum, were refluxed with 80% aqueous ethanol, and evaporated to near dryness, to give a resinous brown gum (550 g.). A solution of the gum in methanol (6 l.) and hydrochloric acid (1 l.) was refluxed for 30 hr. and allowed to cool overnight. Potassium hydroxide (1000 g.) was cautiously added in the minimum of water, and the mixture refluxed with methanol (6 l.) and benzene (1.5 l.), for a further 4 hr. After concentrating to half bulk, the solution was diluted with water (15 l.) and repeatedly extracted with benzene. The golden-yellow extract was dried over anhydrous sodium sulphate, and evaporated to give the non-saponifiable fraction as a pale brown semi-crystalline solid (10.3 g.). Acidification of the alkaline liquors and subsequent extraction with chloroform gave a red acid gum (3.3 g.).

Soyasapogenols A and B.

The non-saponifiable mixture (65 g.), obtained by the above

procedure, was crystallised from benzene (650 c.c.). The solid obtained (25 g.) was collected (mother liquor A) and acetylated using acetic anhydride and pyridine at 100°. A solution of the acetate mixture in light petroleum-benzene (5:1, 800 c.c.) was chromatographed on alumina (1 Kg.). Preliminary elution of the column with light petroleum-benzene mixtures (5:1, 5 l.; 4:1, 3 l.; 3:1, 4 l.) gave fractions which did not crystallise. Elution with light petroleum-benzene (1:1, 9.6 l.) gave crystalline fractions (total, 11g.) which were combined and crystallised from chloroform-methanol to yield soyasapogenol B triacetate as rosettes of needles, m.p. 177-178°, $[\alpha]_D$ + 79° (c,1.2) [Found: C,73.9; H,9.8. Calc. for $C_{36}H_{56}O_6$: C,73.9; H,9.65%] A solution of the triacetate in ether was refluxed with excess lithium aluminium hydride for 1 hr. The product crystallised from chloroform-methanol to give soyasapogenol B as needles, m.p. 260-261°, $[\alpha]_D$ + 90° (c,1.1.).

A solution of sapogenol B (120 mg.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was left overnight at room temperature Isolation of the product in the usual way and crystallisation from chloroform-methanol gave sapogenol B triacetate as needle-rosettes, m.p. and mixed m.p. 179-180°, $[\alpha]_D$ + 77.5° (c,1.0).

Continued elution of the alumina column with light petroleum-benzene (1:2, 16 l.), with benzene (12 l.), and with benzene-ether (20:1, 4 l.), gave fractions which crystallised with difficulty. Benzene-ether (10:1, 11 l.) then eluted crystalline fractions which were combined (total, 6.2 g.) and recrystallised from chloroform-methanol to yield soyasapogenol A tetra-acetate as felted needles,

m.p. 228-229°, $[\alpha]_D$ + 85° (c,1.0) [Found: C,71.1; H,9.1. Calc. for $C_{58}H_{88}O_8$: C,71.0; H,9.1%]. A solution of the tetra-acetate was hydrolysed with 3% methanolic potassium hydroxide for 3 hr. The product crystallised from chloroform-methanol to give soyasapogenol A as fine needles, m.p. 310-313°, $[\alpha]_D$ + 103° (c,0.5). Acetylation of sapogenol A with pyridine and acetic anhydride at 100° regenerated the tetra-acetate as felted needles (from chloroform-methanol), m.p. and mixed m.p. 228-229°, $[\alpha]_D$ + 85°.

Soyasapogenol D and β-Sitosterol.

The benzene mother liquor A (page 95) was concentrated to half-bulk. The amorphous solid (6.0 g.) separating on standing was collected and acetylated using pyridine and acetic anhydride at 100°. A solution of the dry acetylated product in light petroleum-benzene (10:1, 1 l.) was chromatographed on alumina (180 g.). Elution with light petroleum-benzene (10:1, 2 l.) gave amorphous fractions; continued washing with the same solvent mixture (9 l.) eluted crystalline fractions (total, 1.3 g.) m.p.'s between 124 and 130°. These were combined and crystallised from chloroform-methanol to give β-sitosteryl acetate as lustrous plates, m.p. and mixed m.p. 128-129°, $[\alpha]_D$ - 39.5° (c,1.4). Alkaline hydrolysis of the acetate and crystallisation of the product from chloroform-methanol gave β-sitosterol as needles, m.p. and mixed m.p. 135-136°, $[\alpha]_D$ - 34° (c,0.9).

Continued elution of the column with light petroleum-benzene (9:1, 2 l.; 4:1, 3.5 l.; 2:1, 2 l.; 1:1, 4 l.) gave fractions

(total 1.2 g.) which after crystallisation from chloroform-methanol gave soyasapogenol D diacetate as lustrous plates, m.p. 191-192°, $[\alpha]_D$ - 44° (c,1.2). [Found: C,75.3; H,10.2. Calc. for $C_{34}H_{54}O_6$: C,75.2; H,10.0%]. Its infrared spectrum (Nujol) includes a strong band at 1100 $cm.^{-1}$.

A solution of the diacetate in ether was refluxed for 2 hr. with an excess of lithium aluminium hydride. Crystallisation of the product from chloroform-methanol gave soyasapogenol D as granular prisms, m.p. 297-299°, $[\alpha]_D$ - 56° (c,0.6). Acetylation of sapogenol D with pyridine and acetic anhydride at 100° regenerated the diacetate as lustrous plates, (from chloroform-methanol), m.p. 190-191°, $[\alpha]_D$ - 43° (c,0.9)

Soyasapogenol C Diacetate.

The non-saponifiable mixture (60 g.) was crystallised from methanol (200 c.c.). The separating solid (11 g.) was recrystallised from benzene (150 c.c.). The solid was collected and the mother liquor evaporated to dryness. The residue (6.5 g.) was acetylated with pyridine and acetic anhydride at 100°. A solution of the dry acetylated product in light petroleum-benzene (6:1, 500 c.c.) was chromatographed on alumina (250 g.). After elution with light petroleum-benzene (6:1, 2 l.; 4:1, 1 l.), light petroleum-benzene (3:1, 4 l.) gave crystalline fractions (total 1.4 g.) which were combined and crystallised from chloroform-methanol to give soyasapogenol C diacetate as fine needles, m.p.202-203°, $[\alpha]_D$ + 59.5° (c,1.3)

[Found: C,77.7; H,10.2. Calc. for $C_{34}H_{52}O_4$: C,77.8; H,10.0%].

The red acid gum (3.3 g.) obtained on extraction of the acidified saponification product (page 94), was crystallised at 5° from ethyl acetate and light petroleum, to give micro-crystals of a saturated fatty acid, $C_{16}H_{33}COOH$ or $C_{17}H_{35}COOH$, m.p. 52-53° [Found: C,75.76; H,12.9. $C_{17}H_{34}O_2$ requires C,75.50; H,12.67. $C_{18}H_{36}O_2$ requires C,75.99; H,12.76%].

II. Reactions of Soyasapogenol A.

3β:24-21α:22α-bis-isopropylidenedioxy-olean-12-ene. - A solution of sapogenol A (200 mg.) in dry acetone (50 c.c.) and dry ether (200 c.c.) containing concentrated sulphuric acid (1 c.c.) was kept at room temperature for 27 hr. The reaction mixture was diluted with ether, and washed with aqueous sodium hydrogen carbonate and water. Chromatography of the product on alumina and crystallisation from chloroform-methanol gave the bis-isopropylidene-derivative as needles, m.p. 236-238°, $[\alpha]_D$ + 93° (c,1.5) [Found: C,77.6; H,10.6. $C_{36}H_{58}O_4$ requires C,77.9; H,10.5%]. It gives a bright yellow colour with tetranitromethane in chloroform. Infrared absorption: strong bands at 1111 and 1148 $cm.^{-1}$ (acetonide group).[113]

Sapogenol A triacetate. - A solution of sapogenol A (130 mg.) in a cold mixture of pyridine and acetic anhydride (1:1, 10 c.c.) was kept at 0-5° for 17 hr. The product was isolated in the usual way, and its solution in light petroleum-benzene (2:1, 100 c.c.) chromatographed on alumina (5 g.). Elution with light petroleum--benzene (1:3, 7 x 150 c.c.) gave fractions (total, 85 mg.) which crystallised from chloroform-methanol yielding sapogenol A tetra--acetate as needles, m.p. and mixed m.p. 228-230°, $[\alpha]_D$ + 85° (c,1.2). After eluting with benzene (500 c.c.), benzene-ether (20:1, 400 c.c.; 10:1, 500 c.c.; 4:1, 500 c.c.) gave fractions (total 45 mg.) which crystallised from chloroform-methanol to yield sapogenol A triacetate as plates, m.p. 256-258°, $[\alpha]_D$ + 71.7° (c,0.8) [Found: C,71.6; H,9.3. $C_{36}H_{56}O_7$ requires C,72.0; H,9.4%]. Acetylation of the triacetate

using pyridine and acetic anhydride at 100° gave sapogenol A tetra-acetate as needles (from chloroform-methanol), m.p. and mixed m.p. 228-230°, $[\alpha]_D$ + 83° (c,0.6). Chromatography of sapogenol A tetra-acetate on the same preparation of alumina as that used for the separation of the triacetate gave an almost quantitative recovery of tetra-acetate.

Performic Acid Oxidation of Sapogenol A Tetra-acetate. - A solution of sapogenol A tetra-acetate (1.0 g.) in ethyl acetate (40 c.c.) was treated at 45° with hydrogen peroxide (100 vol., 6.5 c.c.) in formic acid (99-100%, 34 c.c.) added dropwise over 3 hr. The solution was kept at 45° for a further 3 hr. and then concentrated to one-third bulk. The crystals (750 mg.) separating were recrystallised from chloroform-methanol to give needles of 3β:21α:22α:24--tetra-acetoxy-12-oxo-oleanane, m.p. 308-310°, $[\alpha]_D$ + 19.6° (c,1.2) [Found: C,69.1; H,8.8. $C_{38}H_{58}O_9$ requires C,69.3; H,8.9%]. It does not give a colour with tetranitromethane in chloroform. Infrared absorption: strong bands at 1706 (carbonyl), 1736, 1258 and 1239 $cm.^{-1}$ (acetate).

