STUDIES IN TERPENOID CHEMISTRY

A thesis presented by

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Abstract

The chemistry of the four known C-27, C-28- triterpenic dicarboxylic acids is reviewed.

The following original contributions are presented:

(i) A partial synthesis of cincholic acid from oleanolic acid.

(ii) A study of the scope of a novel reductive elimination reaction of vicinal esters. In certain instances, to be defined, metals in liquid ammonia have been found to reduce vicinal esters to the corresponding olefine. This reaction was employed in the cincholic acid synthesis.

(iii) The characterization of pyrethrol, a triterpenic alcohol isolated from Pyrethrum extracts.

ACKNOWLEDGEMENTS

I am deeply grateful to Professor D.H.R. Barton F.R.S. for the privilege of working under his guidance and for his help at all times.

To Dr. P.G. Sammes, the author is indebted for his friendly and unfailing interest in this work.

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Thanks are also due to Mr. D. Aldrich and his staff of the Whiffen Laboratory and to Mrs. O.I. Boston for the determination of all the n.m.r. spectra.

Finally I would like to thank Katiça, my wife, for her cooperation and understanding during this work.

I N D E X

Section I

The Chemistry of the C-27, C-28 triterpenic dicarboxylic acids.

Section II

(a) Partial Synthesis of Cincholic acid.

INDEX (Contd.) Page

SECTION I

a) Introduction

The terpenoids constitute a vast number of naturally occurring organic compounds mainly found in the vegetable kingdom. All of them possess a carbon skeleton which can be regarded as derived from two or more isoprene units. Those compounds whose molecular formulae do not contain an integral multiple of 5 carbon atoms, but which are closely related to the terpenes proper are also classified with the terpenes.

The terpenoids are classified on the basis of the carbon content of the molecule - the monoterpenoids (C-10), sesquiterpenoids (C-15), diterpenoids (C-20) and triterpenoids (C-30). The carotenoids (C-40) are conveniently studied as a separate branch of natural product chemistry.

The triterpenoids[1] constitute by far the largest terpenoid class and have been studied for over 100 years. However, it is only within the last two decades that large

strides have been made towards the elucidation of their structures.

The interest of the chemist in these compounds is threefold. Primarily their very structure and the inherent rearrangements possible allow the chemist to study the effects of subtle changes in structure on their conformations and reactions. Secondly they possess an important position in biosynthetic studies, especially their relationship to steroids. Finally, they provide an exciting challenge for chemical synthesis.

The triterpenoids are mainly distributed in vegetables. Amongst the important exceptions are the hydrocarbon squalene, first isolated from shark liver oil, ambrein from ambergris and a number of tetracyclic compounds isolated from wool fat.

The triterpenoids occur in plants as esters, glycosides or in the free state. Few of the glycosides have been adequately studied.

The triterpenoids may be classified into three groups: i) ambrein and squalene. ii) Tetracyclic triterpenoids and iii) Pentacyclic triterpenoids.

Of these, the third group is the largest and is itself normally divided into four further sub-groups. They are those whose structures are related to (i) α-amyrin, (ii) β-amyrin, (iii) lupeol and (iv) hopane.

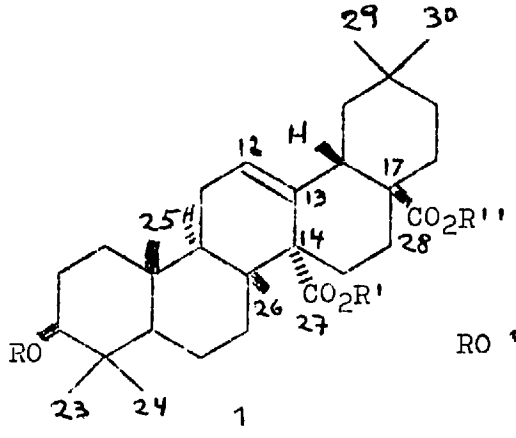
In the following the chemistry of the four known C_{27}, C_{28}- triterpenic dicarboxylic acids is reviewed.

b) Cincholic acid.

Tschesche, Duphorn and Snatzke[2] found that the crude glycoside obtained from Cinchona (Cortex Chinae) contained three glycosides designated A, B and C. Hydrolysis of glycosides A and C afforded the aglycone quinovic acid (2; R=R'=R"=H) with D-glucomethylose and D-glucose respectively. The glycoside B on hydrolysis gave a new acid called cincholic acid[3] (1; R=R'=R"=H) together with D-glucomethylose. These workers also managed to separate the cincholic acid from a crude extract of the total aglycone fraction by partition chromatography. Cincholic acid was found to be an unsaturated triterpenic acid which analysed for $C_{30}H_{46}O_5$ and gave a dimethyl ester and a monoacetate.

On heating, the acid readily lost carbon dioxide, as does quinovic acid[4], to give a pyroacid (3; R=R'=H). The general properties of cincholic acid closely resembled those of quinovic acid.

Dimethyl cincholate was recovered unchanged after treatment for 7 hours with


29
30
12
H
17
13
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H
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28
CO_2R''
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27
CO_2R'
RO
23
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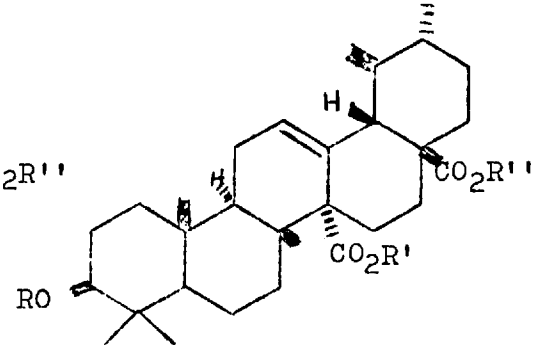
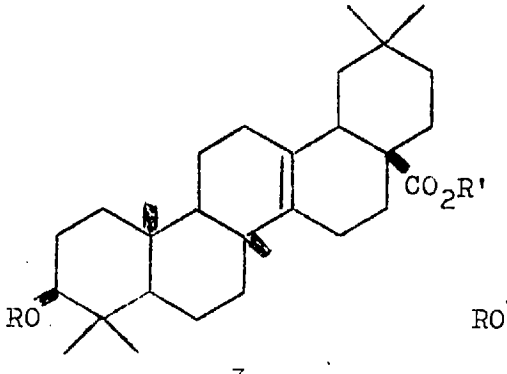
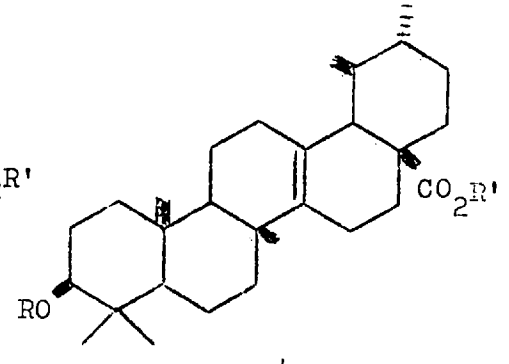
1


H
H
CO_2R''
CO_2R'
RO

2


CO_2R'
RO

3


CO_2R'
RO

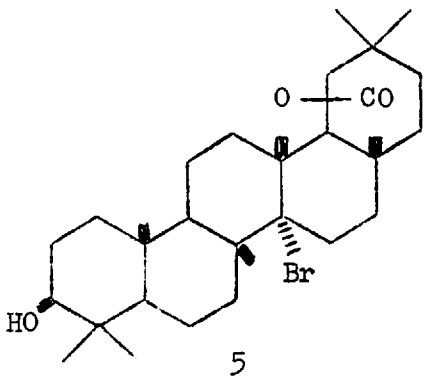
4

refluxing methanolic potassium hydroxide. By analogy with known amyrin esters[5] it was thus improbable that the carboxyl groups were placed at positions 23,29 and 30.

On treatment with bromine, cincholic acid failed to give a bromolactone, as is typical for β-amyrin derivatives[6] having a a double bond at position C-12 and a carboxyl function at C-17. However, the pyrocincholic acid (3) with bromine formed a α-lactone (5) as does pyroquinovic acid[7](6).

Cincholic acid (1=R=R'=R"=H), on treatment with ruthenium tetraoxide, behaved as a Δ^{12}-triterpenoid[3] whilst pyrocincholic acid (3; R=R'=H) was cleaved to give the cyclodecane dione (7).

Dimethyl cincholate acetate (1; R=Ac, R'=R"=CH_3) on treatment with selenium dioxide gave a small yield of the diene (8), a reaction also characteristic of the β-amyrin series[8]. Under the same conditions dimethyl quinovate acetate (2; R=Ac, R'=R"=CH_3) failed to give any diene.



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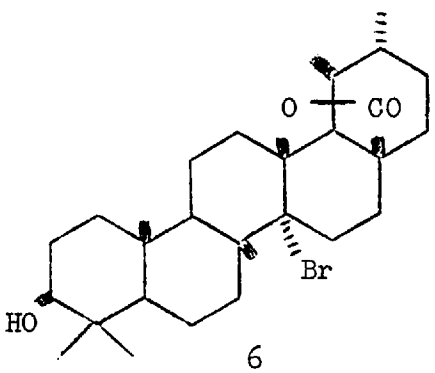

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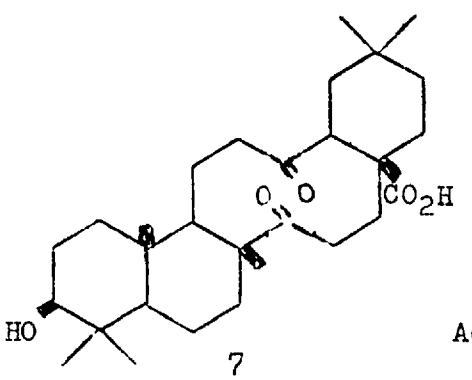

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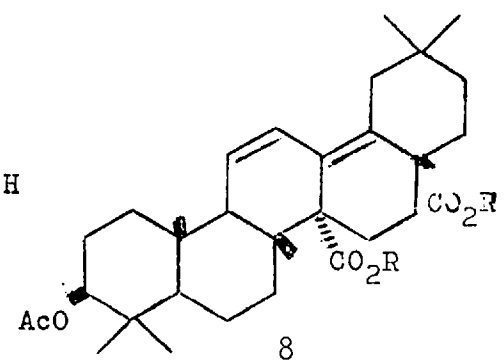

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The infrared spectrum[9] of cincholic acid was found to be similar to oleanolic acid in much the same way that quinovic acid was related to ursolic acid.

With these facts in hand the authors concluded that cincholic acid is the β-amyrin analogue of quinovic acid, the carboxyl group which is removed as carbon dioxide on heating being placed at C-14 and the second group at C-17.

The chemical differences between β-amyrin and α-amyrin are based on the steric effect of the C-19 methyl group in α-amyrin. This exerts a strong shielding and crowding effect on the double bond. In cincholic acid the carboxyl group at C-17 must have a similar effect, because it hinders the formation of the bromolactone[6] and makes difficult the selenium dioxide reaction[10].

The mechanism proposed for the migration of the double bond in quinovic acid[7,8] (2, R=R'=R"=H) from C-12 to C-13(14) during dicarboxylation is considered valid for the

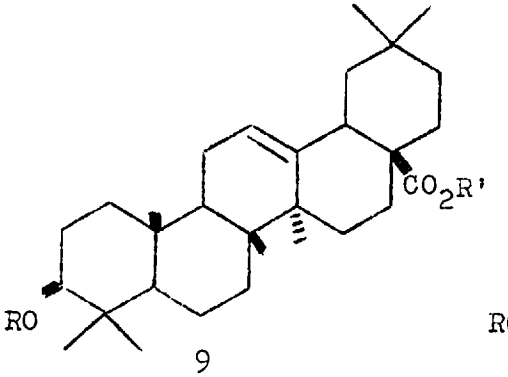
cincholic acid (1, R=R'=R"=H)-pyrocincholic acid (3; R=R'=H) conversion.

The formulation, then, of the bromolactone as (5) would be right and is supported by the molecular rotation differences between cincholic acid - pyrocincholic acid and quinovic acid - pyroquinovic acid.

With these details in hand attempts were made to reduce the carboxyl at C-14 to a methyl group in order to obtain a relation between cincholic acid (1, R=R'=R"=H) and oleanolic acid (9, R=R'=R"=H).

On lithium aluminium hydride reduction of dimethyl cincholate (1; R=H,R'=R"=CH_3), only the C-17 carboxyl group (10; R=R"=H, R'=CH_3) was reduced as also occurred with dimethyl quinovate (2; R=H,R'=R"=CH_3). That the C-17 group was reduced was confirmed by the reduction of methyl oleanolate (9; R=H, R'=CH_3) which gave quantitatively, erythrodiol (11, R=R'=H). It was deduced that the carboxyl group at C-14 is highly hindered because of the cis linkage of ring D and E.

On treatment with lithium iodide in



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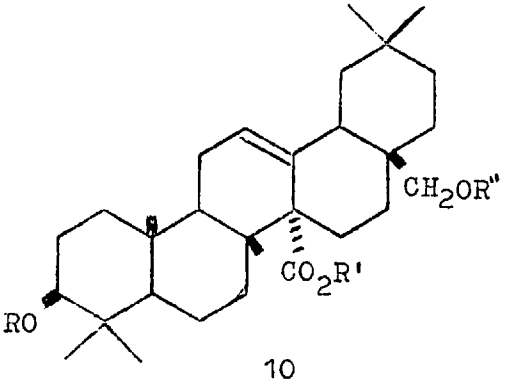

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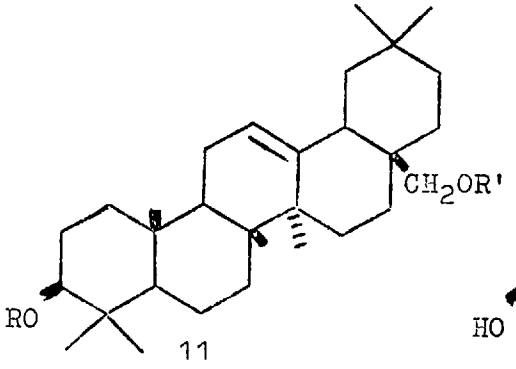

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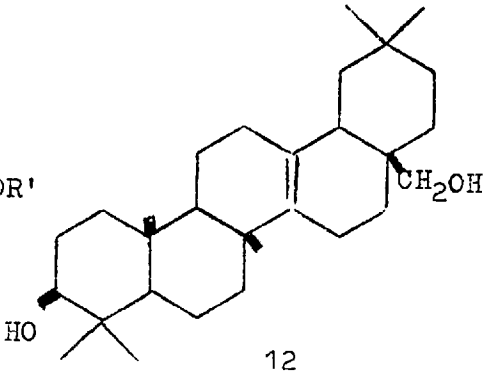


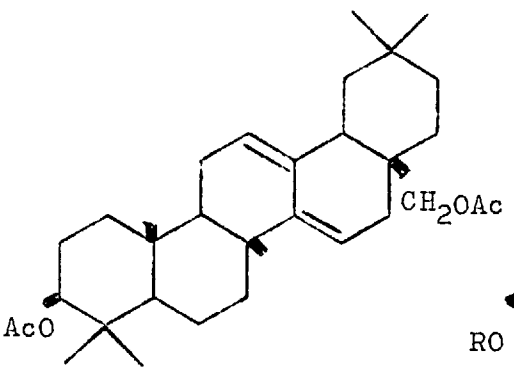
12

Collidine[11] the ester diol (10; R=R"=H, R'=CH_3) gave the acid diol (10; R=R'=R"=H) and the 14-nor diol (12).

The acid diacetate (10; R=R"=Ac,R'=H) on attempted Rosenmund's reduction[12] gave the diene[13] (13) instead of the expected aldehyde.

However, the dimethyl ether of the diol (10; R=R'=R"=CH_3), with lithium iodide in collidine gave the dimethyl ether acid (10; R=R"=CH_3,R'=H) which on treatment with thionyl chloride gave an acid chloride that on lithium aluminium hydride at 0° gave the hydroxy dimethyl ether (14). This hydroxy dimethyl ether was treated with p-toluenesulphonyl chloride to obtain the tosylate which on reduction with lithium aluminium hydride afforded the dimethyl ether of erythrodiol (11; R=R'=CH_3) identical with an authentic specimen.

Thus cincholic acid is 3 β-hydroxy-olean-12-ene-27,28-dioic acid (1; R=R'=R"=H).



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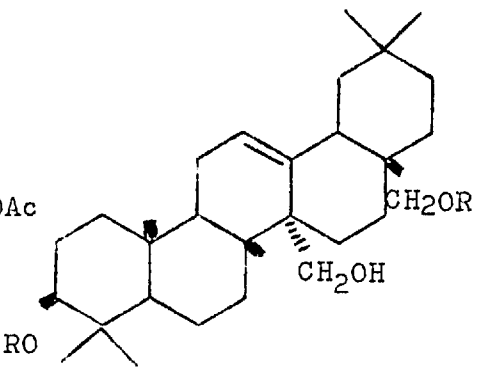


14

C). Quinovic acid

The isolation of quinovic acid from Cinchona bark wasfirst reported by Pelletier and Caventou in 1821 and has been subsequently isolated from Zigophyllum and Mitragyna species by many investigators.

The structure of quinovic acid was assigned as (15; R=R'=R"=H) through the extensive efforts of Wieland[14-19,26-27,32], Ruzicka[20-24,28-30,35-36,38], Jeger[23,30,33,36,38], and their colleagues and finally to Barton and de Mayo[34].

In 1884 Liebermann[25] suggested that quinovic acid was a triterpenoid but evidence for this was not forthcoming until Wieland, Hartmann and Dietrich[26] had pyrolysed quinovic acid with selenium. They obtained the same hydrocarbon which they had isolated previously by a similar treatment of gypsogenin.

Wieland and Erlenbach[27] were able to show that quinovic acid was a monohydroxy dicarboxylic acid of formula $C_{30}H_{46}O_5$. They confirmed that quinovic acid (15; R=R'=R"=H) on heating loses carbon dioxide to give a pyroacid $C_{29}H_{46}O_3$ (16; R=R'=H).

Furthermore, they also confirmed that on

treatment of quinovic acid with sulphuric acid dehydration with loss of one molecule of water occurs to give a new compound called novic acid (17; R=H).