3β:21α:22α:24-Tetra-acetoxy-12-oxo-olean-9(11)-ene. - A solution of bromine (0.38 g.) in glacial acetic acid (25 c.c.) was added over 20 min. to a solution of the saturated 12-ketone (1.4 g.) in glacial acetic acid at 60-63°. The mixture was kept at 100° for 5 hr. and at room temperature overnight. Water was added to the heated solution until crystals formed. After standing, the crystals (1.1 g.) were collected and recrystallised from chloroform-methanol to give 3β:21α:22α:24-tetra-acetoxy-12-oxo-olean-9(11)-ene

as needles, m.p. 275-276°, $[\alpha]_D$ + 72.6° (c,1.0) [Found: C,69.7; H,8.4. $C_{38}H_{56}O_9$ requires C,69.5; H,8.6%]. It does not give a colour with tetranitromethane. Ultraviolet absorption: Maximum at 2470 Å. (ε = 11,000).

A solution of the tetra-acetoxy-αβ-unsaturated ketone (120 mg.) in methanolic potassium hydroxide (3%, 30 c.c.) was refluxed for 2½ hr. The product was isolated by means of ether and acetylated by using pyridine and acetic anhydride at 100°. Crystallisation of the acetylated product from chloroform-methanol gave unchanged 3β:21α:22α:24-tetra-acetoxy-12-oxo-olean-9(11)-ene as needles, m.p. and mixed m.p. 272-274°, $[\alpha]_D$ + 73° (c,1.1)

Selenium Dioxide Oxidation of Tetra-acetoxy-12-oxo-olean-9(11)-ene. - Powdered selenium dioxide (1.5 g.) was added to a solution of the tetra-acetoxy-αβ-unsaturated ketone (900 mg.) in glacial acetic acid (30 c.c.) and the mixture refluxed for 24 hr. The filtered solution was poured into water, and the product isolated in the usual manner, giving a yellow gum (980 mg.). This was purified by chromatography on alumina and crystallised from aqueous methanol to give 3β:21α:22α:24-tetra-acetoxy-12-oxo-taraxera-9(11):14-diene as plates (320 mg.), m.p. 240-241°, $[\alpha]_D$ -1.7° (c,4.1) [Found: C,69.8; H,8.4. $C_{38}H_{54}O_9$ requires C,69.7; H,8.3%]. It gives a pale yellow colour with tetranitromethane in chloroform. Ultraviolet absorption: Maxima at 2440 and 2080 Å. (ε = 11,200 and 8000).

3β:21α:22α:24-Tetrahydroxy-12-oxo-taraxera-9(11):14-diene. - A solution of the tetra-acetoxy-oxo-diene (240 mg.) in 3% methanolic

potassium hydroxide (30 c.c.) was refluxed for 3 hr. The product was isolated by means of a large volume of ether, and crystallised from aqueous methanol to give 3β:21α:22α:24-tetrahydroxy-12-oxo-taraxera-9(11):14-diene as prismatic needles, m.p. 296-298°, $[\alpha]_D$ - 55° (c,1.6 in methanol) [Found: C,74.1; H,9.6. $C_{30}H_{46}O_5$ requires C,74.0; H,9.5%]. It gives a pale yellow colour with tetranitromethane in chloroform. Ultraviolet absorption: Maxima at 2440 and 2080 Å. (ε = 11,000 and 7,000).

Acetylation of the tetrol, using pyridine and acetic anhydride, gave the tetra-acetate as plates (from aqueous methanol), m.p. and mixed m.p. 240-241°, $[\alpha]_D$ - 1.6° (c,2.0)

Mild Selenium Dioxide Oxidation of Sapogenol A Tetra-acetate. - Powdered selenium dioxide (200 mg.) was added to a solution of sapogenol A tetra-acetate (200 mg.) in stabilised acetic acid (30 c.c.) and the mixture refluxed for 1 hr. The hot mixture was filtered and the product isolated by means of ether, and purified by chromatography on alumina and crystallisation from chloroform--methanol to give 3β:21α:22α:24-tetra-acetoxy-oleana-11:13(18)-diene as needles, m.p. 242-243°, $[\alpha]_D$ + 13° (c,1.5) [Found: C,71.2; H,8.8. $C_{38}H_{56}O_8$ requires C,71.2; H,8.8%]. Ultraviolet absorption: Maxima at 2410, 2490 and 2590 Å. (ε = 30,000; 34,400 and 21,800).

A solution of the tetra-acetoxy-diene (240 mg.) in methanolic potassium hydroxide (3%, 30 c.c.) was refluxed for 3 hr. Crystallisation of the product from methanol gave 3β:21α:22α:24-tetrahydroxy-oleana-11:13(18)-diene as plates (175 mg.), m.p. 322-324°, $[\alpha]_D$ - 43°

(c,1.3 in ethanol)[Found: C,76.15; H,10.4. $C_{30}H_{48}O_4$ requires C,76.2; H,10.2%]. Ultraviolet absorption: Maxima at 2310, 2490 and 2590 Å. (ε = 30,000; 34,200 and 21,800).

3β:21α:22α:24-Tetrahydroxy-oleana-11:13(18)-diene Triacetate.-(a) A solution of the tetrahydroxy-diene (125 mg.) in a cold mixture of pyridine (5 c.c.) and acetic anhydride (5 c.c.) was kept at 0-5° for 20 hr. The product was isolated in the usual way, and its solution in light petroleum-benzene (2:1, 100 c.c.) chromatographed on alumina (5 g.). Elution with light petroleum-benzene (1:3, 5 x 130 c.c.) gave fractions (total 60 mg.) which crystallised from chloroform to yield 3β:21α:22α:24-tetra-acetoxyoleana-11:13(18)-diene triacetate as needles, m.p. and mixed m.p. 241-242°, $[\alpha]_D$ + 13° (c,1.0). Continued elution with ether and ether-methanol (20:1) gave fractions (total, 50 mg.) which crystallised from chloroform-methanol to yield 3β:21α:22α:24-tetrahydroxy-oleana-11:13(18)-diene triacetate as rods, m.p. 256-258°, $[\alpha]_D$ - 10.5° (c,1.1.) [Found: C,71.6; H,9.3. $C_{36}H_{54}O_7$ requires C,72.2; H,9.1%]. Ultraviolet absorption: Maxima at 2420, 2500 and 2600 Å. (ε = 30,000; 34,200 and 21,900). Acetylation of the triacetate, using pyridine and acetic anhydride at 100° gave the tetra-acetate as needles (from chloroform-methanol), m.p. and mixed m.p. 241-242°, $[\alpha]_D$ + 12° (c,0.9). Chromatography of tetra-acetoxy-oleana-11:13(18)-diene using the same preparation of alumina as that employed for the separation of the triacetate gave a nearly quantitative recovery of the tetra-acetate.

(b) Powdered selenium dioxide (70 mg.) was added to a solution of sapogenol A triacetate (70 mg.) in stabilised acetic acid (20 c.c.) and the mixture refluxed for 1 hr. The product was isolated by means of ether and its solution in light petroleum-benzene (1:1, 100 c.c.) chromatographed on alumina (4 g.). Elution with ether-methanol (20:1, 3 x 50 c.c.) gave fractions (total, 45 mg.) which crystallised from chloroform-methanol to yield 3β:21α:22α:24-tetra-hydroxy-oleana-11:13(18)-diene triacetate as rods, m.p. and mixed m.p. 256-258°, $[\alpha]_D$ - 9.0 (c,0.5).

Vigorous Selenium Dioxide Oxidation of Sapogenol A Tetra-acetate. - A solution of sapogenol A tetra-acetate (2.1 g.) in benzyl acetate (30 c.c.) was refluxed with powdered selenium dioxide (2.1 g.) for 24 hr. The filtered solution was evaporated to dryness under reduced pressure and the product purified by chromatography on alumina and by crystallisation from chloroform-methanol to yield 3β:21α:22α:24-tetra-acetoxy-12:19-dioxo-oleana--9(11):13(18)-diene (850 mg.) as fine needles, m.p. 265-266°, $[\alpha]_D$ -42.5° (c,1.0) [Found: C,68.4; H,8.0. $C_{38}H_{52}O_{10}$ requires C,68.2; H,7.8%]. It does not give a colour with tetranitromethane in chloroform. Ultraviolet absorption: Maximum at 2780 Å. (ε = 13,600) Infrared absorption (CaF_2 prism): Strong bands at 1745 (acetate), 1715, 1664 and 1626 $cm.^{-1}$ (dioxodiene).

3β:21α:22β:24-Tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene. - A solution of 3β:21α:22α:24-tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (100 mg.) in methanolic potassium hydroxide (3%, 30 c.c.) was refluxed for 2½ hr. The product was isolated

by means of chloroform and acetylated by using acetic anhydride and pyridine at 100°. Crystallisation of the acetylated material from chloroform-methanol gave 3β:21α:22β:24-tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene as prismatic needles, m.p. 330-332° (decomp.), $[\alpha]_D$ - 48° (c,1.8) [Found: C,68.0; H,7.9. $C_{38}H_{52}O_{10}$ requires C,68.2; H,7.8%]. It does not give a colour with tetranitromethane in chloroform. A mixture with the isomeric 3β:21α:22α:24-tetra-acetate had m.p. 254°. Ultraviolet absorption: Maximum at 2780 Å. (ε = 13,200). Infrared absorption (CaF_2 prism): Strong bands at 1754 (acetate), 1712, 1664 and 1626 cm.$^{-1}$ (dioxodiene).

From the mother liquors of the dioxo-3β:21α:22β:24-tetra-acetate, unchanged 3β:21α:22α:24-tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (10 mg.), m.p. and mixed m.p. 263-267°, $[\alpha]_D$ - 44° (c,0.5), was isolated.