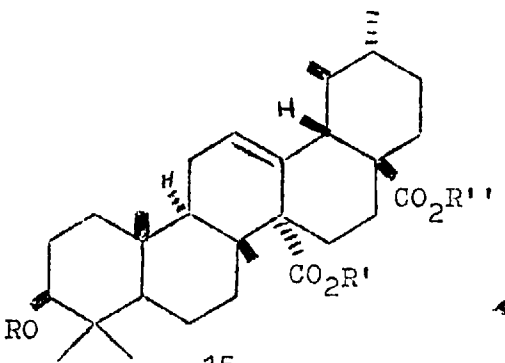
The presence of unsaturation in quinovic acid was detected by Ruzicka and Prelog[28], who found that the dimethyl ester (15; R=H, R'=R"=CH_3) and dimethyl ester acetate (15; R=Ac,R'=R"=CH_3) gave a positive test with tetranitromethane.

That quinovic acid belonged to the α-amyrin class of triterpenes and contains two carboxyl functions at C-14 and C-17 was shown as follows.

Ruzicka and Marxer[29] obtained dimethyl quinovenonedioate (18) by oxidation of dimethyl quinovate (15; R=H,R'=R"=CH_3).

The semicarbozone of this compound on Wolff-Kishner reduction gave dimethyl desoxy-quinovate (19). These reactions showed that the alcohol group is secondary.

The structure of ring A was proved by Ruzicka, Szpilfogel and Jeger[30] when they treated dimethyl quinovate with phosphorus



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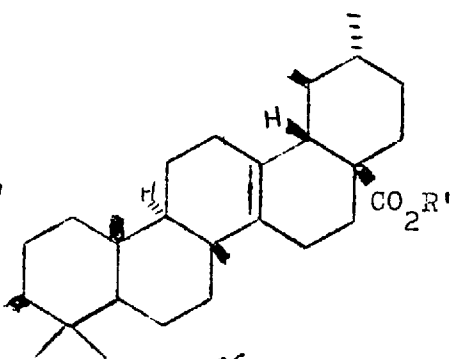

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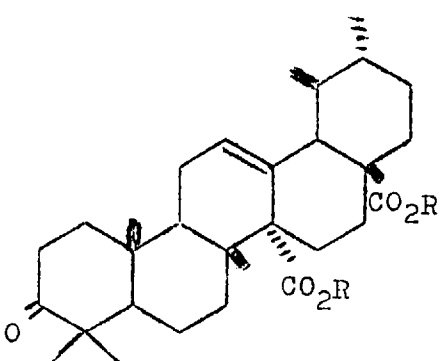

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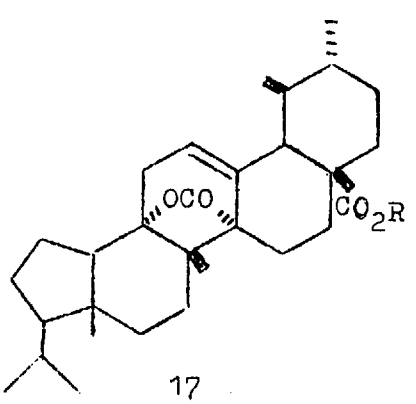

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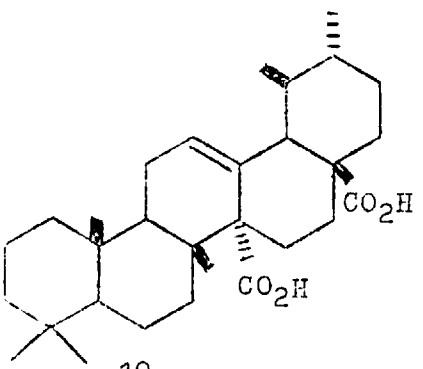
pentachloride to give a diene-ester (20) with loss of water. The ester had two isolated double bonds and on treatment with osmium tetroxide gave a diol (21) which on oxidation gave the keto-ester (22) and acetone, as in the lupeol series[31].

As mentioned above treatment of quinovic acid (15; R=R'=R"=H) with concentrated sulphuric acid gave a new substance called novic acid[25] (17; R=H). Later on Wieland and Hoshino[32] found that novic acid was also prepared by treatment of quinovic acid in acetic acid with zinc chloride. They also suggested that this formation involved a molecular rearrangement because alkaline hydrolysis gave back instead of quinovic acid, an unsaturated compound, anhydroquinovic acid (23; R=R'=H).

On the basis of the observation of Ruzicka, Szpilfogel and Jeger[30] that quinovic acid was converted by treatment with phosphorus pentachloride into a cyclopentane derivative (20) and that the formation of novic acid (17; R=H) required acidic treatment, Jeger[33]



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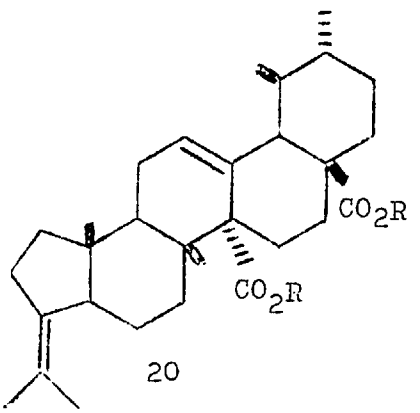

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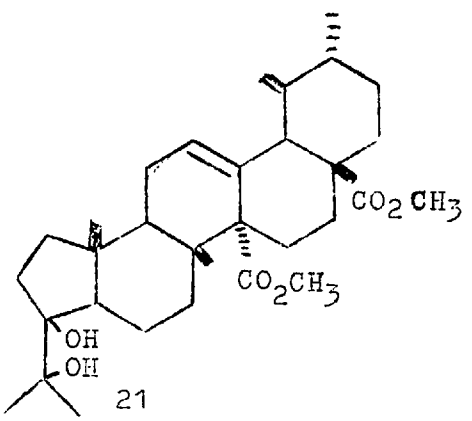

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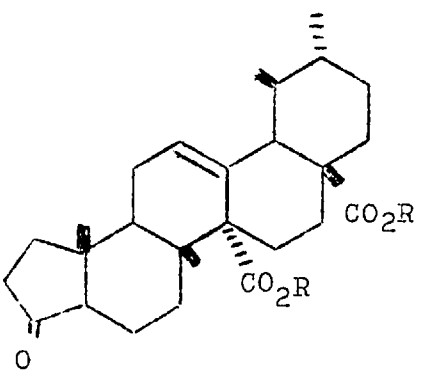

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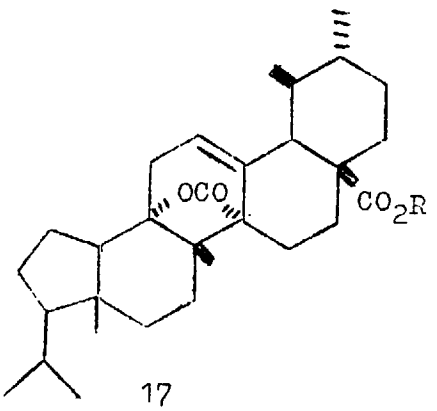
proposed the structure (24) for novic acid. Barton and de Mayo[34] considered this formula improbable for the following reasons. a) It involves a very stable δ -lactone ring. b) The formula is postulated as being formed by non-Markownikoff addition to (25). c) The formula does not explain why the acid anhydride which would be (26) on the basis of (24), of the dicarboxylic acid derived from the nova-quinone (see later), gave two ketones now represented as (27) and 28) on pyrolysis.

They considered that novic acid is more correctly represented by (17; R=H) and that it is formed according to the following mechanism (29). The evidence for the structure (17; R=H) of novic acid was the presence of a γ-lactone band in the infrared, instead of a δ -lactone which Jeger's formula would require and that anhydroquinovic acid (23; R=R'=H) has a very strong positive rotation ($[\alpha]_D$+310°) and λ_{max} 294 mμ (ε 6000). The two facts are in agreement with formula (17; R=H), a pentacyclic triterpenoid in which ring



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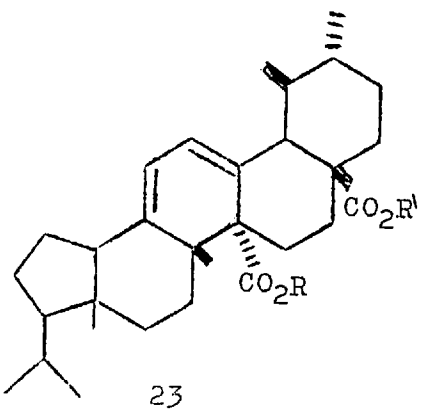

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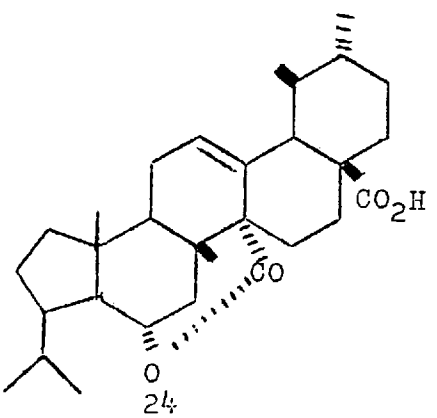

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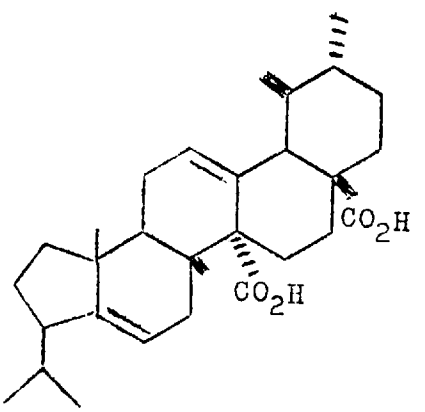

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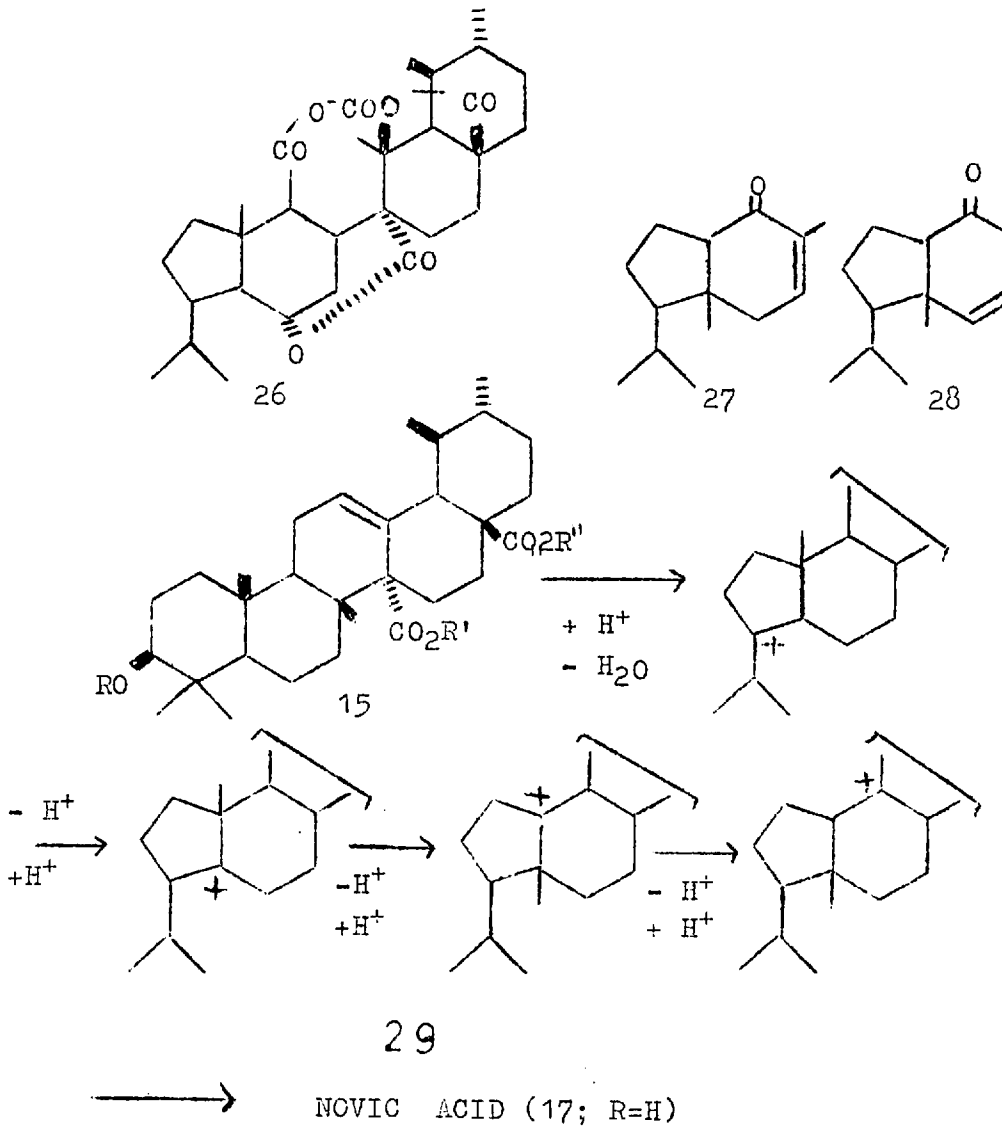
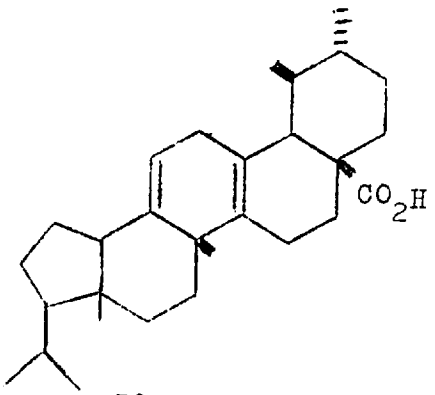

O⁻COO
CO
CO
CO
O
26
O
27
O
28
CO2R''
CO2R'
RO
15
+ H+
- H2O
- H+
+H+
-H+
+H+
- H+
+ H+
29
NOVIC ACID (17; R=H)

C is diunsaturated. Jeger's interpretation for novic acid would give anhydroquinovic acid as (25) with two isolated double bonds. Wieland and Erlenbach[27] showed that anhydroquinovic acid (23; R=R'=H) was β - γ -unsaturated as pyrolysis gave a pyroanhydroquinovic acid which Barton and de Mayo[34] formulated as the non-conjugated diene (30) because it does not show any characteristic ultraviolet absorption.

Wieland and Hoshino[32] found that chromic oxidation of novic acid (17; R=H) gave a yellow substance called novaquinone (31). This was shown to be a 1,2-diketone by oxidation with hydrogen peroxide[26] when a dicarboxylic acid (32) formed. The anhydride of this acid (33) upon pyrolysis[35-36], gave a neutral oil containing two ketones, formulated as (27) and (28). These two ketones provide evidence for the structure of rings A and B in agreement with that of novic acid (17; R=H) given by the work of Barton and de Mayo[34].

The acidic fraction of the pyrolysis[35-36]



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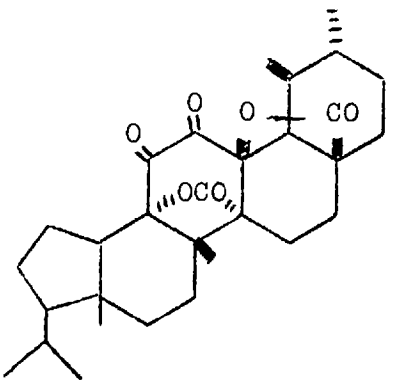

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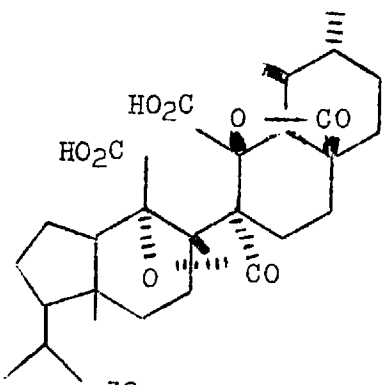

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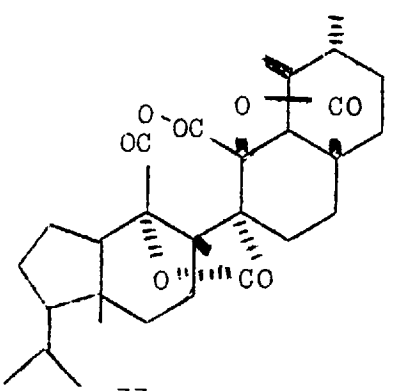

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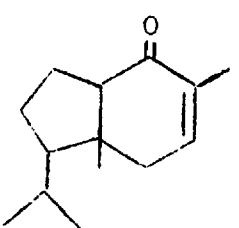

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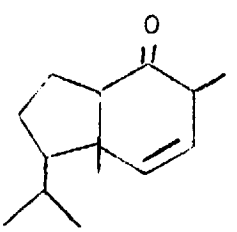

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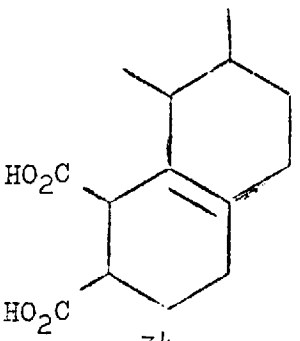
of the anhydride (33) yielded two isomeric acids (34) which gave the same anhydride. Selenium dehydrogenation gave 1,2-dimethyl naphthalene (35) and an anhydride of 1,2-dimethyl naphthalene-7,8-dicarboxylic acid[36] (36).

This series of experiments proved the position of the carboxyl group involved in the lactonization of novic acid (C-27) and the structure of rings D and E.

The position of the unreactive double bond in quinovic acid (15; R=R'=R"=H) was placed at C-12 - C -13; this position is supported by the easy elimination of the carboxyl group at C-14 to give pyroquinovic acid (16; R=R'=H) with a shift of the double bond, as observed in morolic acid by Barton and Brooks[8]

Barton and de Mayo[34] have made a critical study of the position of the carboxyl group placed at C-17 in quinovic acid (15; R=R'=R"=H). They considered equally possible the structure (37).