3β:21α:22α:24-Tetra-acetoxy-12:19-dioxo-olean-9(11)-ene. - A solution of 3β:21α:22α:24-tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (400 mg.) in ethanol (50 c.c.) was refluxed with freshly activated zinc dust (4.0 g.) for 5 hr. The product was isolated in the usual way, and crystallised from chloroform-methanol to yield 3β:21α:22α:24-tetra-acetoxy-12:19-dioxo-olean-9(11)-ene (330 mg.) as lustrous plates, m.p. 274-275°, $[\alpha]_D$ + 108° (c,1.5) [Found: C,68.2; H,8.6. $C_{38}H_{54}O_{10}$ requires C,68.0; H,8.1%]. Ultraviolet absorption: Maximum at 2440 Å. (ε=12,100)

3β:21α:22β:24-Tetra-acetoxy-12:19-dioxo-olean-9(11)-ene. - 3β:21α:22β:24-tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene

(310 mg.) was reduced in ethanol with activated zinc dust as described above. The product crystallised from chloroform-methanol to give 3β:21α:22β:24-tetra-acetoxy-12:19-dioxo-olean-9(11)-ene (220 mg.) as blades, m.p. 239-241°, $[\alpha]_D$ + 153.5° (c,1.0).[Found: C,68.2; H,8.3. $C_{38}H_{54}O_{10}$ requires C,68.0; H,8.1%]. Ultraviolet absorption: Maximum at 2440 Å. (= 12,000).

A sample of the 3β:21α:22β:24-tetra-acetoxy-dioxo-9(11)-ene (10 mg.) was heated at 265° for 20 min. Crystallisation of the product from chloroform-methanol gave 3β:21α:22β:24-tetra-acetoxy--12:19-dioxo-9(11):13(18)-diene as prismatic needles, m.p. and mixed m.p. 326-328°, $[\alpha]_D$ - 45° (c,0.3), $\lambda_{max.}$ 2800 Å. (ε = 10,000).

3β:21α:22β:24-Tetra-acetoxy-12:19-dioxo-18α-olean-9(11)-ene. - (a) A solution of 3β:21α:22α:24-tetra-acetoxy-12:19-dioxo-olean--9(11)-ene (150 mg.) in aqueous methanolic potassium hydroxide (10%, 100 c.c.) was refluxed for 6 hr. in an atmosphere of nitrogen. The product was isolated by means of chloroform and re-acetylated by treatment with pyridine and acetic anhydride at 15° for 16 hr. Crystallisation of the acetylated product from chloroform-methanol gave 3β:21α:22β:24-tetra-acetoxy-12:19-dioxo-18α-olean-9(11)-ene (100 mg.) as needles, m.p. 326-328°, $[\alpha]_D$ + 60.2° (c,0.85) [Found: C,68.0; H,8.2. $C_{38}H_{54}O_{10}$ requires C,68.0; H,8.1%]. Ultraviolet absorption: Maximum at 2430 Å. (ε = 11,400).

(b) 3β:21α:22β:24-Tetra-acetoxy-12:19-dioxo-olean-9(11)-ene (87 mg.) was treated with alkali in an atmosphere of nitrogen and the product reacetylated as described under (a) above. Crystallisation from chloroform-methanol gave 3β:21α:22β:24-tetra-acetoxy-12:19-dioxo-18α--olean-9(11)-ene (60 mg.) as needles, m.p. and mixed m.p. 326-328°,

$[\alpha]_D$ + 61° (c,0.8). Ultraviolet absorption: Maximum at 2430 Å. (ε = 11,200).

When the isomerisations described under (a) and (b) were effected in air, and not in an atmosphere of nitrogen, the product was in each case contaminated with 3β:21α:22β:24-tetra-acetoxy-12:19-dioxo-9(11):13(18)-diene. For example, repetition of experiment (a) in air gave a product, m.p. 326-329°, $[\alpha]_D$ + 52°, $\lambda_{max.}$ 2440 (ε = 8,000) and 2780 Å. (ε = 1,500), which could not be resolved into its components by crystallisation or chromatography.

Attempted Hydrogenolysis of 3β:21α:22α:24-Tetra-acetoxy-12:19-dioxo-olean-9(11)-ene. - A solution of the tetra-acetoxy-dioxo-ene (300 mg.) in glacial acetic acid (100 c.c.) was shaken with hydrogen and platinum catalyst (from 300 mg. PtO_2) for 100 hr. The product was isolated in the usual way and crystallised from chloroform-methanol to give a mixture, $\lambda_{max.}$ 2440 Å. (ε = 8000). Prolonged treatment of the mixture with hydrogen and platinum catalyst achieved a reduction in the amount of non-reacted material, and now had $\lambda_{max.}$ 2440 Å. (ε = 3000). The mixture, however, could not be separated either by crystallisation or by chromatography on alumina. Similar results were obtained when the hydrogenolysis of 3β:21α:22β:24-tetra-acetoxy-12:19-dioxo-olean-9(11)-ene was attempted.

III. Reactions of Soyasapogenol B.

Cold Acetylation of Soyasapogenol B - A solution of sapogenol B (100 mg.) in a cold mixture of pyridine (5 c.c.) and acetic anhydride (5 c.c.) was kept at 0-5° for 17 hr. Isolation of the product in the usual way and chromatography on alumina gave sapogenol B triacetate (105 mg.), crystallising from chloroform-methanol as needle-rosettes, m.p. and mixed m.p. 179-180°, $[\alpha]_D$ + 77.5° (c,1.0).

Performic Acid Oxidation of Sapogenol B Triacetate. - Sapogenol B triacetate (1.0 g.) in ethyl acetate (40 c.c.) was treated at 45° with a solution of hydrogen peroxide (100 vol., 6.5 c.c.) in formic acid (99-100%, 34 c.c.), added dropwise over 3 hr. The solution was kept at 45° for 3 hr. and then concentrated to one-fourth bulk. On cooling, plates separated (430 mg.), m.p. 260-262°. Recrystallisation from chloroform-methanol gave 3β:21α:24-triacetoxy-12-oxo-oleanane as lustrous plates, m.p. 264-265°, $[\alpha]_D$ -4.4° (c,3.1) [Found: C,72.1; H,9.3. Calc. for $C_{36}H_{56}O_7$: C,72.0; H,9.4%]. It does not give a colour with tetranitromethane in chloroform. Tsuda and Kitagawa give m.p. 254-256° for this keto-acetate.[80] Hydrolysis of the triacetate with 3% methanolic potassium hydroxide gave needles of 3β:21α:24-trihydroxy-12-oxo-oleanane (from aqueous methanol), m.p. 258-260°, $[\alpha]_D$ - 10.2° (c,1.5) [Found: C,75.6; H,10.3. Calc. for $C_{30}H_{50}O_4$: C,75.9; H,10.6%]. Tsuda and Kitagawa give m.p. 253-254° for this compound.

A solution of 3β:21α:24-trihydroxy-12-oxo-oleanane (150 mg.) in dry acetone (30 c.c.) was added to a solution of dry ether (150 c.c.) containing sulphuric acid (24 drops). After leaving the

mixture at 17° for 16 hr., the product was worked up in the usual manner, and purified by chromatography on alumina. Subsequent crystallisation from methanol gave fine needles (93 mg.) of 21α-hydroxy-3β:24-isopropylidenedioxy-12-oxo-oleanane m.p. 236-238°, $[\alpha]_D$ - 23.5°. It gives no colour with tetranitromethane. [Found: C,76.7; H,10.5. $C_{33}H_{54}O_4$ requires C,77.0; H,10.6%].

Chromic Acid Oxidation of Sapogenol B Triacetate. - (a) A solution of sapogenol B triacetate (1.0 g.) in stabilised acetic acid (50 c.c.) was treated at room temperature with a solution of chromium trioxide (0.75 g.) in water (0.7 c.c.) and acetic acid (7.5 c.c.), added with stirring over 20 min. After 17 hr., the neutral product was isolated in the usual way and crystallised from chloroform-methanol to give plates (540 mg.), m.p. 242-248°, $[\alpha]_D$ + 30° (c,2.0). Ultraviolet absorption: Maximum at 2480 Å. (ε = 6,000). Attempts to separate the αβ-unsaturated ketone component of this mixture by careful chromatography, by fractional crystallisation and by fractional solution, all failed.
(b) A solution of the triacetate (500 mg.) in stabilised acetic acid (35 c.c.) at 60° was treated with a solution of chromium trioxide (400 mg.) in acetic acid (80%, 8.3 c.c.) added with stirring over 30 min. After 2 hr. the neutral product was isolated and crystallised as above to give micro-plates (204 mg.), m.p. 245-246°. Ultraviolet absorption: Maximum at 2480 Å. (ε = 4,800). The αβ-unsaturated ketone component again could not be purified by crystallisation or chromatography.

A solution of the mixture (300 mg.), m.p. 242-248°, $\lambda_{max.}$ 2480 Å. (ε= 6000), in 3% methanolic potassium hydroxide, was refluxed

for 6 hr. The product, isolated in the usual way, was an amorphous gum (280 mg.). Ultraviolet absorption: End absorption at 2040 Å. (ε = 5000). The gum was acetylated with pyridine and acetic anhydride and a solution of the dry acetylated product in light petroleum-benzene (3:1, 50 c.c.) was chromatographed on alumina (10 g.). Elution with benzene (200 c.c.) gave fractions (total, 150 mg.) which crystallised from chloroform-methanol to give 3β:21α:24-triacetoxy--12-oxo-oleanane as plates, m.p. and mixed m.p. 264-265°, $[\alpha]_D$ - 4.4° (c,1.2) [Found: C,72.3; H,9.3. Calc. for $C_{36}H_{56}O_7$: C,72.0; H,9.4%].

A solution of the mixture (200 mg.) m.p. 242-248°, $\lambda_{max.}$ 2400 (ε = 6000), in acetic acid (20 c.c.) and concentrated hydrochloric acid (2 c.c.) was heated at 100° for 3 hr. The product was isolated in the usual way and crystallised from chloroform-methanol as plates, m.p. 285-300°. Ultraviolet absorption: Maximum at 2420 Å. (ε = 6,000). The mixture could not be separated into its components by crystallisation or by chromatography.

Mild Selenium Dioxide Oxidation of Sapogenol B Triacetate. - A solution of sapogenol B triacetate (1.0 g.) in stabilised acetic acid (150 c.c.) was refluxed with powdered selenium dioxide (1.0 g.) for 1 hr. The product was worked up in the usual way to give a crystalline gum, which was chromatographed on alumina and crystallised from chloroform-methanol to give fine needles of 3β:21α:24-triacetoxy oleana-11:13(18)-diene (920 mg.), m.p. 250-251°, $[\alpha]_D$ - 16° (c,1.0) [Found: C,74.0; H,9.4. $C_{36}H_{54}O_6$ requires C,74.2; H,9.3%]. Ultraviolet absorption: Maxima at 2410, 2490 and 2590 Å. (ε=30,000;

34,400; and 21,800). The triacetoxy-diene was hydrolysed by means of lithium aluminium hydride in ether. The trihydroxy-diene, which was not purified, was dissolved in a cold mixture of acetic anhydride and pyridine (1:1, 12 c.c.), and the solution kept at 0-5° for 17 hr. The product was purified by chromatography on alumina, and by crystallisation from chloroform-methanol to give the triacetoxy-diene as needles, m.p. and mixed m.p. 250-251°, $[\alpha]_D$ - 16.5° (c,1.0), in almost quantitative yield.

3β:21α:24-Triacetoxyolean-13(18)-ene. - A solution of the triacetoxy-diene (94 mg.) in stabilised acetic acid (200 c.c.) was shaken with platinum (from 100 mg. PtO_2) and hydrogen for 36 hr. Crystallisation of the product from chloroform-methanol gave 3β:21α:24-triacetoxyolean-13(18)-ene as fine needles, m.p. 219-221°, $[\alpha]_D$ - 24° (c,1.45) [Found: C,73.6; H,9.6. Calc. for $C_{36}H_{56}O_6$: C,73.9; H,9.65%]. It gives a strong yellow colour with tetranitromethane in chloroform. Ultraviolet absorption: End absorption at 2080 Å. (ε = 12,800). Ruzicka gives m.p. 221-222°, $[\alpha]_D$ - 24° for this compound.[91] Hydrolysis of the triacetate with lithium aluminium hydride in ether gave hexagonal plates of 3β:21α:24-trihydroxyolean-13(18)-ene (from methanol), m.p. 318-320°, $[\alpha]_D$ - 54° (c,0.47 in chloroform-methanol 1:1) [Found: C,78.5; H,11.0. Calc. for $C_{30}H_{50}O_3$: C,78.55; H,11.0%]. Ruzicka gives m.p. 320-321°, $[\alpha]_D$ - 52° for the triol.

21α-Hydroxy-3β:24-isopropylidenedioxyoleana-11:13(18)-diene. A solution of 3β:21α:24-trihydroxyoleana-11:13(18)-diene (400 mg.)

in dry acetone (60 c.c.) and dry ether (300 c.c.) was treated with concentrated sulphuric acid (2 c.c.). After standing at 17° for 18 hr., the mixture was diluted with ether and washed with aqueous sodium hydrogen carbonate. The product was purified by chromatography on alumina and crystallisation from light petroleum to yield the hydroxy-isopropylidene derivative as plates, m.p. 235-236°, $[\alpha]_D$ - 84° (c,0.9) [Found: C,79.8; H,10.6. $C_{33}H_{52}O_3$ requires C,79.8; H,10.55%]. Ultraviolet absorption: Maxima at 2430, 2510 and 2600 Å. (ε = 29,000; 33,700; and 22,500). Infrared absorption: Strong bands at 3484 (hydroxyl), 1155, 1107 and 1093 $cm.^{-1}$ (acetonide).

21-Oxo-3β:24-isopropylidenedioxyoleana-11:13(18)-diene. - The hydroxy-isopropylidene derivative (210 mg.) in pyridine (2 c.c.) was added to a suspension of chromium trioxide (230 mg.) in pyridine (2 c.c.). The mixture was shaken occasionally, left at room temperature for 24 hr. and worked up in the usual way. The crystalline product was chromatographed on alumina and crystallised from chloroform-light petroleum to yield 21-oxo--isopropylidenedioxyoleana-11:13(18)-diene (170 mg.) as rods, m.p. 243-245°, $[\alpha]_D$ - 65° (c,2.8) [Found: C,79.0; H,10.2. $C_{33}H_{50}O_3$ requires C,80.1; H,10.2%]. Ultraviolet absorption: Maxima at 2420, 2500 and 2590 Å. (ε = 25,800; 30,400 and 20,000) Infrared absorption: Strong bands at 1706 (carbonyl), 1155, 1105, 1093 $cm.^{-1}$ (acetonide).

3β:24-Diacetoxy-21-oxo-oleana-11:13(18)-diene. - A solution of the oxo-isopropylidene derivative (130 mg.) in methanol (100 c.c.) containing concentrated hydrochloric acid (25 c.c.) was refluxed for 20 min. The product was worked up in the usual way and acetylated by treatment with acetic anhydride and pyridine at 100°. Crystallisation of the dry acetylated product from methanol gave 3β:24-diacetoxy-21-oxo-oleana-11:13(18)-diene as lustrous plates, m.p. 239-241°, $[\alpha]_D$ - 33° (c,0.8) [Found: C,75.6 H,9.6. $C_{34}H_{50}O_6$ requires C,75.8; H,9.4%]. Ultraviolet absorption: Maxima at 2420, 2520 and 2590 Å. (ε = 25,000; 29,000; and 24,000). Infrared absorption: Strong bands at 1706 (carbonyl), 1735, 1265, 1236 $cm.^{-1}$ (acetate).

A solution of the diacetoxy-oxo-diene (90 mg.) in stabilised acetic acid (25 c.c.) was refluxed with selenium dioxide (90 mg. dissolved in the minimum of water), for 2 hr. The product was worked up in the usual way to give an intractable gum which could not be crystallised even after chromatography and treatment with acetic anhydride and pyridine at 100°. Ultraviolet absorption of the gum ($\lambda_{max.}$ 2420, 2520 and 2600 Å.) indicated it to be essentially unchanged starting material.

Treatment of Sapogenol B with Acetone and Sulphuric Acid. - A solution of sapogenol B (6.0 g.) in dry ether (2 l.) and dry acetone (500 c.c.) containing concentrated sulphuric acid (20 c.c.) was kept at 17° for 60 hr. The product was isolated in the usual way, and chromatographed on alumina. Crystallisation from aqueous

methanol gave 21α-hydroxy-3β:24-isopropylidenedioxyolean-12-ene (3.0 g.) as needle rosettes, m.p. 200-201°, $[\alpha]_D$ + 74° (c,2.2) [Found: C,79.6; H,10.9. $C_{33}H_{54}O_3$ requires C,79.5; H,10.9%]. Infrared absorption: Strong bands at 3509 (hydroxyl), 1151 and 1115 cm.$^{-1}$ (acetonide).

Conversion of Sapogenol B into Sapogenol C. - A solution of the isopropylidene derivative of sapogenol B (120 mg.) in pyridine (20 c.c.) and phosphorus oxychloride (5 c.c.) was refluxed for 2 hr. The product was isolated by means of ether, and its solution in methanolic hydrochloric acid (2N, 5 c.c.) refluxed for 20 min. The crystalline product was isolated by using ether, and acetylated by means of pyridine and acetic anhydride at 100°. The acetylated product was purified by chromatography on alumina and crystallisation from chloroform-methanol to give sapogenol C diacetate as fine needles (66mg.), m.p. and mixed m.p. 200-201°, $[\alpha]_D$ + 59° (c,1.0). The infrared spectrum of this specimen was identical with that of a specimen of sapogenol C diacetate isolated directly from soya bean.

21-Oxo-isopropylidenedioxyolean-12-ene. - The complex prepared from chromium trioxide (3.0 g.) and pyridine (30 c.c.) was added to a solution of the isopropylidene derivative of sapogenol B (3.0 g.) in pyridine (30 c.c.) and the mixture kept for 18 hr. at 17° with occasional shaking. The product was isolated in the usual manner and its solution in light petroleum (300 c.c.) chromatographed on alumina (100 g.). Elution with

the same solvent (2 l.) gave fractions (total 2.18 g.) which crystallised from methanol to yield 21-oxo-3β:24-isopropylidene-dioxyolean-12-ene-as rosettes of stout rods, m.p. 208-209°, $[\alpha]_D$ + 14° (c,0.9) [Found: C,80.1; H,10.7. $C_{33}H_{52}O_3$ requires C,79.8; H,10.55%]. Infrared absorption: Strong bands at 1706 (carbonyl), 1149, 1111 and 1101 cm.$^{-1}$ (acetonide).

Lithium Aluminium Hydride Reduction of 21-Oxo-3β:24-iso-propylidenedioxyolean-12-ene. - A solution of the oxoisopropylidene derivative (1.8 g.) in dry ether was refluxed with an excess of lithium aluminium hydride for 2½ hr. The product was isolated in the usual way and its solution in light petroleum (250 c.c.) chromatographed on alumina (120 g.) Elution with benzene (2.5 l.) gave fractions (total, 800 mg.) which crystallised from methanol to yield 21α-hydroxy-3β:24-isopropylidenedioxyolean-12-ene as needle rosettes, m.p. and mixed m.p. 200-201°, $[\alpha]_D$ + 73° (c,1.1) Benzene (500 c.c.) and then benzene-ether (10:1, 2.5 l.) eluted fractions (800 mg.) which crystallised from aqueous methanol as rosettes, m.p. 127-140°. A solution of these fractions in light petroleum-benzene (1:1, 50 c.c.) was again chromatographed on alumina (70 g.). Elution with light petroleum-benzene (1:9, 125 c.c.) gave a fraction (260 mg.), m.p. 168-170°, $[\alpha]_D$ + 70° (c,0.6), which appeared to be a mixture. Continued elution with the same solvent mixture (1.8 l.) gave a fraction (500 mg.) which after crystallisation from methanol yielded 21β-hydroxy-iso-propylidenedioxyolean-12-ene as needles, m.p. 128-130°, $[\alpha]_D$

+ 58° (c,1.7) [Found: C,79.3; H,11.2. $C_{33}H_{54}O_3$ requires C,79.5; H,10.9%]. Infrared absorption: Identical to that for the 21α-hydroxyisopropylidene derivative, with bands at 3509 (hydroxyl), 1151 and 1115 cm.$^{-1}$ (acetonide).