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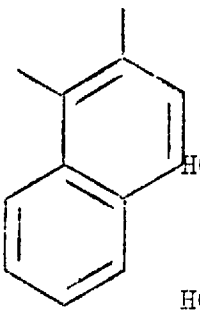

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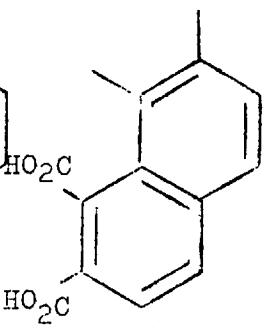

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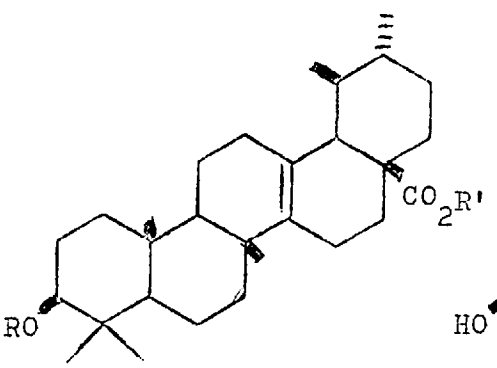

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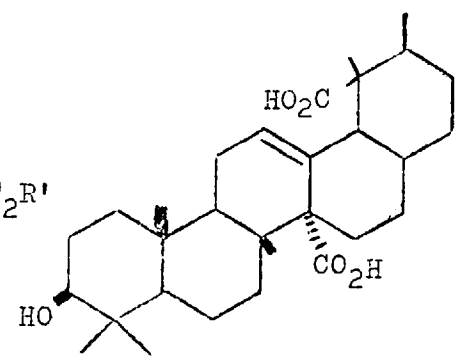
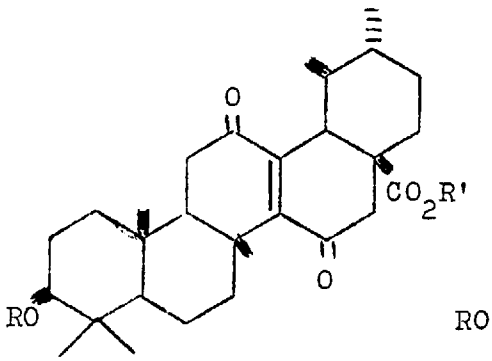


37

However, by the following series of experiments they proved that (15; R=R'=R"=H) is the right interpretation.

Oxidation of methyl pyroquinovate acetate (16; R=Ac,R'=CH_3) gave methyl diketo-pyroquinovate acetate (38; R=Ac,R'=CH_3) which on zinc-acetic acid reduction afforded the dihydro derivative (39; R=Ac,R'=CH_3). Bromination of the methyl diketopyroquinovate acetate (38; R=Ac, R'=CH_3) gave the dibromo compound (40; R=Ac,R'=CH_3) previously prepared by Wieland, Hartmann and Dietrich[26]. Barton and de Mayo assigned to it the formula (40; R=Ac,R'=CH_3) because when they treated this compound with zinc-acetic acid it gave the methyl dihydro-diketopyroquinovate acetate (39; R=Ac,R'=CH_3) which was oxidised back to the ene-dione by digestion with potassium methoxide. Re-esterification and re-acetylation gave back methyl diketopyroquinovate acetate (38; R=Ac,R'=CH_3). This evidence showed that bromination had occurred without any molecular rearrangement.

When methyl dibromodiketopyroquinovate



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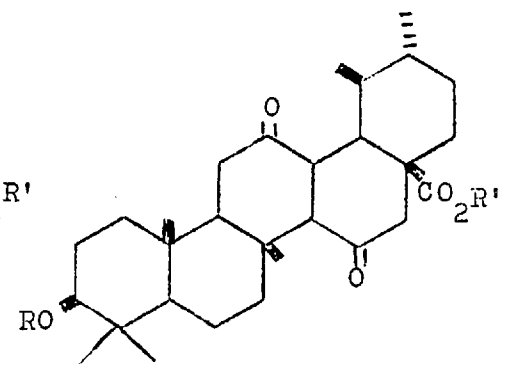

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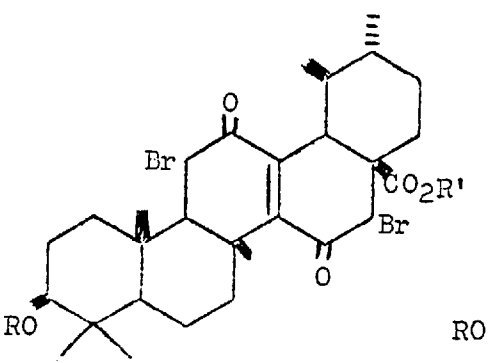

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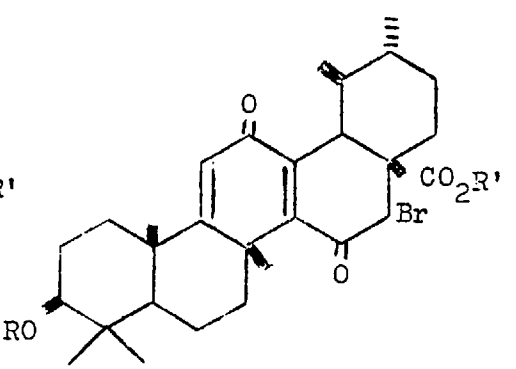
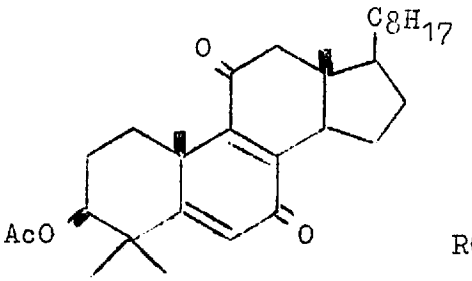


41

acetate (40; R=Ac,R'=CH_3) was treated with silver nitrate and pyridine or with refluxing pyridine or collidine it gave methyl monobromo diketopyroquinovate acetate (41; R=Ac, R'=CH_3). They assigned this structure from a comparison of the infrared and ultraviolet spectra with those of 7,11-diketo lan osta-5,8-dienyl acetate[37] (42).

Upon vigorous treatment by collidine at 210°, in a sealed tube, both the dibromo (40; R=Ac,R'=CH_3) and the monobromo compounds (41; R=Ac,R'=CH_3) were converted into the phenol (43; R=R'=Ac), characterized as its diacetate and through its infrared and ultraviolet spectral properties.

The resistance of the dehydrobromination, except under forcing conditions is explained much better by the formula based on quinovic as (15; R=R'=R"=H) instead of (37). More decisive evidence was obtained when dibromo-diketopyroquinovid acid acetate (40; R=Ac,R'=H) after treatment with silver nitrate and pyridine at room temperature, furnished the phenol (43). In this reaction the quinovic



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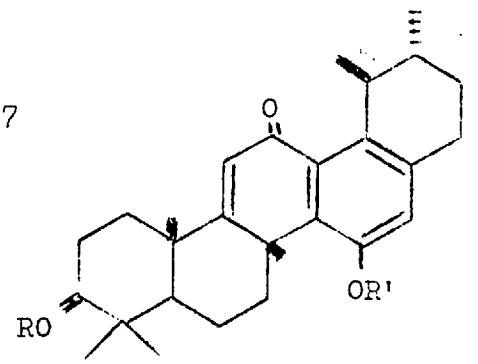

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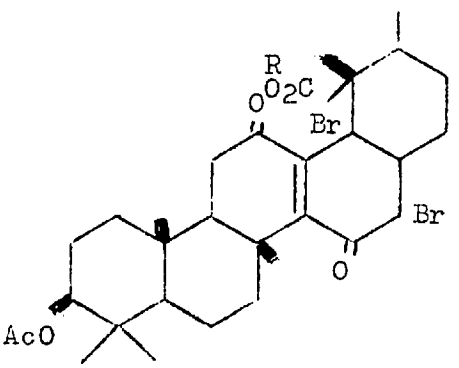

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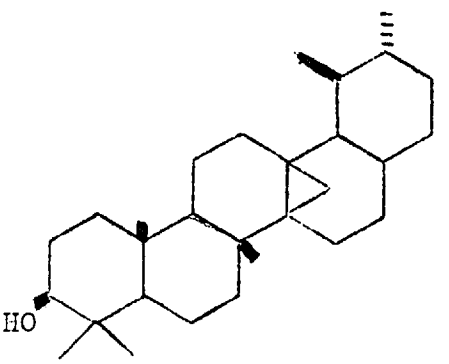

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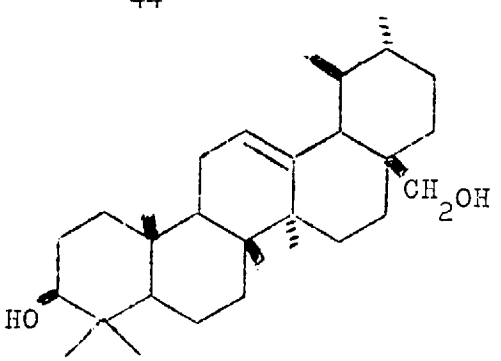


46

acid formulated as (37) would not give a phenol since its dibromodiketo acid derivative would be (44), whilst structure (15; R=R'=R"=H) for quinovic acid can explain this behaviour.

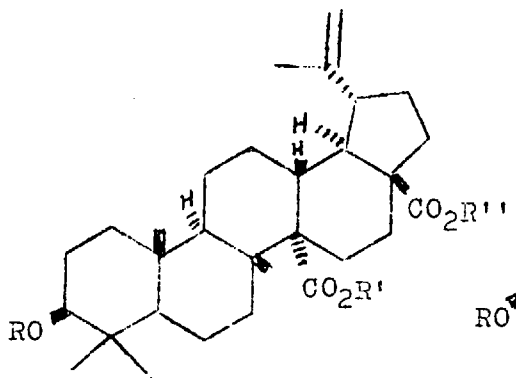
Finally Zürcher, Jeger and Ruzicka[38] completely settled the structure of quinovic acid (15; R=R'=R"=H) with the preparation of uvaol (46) from quinovic acid via phyllantol (45).

Thus quinovic acid is 3 β -hydroxyursan-12-ene-27,28-dioic acid (15; R=R'=R"=H).

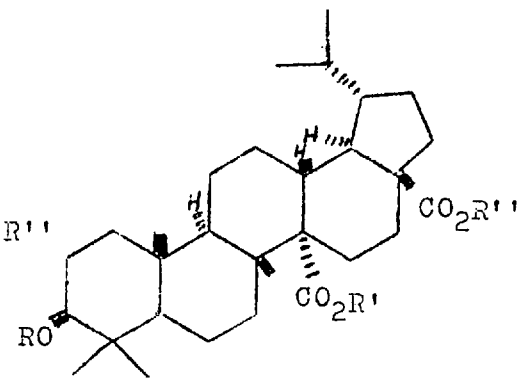
d) Melaleucic acid.

This acid was isolated, along with betulinic acid from Melaleuca species[39]. It is a hydroxy dicarboxylic acid, forming monoacyl derivatives and a dimethyl ester. Dimethyl melaleucate (47; R=H;R'=R"=CH_3) contains a methylene group which absorbs in its infrared spectrum at 885 cm^{-1}. This methyl ester (47; R=H,R'=R"=CH_3) is reduced catalytically to dimethyl dihydromelaleucate (48; R=H,R'=R"=CH_3) and with osmium tetraoxide it affords a glycol (49; R=H,R'=R"=CH_3) which on oxidation with periodate gave a methyl ketone (50; R=H,R'=R"=CH_3).

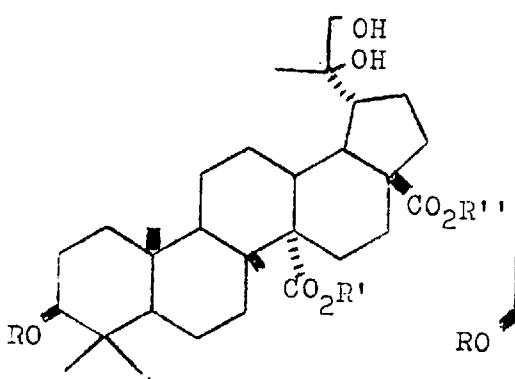
This demonstrates the presence of an isopropenyl group in dimethyl melaleucate. The attachment of the isopropenyl group to a five membered ring was demonstrated by degradation of the methyl ketone acetate (50; R=Ac, R'=R"=CH_3), by the method of Voser, Jeger and Ruzicka[40], to an acetoxytrisnorketone, which contained a five membered-ring carbonyl with a neighbouring methylene group.


H
H
H
CO_2R''
CO_2R'
RO

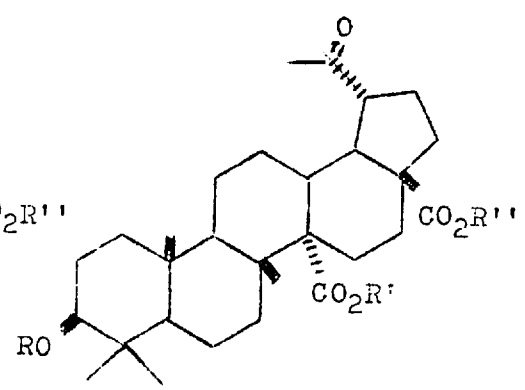
47


H
H
CO_2R''
CO_2R'
RO

48


OH
OH
CO_2R''
CO_2R'
RO

49

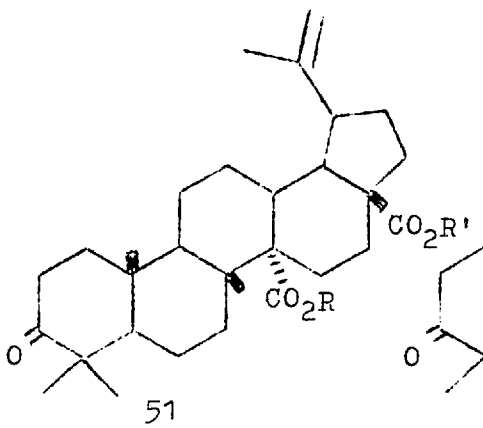

O
CO_2R''
CO_2R'
RO

50

From this it appeared that melaleucic acid is a lupane derivative. This is supported by the fact that the keto-ester obtained by oxidation of dimethyl melaleucate, gave a positive Zimmermann test[6], indicating that the hydroxy group is attached at C-3. Spectroscopic evidence showed that this hydroxyl was attached in the β-position and also that the keto acid (51; R=R'=H) forms a δ-lactone (52; R=H) on treatment with acid, as does betulinic acid[41], showing that one carboxyl group must be at C-17.

Chopra et al.[42] has shown by n.m.r. that the other carboxyl group was placed at C-14, since the signals of the C-27 and C-28 methyl groups in melaleucic acid were absent on comparison to the n.m.r. information found by Lehn and Ourrison[43] in the lupane series.

The lactone formed by acid treatment of methyl dihydromelaleucate (48; R=H,R'=R"=CH_3) first prepared by Arthur et al.[39], was later assigned the structure[42] (54; R=H). On alkaline hydrolysis this lactone did not give back



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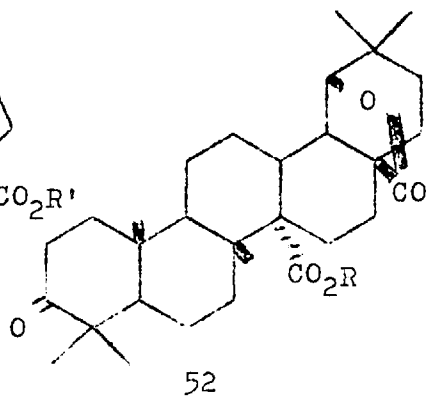

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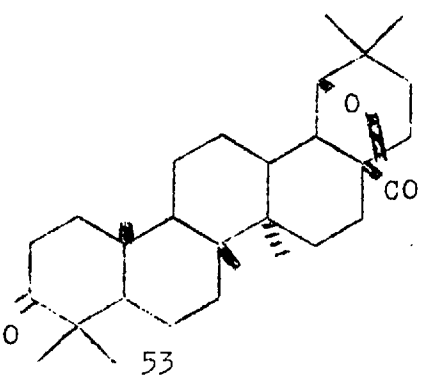

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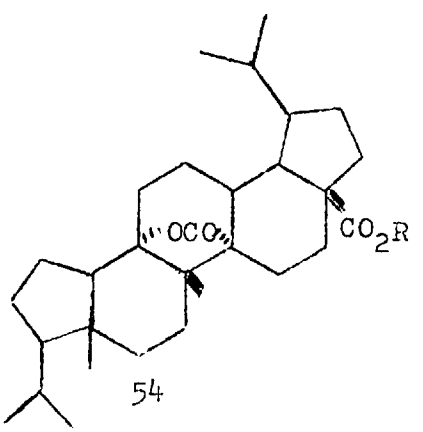

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dihydromelaleucicacid, yielding instead an unsaturated acid containing a trisubstituted double bond. This acid was readily converted back to the original lactone (54; R=H) by treatment with acid. Hence it was concluded that a rearrangement had taken place during the initial lactonization. This is already known in the quinovic acid - novic acid transformation[34] and it was suggested by Barton that a similar rearrangement could take place in this system.

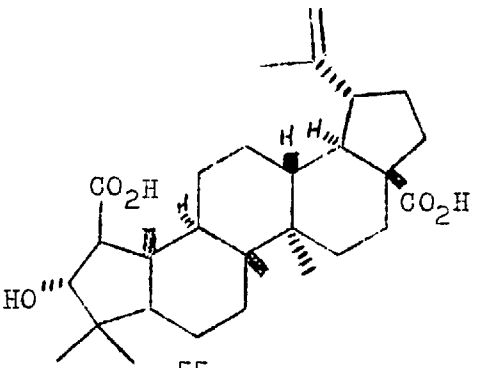
Thus melaleucic acid (47; R=R'=R"=H) was formulated by Chopra et al.[42] as 3β - hydroxylup-20(29)-ene-27,28-dioic acid. This structure was further confirmed by an X-ray crystallographic investigation of dimethyl melaleucate iodoacetate.

e) Ceanothenic acid.

Investigations on the triterpenic constituents of Ceanothus americanus were first initiated by Julian, Pikl and Dawson[44].