3β:21β:24-Triacetoxyolean-12-ene. - A solution of the 21β-hydroxy-isopropylidene derivative (500 mg.) in methanol (150 c.c.) and concentrated hydrochloric acid (37.5 c.c.) was refluxed for 15 min. The mixture was diluted with water and the crystalline product isolated by means of ether. The hydrolysis product was acetylated by treatment with acetic anhydride and pyridine for 48 hr. at 17°. The acetylated product was isolated in the usual way and purified by chromatography on alumina and crystallisation from chloroform-methanol from which 3β:21β:24-triacetoxyolean-12-ene separated as blades, m.p. 214-215°, $[\alpha]_D$ + 64.5° (c,2.5) [Found: C,74.0; H,9.7. $C_{36}H_{56}O_6$ requires C,73.9; H,9.65%]. Infrared absorption: Identical to that for sapogenol B triacetate, with bands at 1730, 1259 and 1242 cm.$^{-1}$ (acetate).

Vigorous Selenium Dioxide Oxidation of Sapogenol B Triacetate. - A solution of sapogenol B triacetate (1.8 g.) in benzyl acetate (35 c.c.) was refluxed with powdered selenium dioxide (1.8 g.) for 21 hr. The product was isolated in the usual way, and purified by chromatography on alumina. Subsequent crystallisation of the product from chloroform-light petroleum and from chloroform-methanol gave 3β:21α:24-triacetoxy-12:19--dioxo-oleana-9(11):13(18)-diene (800 mg.) as rods, m.p.274-275°,

$[\alpha]_D$ - 52° (c,2.5) [Found: C,70.6; H,8.5, Calc. for $C_{36}H_{50}O_8$: C,70.8; H,8.25%]. Ultraviolet absorption: Maximum at 2780 Å. (ε = 12,800). Infrared absorption: Strong bands at 1736 (acetate) 1695, 1660, 1626, (dioxodiene), 1256 and 1236 $cm.^{-1}$ (acetate). Meyer, Jeger, and Ruzicka give m.p. 267.5-268°, $[\alpha]_D$ - 48° for this compound.

3β:21α:24-Trihydroxy-12:19-dioxo-oleana-9(11):13(18)-diene. - A solution of the dioxodiene triacetate (500 mg.) in 3% methanolic potassium hydroxide (100 c.c.) was refluxed for 2½ hr. The product was isolated by means of chloroform, and crystallised from aqueous methanol to give 3β:21α:24-trihydroxy-12:19-dioxo--oleana-9(11):13(18)-diene (405 mg.) as plates, m.p. 301-303°, $[\alpha]_D$ - 145° (c,0.4 in pyridine). Ultraviolet absorption: Maximum at 2800 Å. (ε = 11,000). Meyer, Jeger and Ruzicka, give m.p. 300-301°, $[\alpha]_D$ - 145° for this compound. Acetylation of the triol was carried out by using pyridine and acetic anhydride at 100°. Subsequent crystallisation gave 3β:21α:24-triacetoxy--12:19-dioxo-oleana-9(11):13(18)-diene as rods (from chloroform methanol), m.p. and mixed m.p. 273-274°, $[\alpha]_D$ - 52° (c,2.6). Ultraviolet light absorption: Maximum at 2780 Å. (ε = 12,800).

3β:21α:24-Triacetoxy-12:19-dioxo-olean-9(11)-ene. - A solution of the dioxodiene triacetate (570 mg.) in ethanol (50 c.c.) was refluxed with freshly activated zinc dust (5 g.) for 5 hr. The product was isolated in the usual way, and crystallised from chloroform-methanol to give 3β:21α:24-triacetoxy-

-12:19-dioxo-olean-9(11)-ene as plates, m.p. 265-267°, $[\alpha]_D$ + 126.5° (c,1.1) [Found: C,70.5; H,8.6. $C_{36}H_{52}O_6$ requires C,70.6; H,8.6%]. Ultraviolet absorption: Maximum at 2460 Å. (ε = 12,000)

3β:21α:24-Triacetoxy-19-oxo-olean-9(11)-ene. - A solution of 3β:21α:24-triacetoxy-12:19-dioxo-olean-9(11)-ene (340 mg.) in glacial acetic acid (230 c.c.) was shaken with hydrogen and platinum catalyst (from 200 mg. PtO_2) for 20 hr. The product was isolated in the usual manner and crystallised from chloroform-methanol to give 3β:21α:24-triacetoxy-19-oxo-olean-9(11)-ene as plates, m.p. 225-226°, $[\alpha]_D$ + 102.5° (c,1.1) [Found: C,72.2; H,9.0. $C_{36}H_{54}O_7$ requires C,72.2; H,9.1%]. It gives a yellow colour with tetranitromethane in chloroform.

3β:21α:24-Triacetoxy-19-oxo-18α-olean-9(11)-ene. - A solution of 3β:21α:24-triacetoxy-19-oxo-olean-9(11)-ene (220 mg.) in glacial acetic acid (35 c.c.) containing concentrated hydrochloric acid (1 c.c.) was heated at 100° for 1 hr. After evaporating the mixture to dryness, the product was crystallised from chloroform-methanol to give 3β:21α:24-triacetoxy-19-oxo-18α-olean-9(11)-ene as rods, m.p. 235-236°, $[\alpha]_D$ + 98° (c,1.0) [Found: C,72.0; H,9.3. $C_{36}H_{54}O_7$ requires C,72.2; H,9.1%].

Treatment of 3β:21α:24-Triacetoxy-19-oxo-olean-9(11)-ene with Lithium Aluminium Hydride. - A solution of the triacetoxy ketone (220 mg.) in dry ether (100 c.c.) was refluxed with a suspension of lithium aluminium hydride (1 g.) for 2½ hr. The product was isolated in the usual manner, to give a crystalline

material (200 mg.) which was dissolved in dry ether (150 c.c.) and dry acetone (30 c.c.), containing sulphuric acid (30 drops), and left at 17° for 40 hr. with occasional shaking. A solution of the product, in light petroleum-benzene (1:1, 100 c.c.) was chromatographed on alumina (30 g.). Elution with benzene-ether (1:1, 150 c.c.) gave fractions (total 163 mg.), which crystallised from light petroleum-chloroform as fine needles of 19α:21α--dihydroxy-3β:24-isopropylidenedioxyolean-9(11)-ene, m.p. 324-326°, $[\alpha]_D$ + 69° (c,1.1) [Found: C,76.7; H,10.5. $C_{33}H_{54}O_4$ requires C,77.0; H,10.6%]. Infrared absorption: Bands at 3484 (hydroxyl), 1151, 1111, and 1101 $cm.^{-1}$ (acetonide)

Treatment of 3β:21α:24-Triacetoxy-19-oxo-18α-olean-9(11)-ene with Lithium Aluminium Hydride. - A solution of the 18α-triacetoxy ketone (184 mg.) was treated with lithium aluminium hydride as described above, to give the tetrahydroxy-derivative (140 mg.), which was not further purified. A solution of the tetrol in dry acetone (30 c.c.) was mixed with dry ether (150 c.c.) and sulphuric acid (30 drops), and left at 17° for 24 hr. A solution of the product in light petroleum-benzene (1:1, 150 c.c.) was chromatographed on alumina (6 g.). Elution with benzene-ether (10:1, 200 c.c.) gave a fraction (102 mg.) which crystallised from aqueous-methanol as needles of 19β:21α-dihydroxy-3β:24-isopropylidenedioxy-18α-olean-12-ene, m.p. 332-335°, $[\alpha]_D$ + 74.5° (c,0.7). A mixture with the 18β-isomeric dihydroxyisopropylidene derivative had m.p. 316°. [Found: C,76.8; H,10.7. $C_{33}H_{54}O_4$ requires C,77.0; H,10.6%].

Infrared absorption: Identical to that of the 18β-dihydroxy--isopropylidene derivative.

Vigorous Selenium Dioxide Oxidation of 3β:21β:24-Triacetoxy-olean-12-ene. - A solution of the triacetate (450 mg.) in benzyl acetate (10 c.c.) was refluxed with selenium dioxide (400 mg.) for 18 hr. The reaction product was isolated in the usual way and purified by chromatography on alumina followed by crystallisation from chloroform-light petroleum to yield 3β:21β:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene as needles, m.p. 241-242°, $[\alpha]_D$ - 57° (c,1.1). A mixture with 3β:21α:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene had m.p. 230°. [Found: C,70.6; H,8.1. $C_{36}H_{56}O_8$ requires C,70.8; H,8.25%]. Ultraviolet absorption: Maximum at 2800 Å. (ε = 12,600). Infrared absorption: Identical to that for 3β:21α:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)--diene, with strong bands at 1736 (acetate), 1695, 1660, 1626, (dioxodiene), 1256 and 1236 $cm.^{-1}$ (acetate).

Conversion of 3β:21β:24-Triacetoxy-12:19-dioxo-olean-9(11):13(18)-diene into 3β:21α:24-Triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene. - A solution of the 3β:21β:24-triacetoxy-dioxodiene (120 mg.) in 3% methanolic potassium hydroxide (100 c.c.) was refluxed for 2½ hr. Isolation of the product by means of chloroform yielded a crystalline solid which was treated with pyridine and acetic anhydride at 100° for 1 hr. The acetylated product crystallised from chloroform-light petroleum to give 3β:21α:24-triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (90 mg.) as rods, m.p. and mixed m.p. 274-275°, $[\alpha]_D$ - 52° (c,1.4).

Ultraviolet absorption: Maximum at 2780 Å. (ϵ = 12,700).

Treatment of the Dioxodiene Derivative of Sapogenol B with Acetone and Sulphuric Acid. - A solution of 3β:21α:24-trihydroxy-12:19-dioxo-oleana-9(11):13(18)-diene (380 mg.) in dry acetone (50 c.c.) and dry ether (200 c.c.) containing concentrated sulphuric acid (2 c.c.) was kept at 17° for 60 hr. The product was isolated in the usual way, and purified by chromatography on alumina. Crystallisation from chloroform-light petroleum gave 21α-hydroxy-3β:24-isopropylidenedioxy-12:19-dioxo-oleana-9(11):13(18)-diene (250 mg.), as needles, m.p. 288-290°, $[\alpha]_D$ - 77° (c,1.2) [Found: C,75.6; H,9.4. $C_{33}H_{48}O_5$ requires C,75.5; H,9.2%]. Ultraviolet absorption: Maximum at 2800 Å. (ϵ = 12,000). Infrared absorption: Strong bands at 3484 (hydroxyl), 1698, 1667, 1616 (dioxodiene), 1103 $cm.^{-1}$ (acetonide).