De Mayo and Starratt[45-46], whilst working on the isolation of ceanothic acid (55) from Ceanothus americanus also isolated a number of simple aromatic acids together with lupeol, betulin, betulinic acid and a new acid they called ceanothenic acid (56; R=R'=H). Ceanothenic acid analysed for $C_{29}H_{42}O_4$ or $C_{30}H_{44}O_4$ and was a dicarboxylic acid.

It gave a dimethyl ester converted to the acid by treatment with lithium iodide in collidine[11] and gave a tetrahydro acid (57; R=R'=H) on hydrogenation over platinum oxide, which, on methylation gave a dimethyl ester. Both the latter ester (57; R=R'=CH_3) and the acid (57; R=R'=H) were optically transparent in the ultraviolet absorption region of 195-220 mμ and gave no colour with tetranitromethane. Thus, ceanothenic acid (56; R=R'=H) contained two ethylenic linkages and was pentacyclic. The tetrahydro acid (57; R=R'=H)



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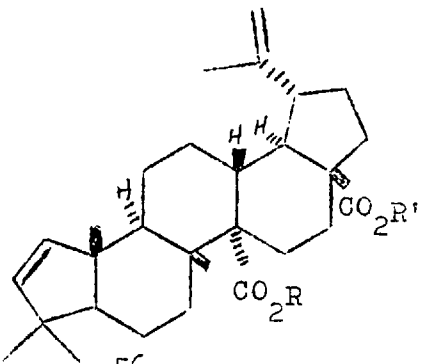

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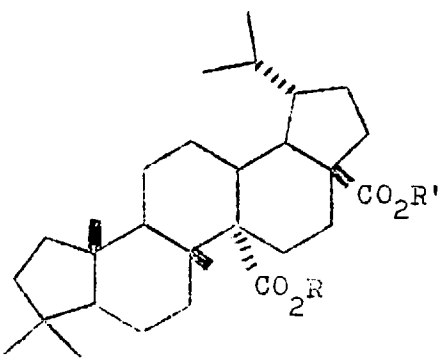

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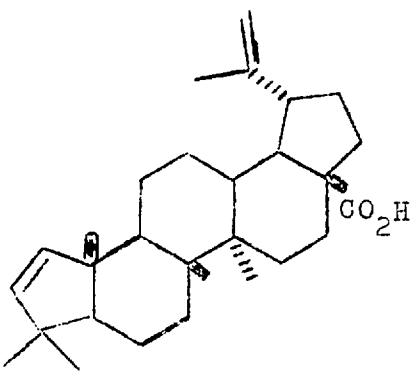


58

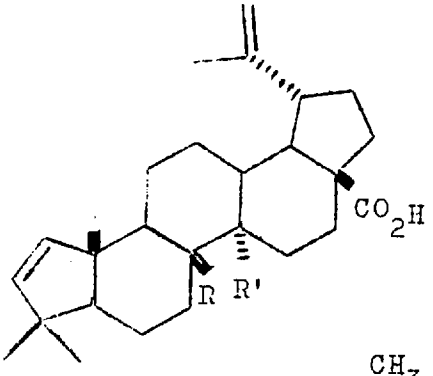
was converted into the acid chloride and then treated with lithium aluminium hydride to give the saturated diol, characterized as the diacetate and, by oxidation as the dialdehyde.

In its infrared absorption spectrum ceanothenic acid (56; R=R'=H) had two absorption bands, at 893 and 753 cm^{-1}. The former band was in the region expected for methylene in an isopropenyl group. This assignment was confirmed by the production of formaldehyde upon ozonolysis of ceanothenic acid and by the presence, in the n.m.r. spectrum of its dimethyl ester, of bands at τ 5.29 and 5.41(two multiplets, 2H, $>C=CH_2$) and 8.34 (singlet, 3H) for the methylene and methyl of the isopropenyl group. The band at 753 cm^{-1} was due to a cis disubstituted ethylenic linkage. In the n.m.r. spectrum there were two doublets, at τ 4.66 and 4.11 (J 5.7 cps.)(2H), in accord with the presence of vicinal vinyl hydrogen atoms. The similarity of this pattern to that obtained from the compound (58), derived from ceanothic

acid (55) by dehydration and decarboxylation, was indicative of a like environment for the double bond in the ceanothenic acid. The molecular weight of ceanothenic acid, determined mass spectroscopically on the dimethyl ester of the tetrahydro acid (57; R=R'=H), indicated that it contains only 29 carbon atoms. The latter with former n.m.r. evidence indicated that ring A was contracted. At this point the authors considered as a possible structure (59; Ror R'=CO_2H) and sought chemical evidence in confirmation.

Methyl ceanothenate, on treatment with osmium tetroxide, gave the expected tetrol. Cleavage with periodic acid in methanol solution, with the appropriate oxygen uptake, gave a crystalline product differing from that expected by the elements of methanol. Finally, any aldehydic structure could be rejected from the observed n.m.r. spectrum. The presence of the acetyl group at C-19 was indicated by a singlet (3H) at τ 7.84 but a singlet at τ 6.57 showed the presence of a methoxyl

function. In addition there were bands (1 H each) at τ 5.84 and 5.43 both indicative of a methine next to an oxygen function. This pattern was compatible with acetal and hemiacetal hydrogens of the structures (60) and (61). That the hemiacetal structure can equilibrate with the dialdehyde form was demonstrated by the formation, under acidic conditions, of the tris-2,4-dinitrophenylhydrazone. When cleavage of the tetrol was carried out in aqueous dioxan, the non-crystalline product, showed bands at τ 0.85 and 0.73 compatible with aldehydes bearing no α hydrogen atoms. The infrared spectrum showed hydroxyl absorption at 3360 cm^{-1}, probably due to some of the tautomeric hemiacetal (62). Treatment with 2,4-dinitrophenylhydrazine in acid gave the same tris-2,4-dinitrophenylhydrazone as obtained previously. When the hemiacetal (62) was heated in aqueous methanol containing 2% sodium hydroxide followed by acidification a δ lactone formed, either (63) or (64). The formation of the



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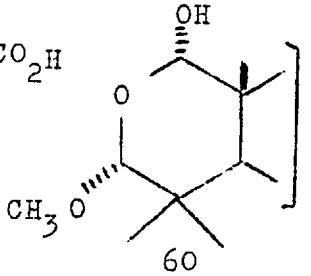

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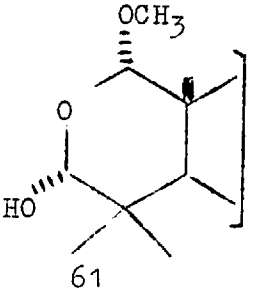

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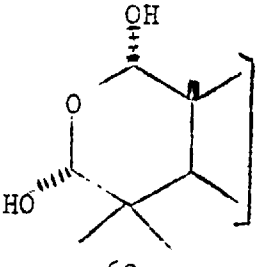

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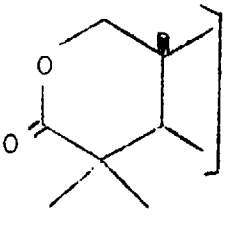

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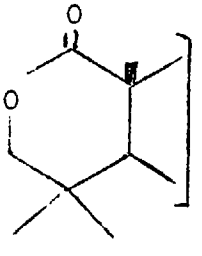

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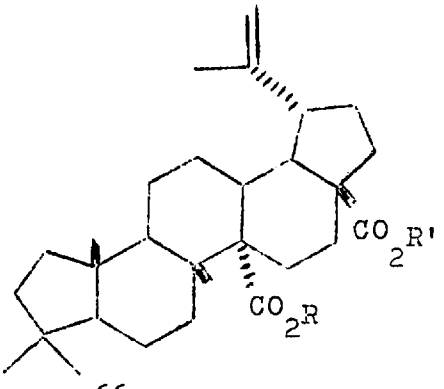
lactone by an internal Cannizzaro reaction, has analogy in the chemistry of the monoterpenoid iridodial[47]. The transformations described established the nature of ring A and excluded from consideration positions 23, 24 and 25 for the unplaced carboxyl group. Had this carboxyl group been present at any one of these points a very facile deformylation would have been expected in the cleavage product, in view of its nature as an α formyl ester, on treating to the conditions of the Cannizzaro reaction. Two positions C-8 and C-14 remained for the second carboxyl function.

In order to decide at which of the two possible positions the carboxyl group was placed an approach was used in which a function was introduced into ring C so that selective decarboxylation could be effected. The introduction of such a group lay in the known conversion of substances of the lupeol series into that of β -amyrin by acid catalysed expansion of the ring E, with, in the case of substances having a carboxyl at C-17, subsequent lactonization[41]. When a solution of ceanothenic acid was treated with acid,

a neutral product was obtained involving both carboxyl groups in the lactonization (65). The infrared and n.m.r. spectra proved that the methylene group was not present. Since the formation of the dilactone (65) involved rearrangement in both ring E and ring A no relevant information could be obtained from it. (The structure of this dilactone is discussed below). Thus to selectively introduce a function into ring C the prerequisite was to first remove the unsaturation of ring A. A very elegant method for the selective reduction of this double bond was achieved using diimide as reducing agent, which left the methylene group untouched. The dihydroceanothenic acid (66; R=R'=H) formed, after acid treatment gave the acid lactone (67; R=H) which was reduced to the diol (68) with lithium aluminium hydride. Dehydration and selenium dioxide oxidation gave only a small amount of material having unsaturation in ring C. At this point in the work, it was reported that the oxidation of compounds of the lupeol-betulin class with mercuric acetate gives the corresponding C-12 olefine.[48] Oxidation of dimethyl dihydroceanothenate (66;R=R'=CH_3) gave the olefine (69) which, on selective hydrogenation, gave the compound (70; R=R'=CH_3) with the isopropenyl group saturated.