12:19:21-Trioxo-3β:24-isopropylidenedioxyoleana-9(11):13(18)-diene. - A solution of the 21α-hydroxy-isopropylidene derivative (800 mg.) in pyridine (8 c.c.) was added to a suspension of chromium trioxide (800 mg.) in pyridine (8 c.c.). The mixture was shaken occasionally, and left at 17° for 24 hr. Isolation of the product in the usual way gave 12:19:21-trioxo-3β:24-isopropylidenedioxyoleana-9(11):13(18)-diene (750 mg.) as needles (from chloroform-light petroleum), m.p. 282-284°, $[\alpha]_D$ - 208° (c,1.1) [Found: C,75.8; H,9.1. $C_{33}H_{46}O_5$ requires C,75.8; H,8.9%]. Ultraviolet absorption: Maximum at 2800 Å. (ϵ = 13,000)

Infrared absorption: Strong bands at 1733 (carbonyl), 1698, 1645, 1623, 1316 (dioxodiene), 1152, 1116, 1101 cm.$^{-1}$ (acetonide).

3β:24-Diacetoxy-12:19:21-trioxo-oleana-9(11):13(18)-diene. - The isopropylidene derivative of the trioxodiene (750 mg.) in methanol (100 c.c.) containing concentrated hydrochloric acid (25 c.c.) was refluxed for 15 min. The product was isolated using ether, and acetylated by treatment with pyridine and acetic anhydride at 100° for 3 hr. Crystallisation of the acetylated product from chloroform-light petroleum gave 3β:24-diacetoxy-12:19:21-trioxo-oleana-9(11):13(18)-diene (500 mg.) as needles, m.p. 203-205°, $[\alpha]_D$ - 131° (c,2.3) [Found: C,72.0; H,8.4. $C_{34}H_{46}O_7$ requires C,72.05; H,8.2%]. Ultraviolet absorption: Maximum at 2800 Å. (ε = 12,000). Infrared absorption: Strong bands at 1736 (acetate), 1709 (carbonyl), 1667, 1613 (dioxodiene), and 1232 cm.$^{-1}$ (acetate).

Addition of methanolic potassium hydroxide (10%, 30 c.c.) to a methanol solution of the diacetoxy-trioxodiene (70 mg.) gave a deep orange colour. The solution was refluxed for 1 hr. and diluted with water. The product was not extracted from the alkaline solution by means of ether. Acidification of the aqueous solution with hydrochloric acid precipitated a solid which was isolated by means of ether as an amorphous solid. Attempts to crystallise this acid and to obtain a crystalline derivative by acetylation and by esterification with diazomethane, all failed.

Treatment of the amorphous acid with chloroform gives an intense blue-green solution.

Treatment of 3β-Acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene with Alkaline Hydrogen Peroxide. - A solution of the dioxodiene (500 mg.) in methanolic potassium hydroxide (20%, 100 c.c.) was treated at 45° with hydrogen peroxide (30%, 30 c.c.) added over 30 min. The solution was then refluxed for a further 30 min., and the product worked up in the usual way. Acetylation of the neutral product (480 mg.) with pyridine and acetic anhydride at 100°, and crystallisation from chloroform-light petroleum gave unchanged 3β-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (490 mg.), m.p. and mixed m.p. 242-243°, $[\alpha]_D$ - 92° (c,3.3).

Treatment of 3β:24-Diacetoxy-12:19:21-trioxo-oleana-9(11):13(18)-diene with Alkaline Hydrogen Peroxide. - A solution of the trioxodiene (210 mg.) in methanolic potassium hydroxide (20%, 50 c.c.) was treated with hydrogen peroxide as described above. The reaction mixture was poured into an excess of water, and an acid and neutral product (each 100 mg.) extracted in the usual way. Acetylation of the neutral product by means of pyridine and acetic anhydride at 100°, and subsequent crystallisation of the acetylated product, gave felted needles of unchanged trioxodiene (from chloroform-light petroleum), m.p. and mixed m.p. 202-203°, $[\alpha]_D$ - 131° (c,0.7). The acid product failed to crystallise, even after acetylation. It showed maximal absorption at 2050, 2250 and 2600 Å. (ε = 10,700; 7,800; and 9,100).

IV. Reactions of Soyasapogenol C.

Osmium Tetroxide Oxidation of Sapogenol C Diacetate. - Sapogenol C diacetate (850 mg.) in pyridine (20 c.c.) was mixed with a solution of osmium tetroxide (600 mg.) in pyridine (6 c.c.) and the mixture kept in the dark for 14 days. The mixture was diluted with ether (150 c.c.) and after the addition of lithium aluminium hydride (2.0 g.), refluxed for 1 hr. The product was isolated in the usual way and acetylated using acetic anhydride and pyridine. A solution of the acetylated product (900 mg.) in light petroleum-benzene (10:1, 200 c.c.) was chromatographed on alumina (30 g.). The column was washed successively with light petroleum-benzene mixtures (9:1, 320 c.c.; 6:1, 1120 c.c.; 7:3, 2720 c.c.; 3:2, 1280 c.c.; 2:3, 800 c.c.; 1:4, 320 c.c.), with benzene (800 c.c.) and finally with benzene-ether (3:1, 440 c.c.) to give fractions (50 x 160 c.c.). Evaporation of fractions 3-8 gave a solid (170 mg.) which was crystallised from chloroform-methanol to furnish unchanged sapogenol C diacetate as fine needles, m.p. and mixed m.p. 203-204°, $[\alpha]_D$ + 59°. Fractions 43-4 furnished sapogenol A tetra-acetate (70 mg.) as needles (from chloroform-methanol)m.p. and mixed m.p. 228-230°, $[\alpha]_D$ + 89°. Fractions 9-42 were evaporated and the crystalline residues combined (total 600 mg.) and rechromatographed on alumina (20 g.). The column was washed successively with light petroleum-benzene mixtures (4:1, 800 c.c.; 3:7, 600 c.c.; 1:1, 1200 c.c.; 1:2, 1000 c.c.; 1:4, 2600 c.c.), with benzene (1 l.) and with benzene-ether

(19:1, 2 l.) to give fractions (40 x 200 c.c.). Fractions 20-38 were evaporated and the combined solids (330 mg.) crystallised from chloroform-methanol to give 3β:21β:22β:24-tetra-acetoxyolean-12-ene as needles, m.p. 228-229°, $[\alpha]_D$ + 41° (c,1.1). Meyer, Jeger, and Ruzicka give m.p. 226-227°, $[\alpha]_D$ + 38° for the stereoisomeric sapogenol A tetra-acetate.[90] The crude solids obtained from fractions 16-33 showed selective absorption at 2800 Å. (ε= 1000). During recrystallisations, absorption at 2800 Å. gradually diminished to (ε=50).

Vigorous Selenium Dioxide Oxidation of the Steroisomeric Sapogenol A tetra-acetate. - A solution of 3β:21β:22β:24-tetra-acetoxyolean-12-ene (300 mg.) in benzyl acetate (10 c.c.) was refluxed for 24 hr. with powdered selenium dioxide (250 mg.). The product was isolated in the usual manner, and purified by chromatography on alumina and crystallisation from chloroform-methanol to give 3β:21β:22β:24-tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (150 mg.), as prisms, m.p. 212-214°, $[\alpha]_D$ - 95° (c,0.9) [Found: C,68.4; H,7.6. $C_{36}H_{52}O_{10}$ requires C,68.2; H,7.8%]. Ultraviolet absorption: Maximum at 2780 Å. (ε = 13,000). Infrared absorption (CaF_2 prism): Strong bands at 1759, 1741 (acetate), 1703, 1660, 1621 $cm.^{-1}$ dioxodiene).

3β:21α:22β:24-Tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene. - A solution of 3β:21β:22β:24-tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (100 mg.) in methanolic potassium hydroxide (3%, 30 c.c.) was refluxed for 2½ hr. The product was

isolated by means of chloroform and acetylated by using pyridine and acetic anhydride at 100°. The acetylated product was crystallised from chloroform-methanol to give 3β:21α:22β:24-tetra-acetoxy--12:19-dioxo-oleana-9(11):13(18)-diene (40 mg.), as prisms, m.p. and mixed m.p. 330-332P (decomp.), $[\alpha]_D$ - 48° (c,0.6). Ultraviolet absorption: Maximum at 2780 Å. (ε = 13,200).

From the chloroform-methanol mother liquor, unchanged 3β:21β:22β:24-tetra-acetoxy-12:19-dioxo-oleana-9(11):13(18)-diene (40 mg.) was isolated, m.p. and mixed m.p. 210-212°, $[\alpha]_D$ - 95° (c,0.95).

Vigorous Selenium Dioxide Oxidation of Sapogenol C Diacetate. - A solution of sapogenol C diacetate (250 mg.) in benzyl acetate was refluxed with powdered selenium dioxide (300 mg.) for 18 hr. The product was purified by chromatography on alumina and crystallisation from light petroleum-chloroform to give the dioxodiene derivative as needles, m.p. 217-218°, $[\alpha]_D$ - 78.5° (c,0.8) [Found: C,74.2; H,8.5. $C_{34}H_{46}O_6$ requires C,74.15; H,8.4%]. It does not give a colour with tetranitromethane in chloroform. Ultraviolet absorption: Maximum at 2740 Å. (ε = 12,500). Infrared absorption: Bands at 1742 (acetate), 1704, 1667, 1626 (dioxodiene), 1235 $cm.^{-1}$ (acetate).

Reduction of the Dioxodiene Derivative of Sapogenol C Diacetate. - The dioxodiene derivative (70 mg.) in ethanol (10 c.c.) was refluxed with freshly activated zinc dust (1 g.) for 5 hr. The product was isolated in the usual way, and crystallised from chloroform-methanol to give the dihydro-derivative (45 mg.) as plates, m.p. 266-268°, $[\alpha]_D$ + 106° (c,0.8) [Found: C,74.0; H,8.9. $C_{34}H_{48}O_6$ requires C,73.9; H,8.75%]. The dihydro-derivative does not give a colour

with tetranitromethane in chloroform. Ultraviolet spectrum: Maximum at 2450 Å. (ε = 12,600). Infrared spectrum : Bands at 1739 (acetate), 1721 (carbonyl), 1667 (αβ-unsaturated ketone), 1258 and 1242 $cm.^{-1}$.

3β:24-Diacetoxyolean-12-ene. - A solution of sapogenol C diacetate (250 mg.) in glacial acetic acid (100 c.c.) was shaken with hydrogen in the presence of platinum catalyst (from 250 mg. PtO_2) for 18 hr. The product was isolated in the usual way and crystallised from methanol-chloroform to give 3β:24-diacetoxyolean--12-ene as needles, m.p. 188-189°, $[\alpha]_D$ + 82° (c,1.1)[Found: C,77.7; H,10.6. Calc. for $C_{34}H_{54}O_4$: C,77.5; H,10.3%]. Meyer, Jeger, and Ruzicka give m.p. 194-195°, $[\alpha]_D$ + 80° for this compound.

Vigorous Selenium Dioxide Oxidation of Dihydrosapogenol C Diacetate. - A solution of the dihydro-diacetate (200 mg.) in benzyl acetate (10 c.c.) was refluxed with powdered selenium dioxide (300 mg.) for 20 hr. The product was purified by chromatography on alumina and crystallisation from light petroleum to give 3β:24-diacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene as needles, m.p. 247-248°, $[\alpha]_D$ - 77° [Found: C,73.9; H,9.0. $C_{34}H_{48}O_6$ requires C,73.9; H,8.75%]. It does not give a colour with tetranitromethane in chloroform. Ultraviolet absorption: Maximum at 2760 Å. (ε = 13,000)

3β:24-Diacetoxy-12:19-dioxo-olean-9(11)-ene. - A solution of the dioxodiene from dihydrosapogenol C diacetate (70 mg.) was

reduced with zinc dust in ethanol in the usual way. The product was crystallised from chloroform-methanol to give 3β:24-diacetoxy-12:19-dioxo-olean-9(11)-ene as laminae, m.p. 249-251° (decomp.), $[\alpha]_D$ + 120° (c,1.0) [Found: C,73.7; H,9.3. $C_{34}H_{50}O_6$ requires C,73.6; H,9.1%]. It does not give a colour with tetranitromethane in chloroform. Ultraviolet absorption: Maximum at 2450 Å. (ε = 12,700). Infrared absorption: Bands at 1739 (acetate), 1709 (carbonyl), 1667 (αβ-unsaturated ketone), 1258 and 1245 $cm.^{-1}$ (acetate).

3β:24-Diacetoxy-19-oxo-olean-9(11)-ene. - (a) A solution of the dihydro-dioxodiene derivative of sapogenol C diacetate (30 mg.) in acetic acid (50 c.c.) was shaken with hydrogen and platinum (from 250 mg. PtO_2) for 4 days. The product was isolated in the usual way and purified by chromatography on alumina. Crystallisation from chloroform-methanol yielded 3β:24-diacetoxy-19-oxo-olean-9(11)-ene (42 mg.) as plates, m.p. 237-239°, $[\alpha]_D$ + 104° (c,0.9) [Found: C,75.8; H,10.0. $C_{34}H_{52}O_6$ requires C,75.5; H,9.7%]. It gives a pale yellow colour with tetranitromethane in chloroform. Infrared absorption: Strong bands at 1739 (acetate), 1712 (carbonyl), 1267, 1258, 1242 $cm.^{-1}$ (acetate).

(b) Hydrogenolysis of 3β:24-diacetoxy-12:19-dioxo-olean-9(11)-ene by shaking its solution in acetic acid with hydrogen and platinum for 4 days gave 3β:24-diacetoxy-19-oxo-olean-9(11)-ene as plates (from methanol), m.p. and mixed m.p. 238-239°, $[\alpha]_D$ + 102° (c,0.8). Infrared absorption: Identical to that obtained according to (a) above.

V. Reactions of Soyasapogenol D.

Attempted Hydrogenolysis of Sapogenol D Diacetate. - A solution of sapogenol D diacetate (550 mg.) in glacial acetic acid (100 c.c.) was shaken with hydrogen and platinum (from 250 mg. PtO_2) for 48 hr. Crystallisation of the product from ether-methanol recovered the diacetate as lustrous plates, m.p. and mixed m.p. 191-192°, $[\alpha]_D$ - 44° (c,1.0).

Sapogenol D diacetate was also recovered almost quantitatively after treatment with zinc in ethanol, and zinc in acetic acid.

Mild Selenium Dioxide Oxidation of Sapogenol D Diacetate. - (a) A solution of the diacetate (440 mg.) in benzyl acetate (15 c.c.) was refluxed with powdered selenium dioxide (500 mg.) for 3½ hr. The product was isolated in the usual way, and its solution in light petroleum-benzene (20:1, 250 c.c.) chromatographed on alumina (15 g.). Elution with benzene:ether (20:1, 1500 c.c.) gave fractions (total 280 mg.) which crystallised from chloroform-methanol to give the diacetoxy-dioxodiene as prisms, m.p. and mixed m.p. 255--256°, $[\alpha]_D$ - 76° (c,1.0). Ultraviolet absorption: Maximum at 2780 Å. (ε = 11,700).

(b) A solution of the diacetate (165 mg.) in redistilled acetic acid (10 c.c.) was refluxed with powdered selenium dioxide (165 mg.) for 10 hr. The product was isolated in the usual way, and its solution in light petroleum-benzene (10:1, 250 c.c.) chromatographed on alumina (6 g.). Elution with light petroleum-benzene (3:2, 1 l., 1:1, 360 c.c.; 2:3, 320 c.c.) and benzene (320 c.c.), gave fractions

(total 100 mg.) m.p. 170-178°, which showed maximal absorption at 2080 (ε = 7,000), 2420, 2500 (ε = 11,000) and 2600 Å. The mixture could not be separated into its components by crystallisation. Continued elution with benzene-ether (4:1, 240 c.c.) gave fractions (50 mg.) which crystallised from chloroform-methanol to give the diacetoxy-dioxodiene as prisms, m.p. and mixed m.p. 256-258°, $[\alpha]_D$ -79.5 (c,1.1). Ultraviolet absorption: Maximum at 2780 Å. (ε = 11,500).

(c) A solution of the diacetate (200 mg.) in redistilled acetic acid (30 c.c.) was treated with selenium dioxide (200 mg.) in water (1 c.c.), and refluxed for 26 hr. The product was isolated in the usual way, and its solution in light petroleum-benzene (3:1, 150 c.c.) chromatographed on alumina (6 g.). Elution with light petroleum benzene (1:1, 1 l.) gave fractions (33 mg.) which crystallised from chloroform-methanol to give needles, m.p. 243-245°, $[\alpha]_D$ - 19° (c,0.6), which showed no depression of melting point on mixture with 3β:21α:24-triacetoxyoleana-11:13(18)-diene. Ultraviolet absorption: Maxima at 2410, 2500 (ε = 23,000) and 2610 Å. Continued elution with benzene-ether (4:1, 600 c.c.) gave fractions (54 mg.) which crystallised from chloroform-methanol to give 3β:21α:24--triacetoxy-12:19-dioxo-oleana-9(11):13(18)-diene as rods, m.p. and mixed m.p. 271-273°, $[\alpha]_D$ - 51° (c,1.6). Ultraviolet absorption: Maxima at 2780 Å. (ε = 12,500).

Chromic Acid Oxidation of Sapogenol D Diacetate. - A solution of the diacetate (300 mg.) in stabilised acetic acid (15 c.c.) was treated at room temperature with chromium trioxide (225 mg.) in stabilised acetic acid (15 c.c.) added with stirring over 20 min. The mixture was allowed to stand overnight at 17°, after which methanol was added, and the product worked up in the usual way, giving a non-crystalline gum (290 mg.). A solution of the gum in light petroleum-benzene (10:1, 150 c.c.) was chromatographed on alumina (8 g.). Elution with light petroleum-benzene (2:3, 750 c.c.) gave a fraction (60 mg.) which crystallised from chloroform- methanol to give 3β:24-diacetoxy-19-oxo-oleanoxer-13(18)-ene as plates, m.p. 229-231°, $[\alpha]_D$ - 112° (c,1.0) [Found: C,73.2; H,9.1. $C_{34}H_{52}O_6$ requires C,73.3; H,9.4%]. It does not give a colour with tetranitromethane in chloroform. Ultraviolet absorption: Maximum at 2540 Å. (ε = 7,000). Infrared absorption: Strong bands at 1724 (acetate), 1667 (αβ-unsaturated ketone), 1093 $cm.^{-1}$ (oxide ring).

A solution of the αβ-unsaturated ketone (20 mg.) in glacial acetic acid (30 c.c.) containing concentrated hydrochloric acid (1 c.c.) was heated at 100° for 30 min. Crystallisation of the product from chloroform-methanol recovered unchanged starting material as plates, m.p. and mixed m.p. 226-228°, $[\alpha]_D$ - 108° (c,0.5).

A solution of the αβ-unsaturated ketone (30 mg.) in glacial acetic acid (100 c.c.) was shaken with hydrogen and platinum catalyst (from 200 mg. PtO_2) for 100 hr. The product was isolated in the

usual manner and purified by chromatography on alumina to give sapogenol D diacetate (15 mg.) as lustrous plates (from chloroform methanol), m.p. and mixed m.p. 192-194°, $[\alpha]_D$ - 42° (c,0.5).

Performic Acid Oxidation of Sapogenol D Diacetate. - A solution of sapogenol D diacetate (100 mg.) in ethyl acetate (4 c.c.) was treated at 45° with hydrogen peroxide (100 vol., 0.7 c.c.) in formic acid (99-100%, 3.4 c.c.), added dropwise over 3 hr. The solution was kept at 45° for a further 3 hr. and then concentrated to low bulk. The crystals (30 mg.) which separated were recrystallised from chloroform-methanol to give 3β:24-diacetoxy-13:18-epoxy-oleanoxerane as plates, m.p. 234-235°, $[\alpha]_D$ - 4.8° (c,1.1) [Found: C,73.3; H,9.9. $C_{34}H_{54}O_6$ requires (C,73.1; H,9.7%]. It does not give a colour with tetranitromethane in chloroform. Infrared absorption: Acetate bands at 1736, 1256 and 1241 $cm.^{-1}$.

Similar treatment of 3β-acetoxyolean-13(18)-ene gave 3β-acetoxy--13:18-epoxyoleanane as needles, m.p. 268-269°, $[\alpha]_D$ + 5.5° (c,1.7) [Found: C,79.5; H,11.1. Calc. for $C_{32}H_{52}O_3$: C,79.3; H,10.8%]. Ruzicka et al. give m.p. 2 -2 ° for this compound.[104]

A solution of 3β:24-diacetoxy-13:18-epoxy-oleanoxerane (19 mg.) in glacial acetic acid (30 c.c.) containing concentrated hydrochloric acid (1 c.c.) was heated at 100° for 30 min. Crystallisation of the product from chloroform-methanol gave fine-needles, m.p. 207-209°. It gives a positive Beilstein test for chlorine, and exhibits maximal absorption at 2430, 2520 and 2610 Å. (ε = 24,400; 28,000;

and 18,000). The infrared spectrum shows no band at 1100 cm.$^{-1}$. Similar treatment of 3β-acetoxy-13:18-epoxy-oleanane yields 3β-acetoxyoleana-11:13(18)-diene, m.p. and mixed m.p. 227-229°, $[\alpha]_D$ -62° (c,1.2), $\lambda_{max.}$ 2420, 2510 and 2600 Å. (ε=28,000; 31,000 and 20,000).

Acid Rearrangement of Sapogenol D Diacetate. - A solution of the diacetate (150 mg.) in glacial acetic acid (35 c.c.) containing concentrated hydrochloric acid (1 c.c.) was heated at 100° for 20 min. Crystallisation of the product from chloroform-methanol gave 21-chloro-3β:24-diacetoxyolean-13(18)-ene as needles, m.p. 204-206°, $[\alpha]_D$ - 46° (c,1.1) [Found: C,72.5; H,9.6; Cl,6.3. Calc. for $C_{34}H_{54}O_4Cl$: C,72.8; H,9.5; Cl,6.0%]. Ruzicka et al. give m.p. 201.5-202°, $[\alpha]_D$ - 44° for this compound.

Vigorous Selenium Dioxide Oxidation of Sapogenol D Diacetate. - A solution of sapogenol D diacetate (500 mg.) in benzyl acetate (10 c.c.) was refluxed with powdered selenium dioxide (500 mg.) for 17 hr. The product was isolated in the usual way, and purified by chromatography. Crystallisation from chloroform-methanol gave the dioxodiene derivative (300 mg.) as prisms, m.p. 257-258°, $[\alpha]_D$ - 78° (c,1.0) [Found: C,71.7; H,8.7. Calc. for $C_{34}H_{48}O_7$: C,71.8; H,8.5%]. It does not give a colour with tetranitromethane in chloroform. Ultraviolet absorption: Maximum at 2780 Å. (ε - 11,700). Infrared absorption: Strong bands at 1730 (acetate), 1690, 1660, 1613, (dioxodiene), 1250, 1236 (acetate) and 1092 cm.$^{-1}$ (oxide ring).

Ruzicka, *et al.* give m.p. 253-254°, $[\alpha]_D$ - 79° for this compound.

A solution of the dioxodiene (100 mg.) in glacial acetic acid (35 c.c.) containing concentrated hydrochloric acid (1 c.c.), was heated at 100° for 30 min. Crystallisation of the product from chloroform-methanol gave unchanged starting material, m.p. and mixed m.p. 256-257°, $[\alpha]_D$ - 77° (*c*,0.8). The dioxodiene was also found to be stable to pyridine and acetic anhydride at 100°.

Zinc-Ethanol Reduction of the Dioxodiene from Sapogenol D Diacetate. - A solution of the dioxodiene (110 mg.) in ethanol (15 c.c.) was refluxed with activated zinc dust (1 g.) for 5 hr. Crystallisation of the product from chloroform-methanol yielded 3β:24-diacetoxy-12:19-dioxo-oleanoxer-9(11)-ene as needles, m.p. 216-217°, $[\alpha]_D$ + 150° (*c*,1.1) [Found: C,71.6; H,8.8. $C_{34}H_{50}O_7$ requires C,71.55; H,8.5%]. Ultraviolet absorption: Maximum at 2450 Å. (ε = 12,000). Infrared absorption: Strong bands at 1733 (acetate), 1667 (αβ-unsaturated ketone), and 1099 $cm.^{-1}$ (oxide ring).

A solution of the dihydro-dioxodiene (90 mg.) in glacial acetic acid (35 c.c.) containing concentrated hydrochloric acid (1 c.c.) was heated at 100° for 20 min. Crystallisation of the product from chloroform-methanol gave needles, m.p. 230-231°, $[\alpha]_D$ + 174° (*c*,1.1). Ultraviolet absorption: Maxima at 2200 and 3200 Å. (ε=5,000 and 12,400). Treatment of the product with pyridine and acetic anhydride (1:1) at 100° for 2 hr. gave a gum, $\lambda_{max.}$ 2030, 2280 and 2760 Å. (ε = 9,000; 6,000; and 10,600).

3β-Acetoxy-12:19-epoxyoleana-9(11):12(18)-triene. - A solution of 3β-acetoxy-12:19-dioxo-olean-9(11)-ene (600 mg.) in glacial acetic acid (70 c.c.) containing concentrated hydrochloric acid (2 c.c.) was heated at 100° for 10 min. Crystallisation of the product from chloroform-methanol gave 3β-acetoxy-12:19-epoxyoleana--9(11):12:18-triene (550 mg.) as needles, m.p. and mixed m.p. 180-181°, $[\alpha]_D$ + 170° (c,1.65) [Found: C,80.1; H,9.8. Calc.for $C_{32}H_{46}O_3$: C,80.3; H,9.7%]. McKean gives m.p. 180-181°, $[\alpha]_D$ + 170°. Crystallisation of the epoxy-triene from chloroform-msthanol containing a trace of mineral acid gives a mixture containing dioxodiene, $\lambda_{max.}$ 2430, 2780 Å. (ε = 8000 and 6000). On leaving a methanol solution of the mixture exposed to the atmosphere for one week, crystals of 3β-acetoxy-12:19-dioxo-oleana-9(11):13(18)--diene were obtained, m.p. and mixed m.p. 241-242°, $[\alpha]_D$ - 91° (c,1.1), $\lambda_{max.}$ 2780 Å. (ε = 11,800).

Hydrogenolysis of the Dihydro-Dioxodiene Derivative. - A solution of 3β:24-diacetoxy-12:19-dioxo-oleanoxer-9(11)-ene (240 mg.) in glacial acetic acid (75 c.c.) was shaken with hydrogen and platinum catalyst (from 200 mg. PtO_2) for 20 hr. Crystallisation of the product gave plates of 3β:24-diacetoxy-19-oxo-oleanoxer-9(11)-ene (from chloroform-methanol), m.p. 208-209°, $[\alpha]_D$ + 109° (c,1.0) [Found: C,73.45; H,9.35. $C_{34}H_{52}O_6$ requires C,73.3; H,9.4%]

Treatment of the diacetoxy-ketone with acetic acid (35 c.c.) containing hydrochloric acid (1 c.c.) for 1 hr. at 100° gave

3β:24-diacetoxy-19-oxo-18α-oleanoxer-9(11)-ene as needles (from chloroform-methanol), m.p. 221-222°, $[\alpha]_D$ + 93.5° (c,1.6) [Found: C,73.4; H,9.5. $C_{34}H_{52}O_6$ requires C,73.3; H,9.4%]. It gives a pale yellow colour with tetranitromethane in chloroform.

3β:24-Diacetoxy-19-Hydroxy-18α-oleanoxer-9(11)-ene. - A solution of 3β:24-diacetoxy-19-oxo-18α-oleanoxer-9(11)-ene (93 mg.) in dry ether (30 c.c.) was refluxed with a suspension of lithium aluminium hydride (300 mg.) for 2½ hr. The product was isolated avoiding the use of mineral acid, and acetylated with pyridine and acetic anhydride at 17° for 16 hr. Crystallisation of the acetylated product from chloroform-methanol gave 3β:24-diacetoxy-19-hydroxy--18α-oleanoxer-9(11)-ene as rods, m.p. 257-259°, $[\alpha]_D$ + 80° (c,0.8) [Found: C,72.9; H,9.5. $C_{34}H_{54}O_6$ requires C,73.1; H,9.7%]. It gives a yellow colour with tetranitromethane. Infrared absorption: Bands at 3509 (hydroxyl), 1739, 1235 (acetate), and 1099 $cm.^{-1}$ (oxide ring).

3β:24-Diacetoxyoleanoxera-9(11):18-diene. - A solution of the hydroxy-diacetate (50 mg.) in dry pyridine (10 c.c.) containing phosphorus oxychloride (1.5 c.c.) was refluxed for 2 hr. The product was isolated in the usual way and purified by chromatography on alumina. Crystallisation from chloroform-methanol gave 3β:24--diacetoxyoleanoxera-9(11):18-diene as needles, m.p. 165-167°, $[\alpha]_D$ + 70° (c,0.4)[Found: C,75.6; H,9.9. $C_{34}H_{52}O_5$ requires C,75.5; H,9.7%]. Infrared absorption: Strong bands at 1721 (acetate) and 1090 $cm.^{-1}$ (oxide ring).

A solution of the diacetoxy-diene (15 mg.) in acetic acid (7 c.c.) containing concentrated hydrochloric acid (0.2 c.c.) was heated at 100° for 10 min. Crystallisation of the product from chloroform-methanol gave unchanged starting material as needles, m.p. and mixed m.p. 165-167°. A solution of the diacetoxy-diene (50 mg.) in acetic acid (30 c.c.) containing hydrochloric acid (3 c.c.) was similarly treated for 6 hr. The product was isolated in the usual manner and purified by chromatography. Crystallisation from chloroform-methanol gave fine needles of a chloro-diene m.p. 215-215.5°, $[\alpha]_D$ - 61° (c,0.5). Ultraviolet absorption: Maxima at 2440, 2520 and 2620 Å. (ε = 25,000; 29,000; and 18,000). Infrared absorption: Acetate band at 1739 $cm.^{-1}$; no oxide band at 1100 $cm.^{-1}$. It gives a positive colour with the Beilstein test. Lack of material prevented further investigation.

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