This dimethyl ester (70;R=R'=CH_3) was treated with lithium iodide[11] to give the



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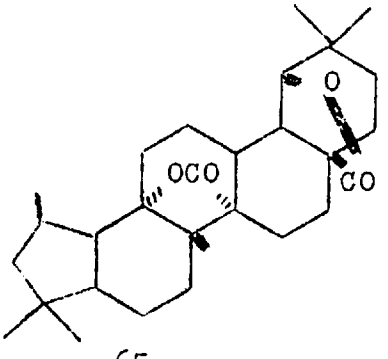

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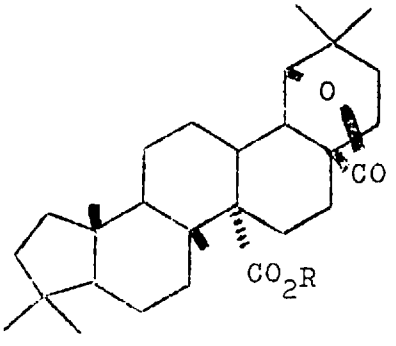

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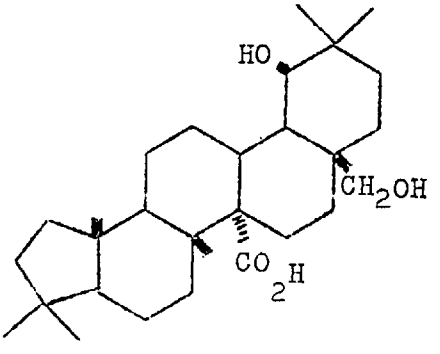

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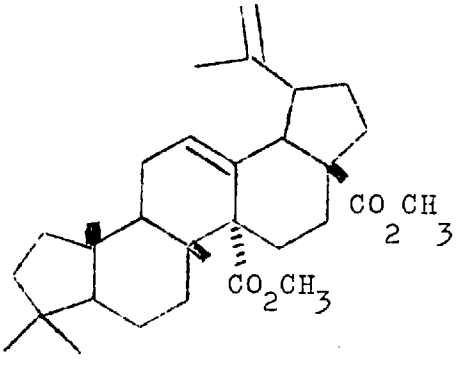
corresponding diacid (70; R=R'=H) which evolved gas at its melting point, as expected of a β,γ-unsaturated acid[8], yielding the pyro-acid (71).

Further information on the position of the second carboxyl function was found when oxidation of the diester (70; R=R'=CH_3) gave the 11 ketone (72; R=R'=CH_3) which, on hydrolysis, gave the dicarboxylic acid monoester (72; R=H,R'=CH_3). This acid, on heating at 145°, decarboxylated giving the keto ester (73).

These results were accounted for by placing the second carboxyl group at C-14. The ethylenic linkage of compound (73) was placed at C-12 - C-13 because it retained its α,β-unsaturated ketone moiety.

With this clear idea about the structure of the ceanothenic acid (56; R=R'=H) the authors were able to find out the structure of the dilactone (65). Since the direct lactonization from C-27 to C-1 or C-3 is sterically impossible, rearrangement must occur on dilactonization[34]. Two structures may be considered (65) and (74), the formation of



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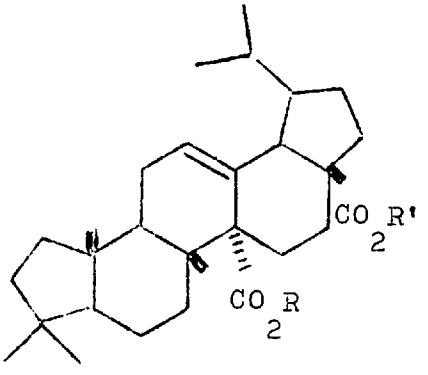

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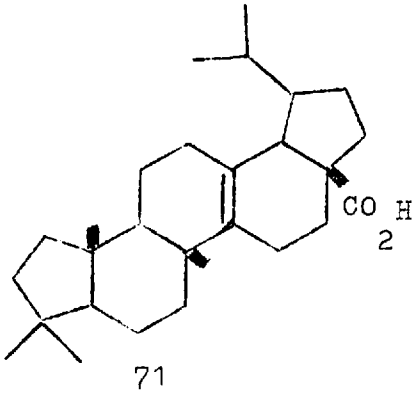

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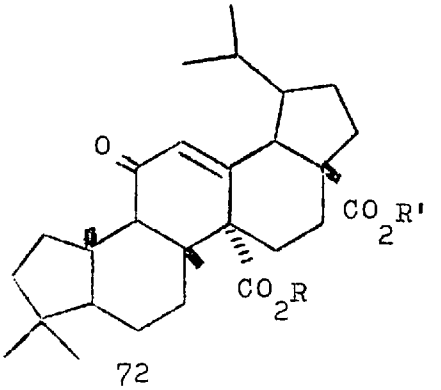

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both of which involves only generation of tertiary carbonium ions after initial migration. These are derived by protonation at either end of the cyclo pentene ethylenic linkage.

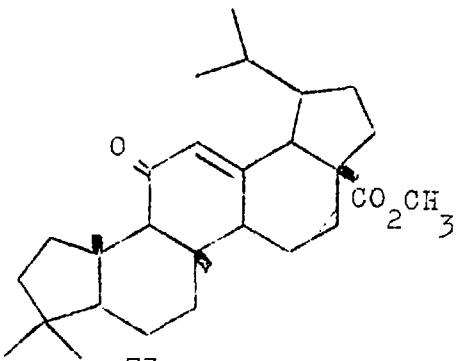
These two formulations differ in the number of potential acetic acid molecules obtained by Kuhn-Roth oxidation.

The monolactone (67; R=H) has two tertiary methyl groups and two geminal dimethyl groups, as has the dilactone (65).

The alternative dilactone (74), however, has four methyl groups and one geminal dimethyl group. Since geminal dimethyl functions are known to give only a small amount of acetic acid (74) would be expected to give a greatly increased Kuhn)Roth yield of acetic acid over the monolactone (67; R=H). In fact the monolactone gave 2.3 C-Me and the dilactone 2.2.

On this basis (74) could be excluded. The position of the (27→9)-lactone is that

found in the acid catalysed lactone formed from quinovic acid - novic acid[34].



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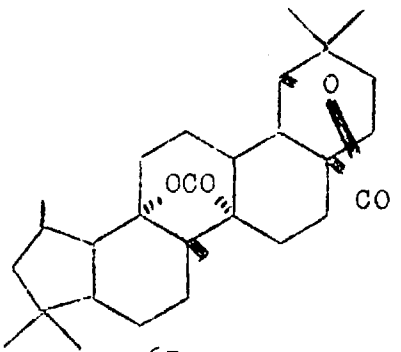

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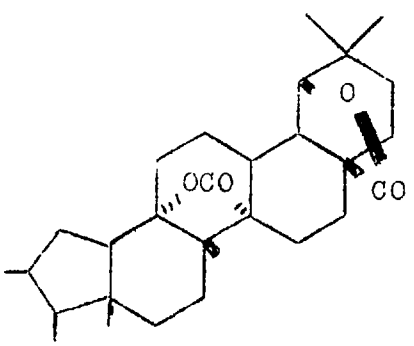

74

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SECTION II

The principal objects of the present work were twofold, the characterization of pyrethrol, a triterpenic constituent of pyrethrum extract, and the development of a synthetic route to the triterpene cincholic acid. The latter problem was the major objective and will be discussed initially. The scope of a new reductive elimination reaction is also discussed.

(a) Partial Synthesis of Cincholic Acid.

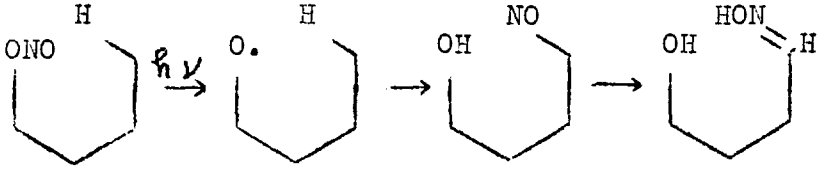
(i) Introduction

Cincholic acid (I; R=R'=R''=H) is related to oleanolic acid[1] (II; R=R'=H), the only difference being the presence of a carboxyl function at C_{14} instead of the methyl group as in oleanolic acid.

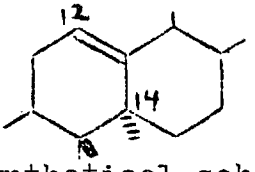
It was considered that if the methyl group at C_{14} in oleanolic acid could be functionalized then a synthetic route to cincholic acid would be opened.

One of the best methods of attacking methyl groups is by means of the Barton reaction[2].

This reaction requires a hydroxyl group in the δ-position to the methyl group. Providing steric conditions are satisfied photolysis of the nitrite ester of such a system would lead to an oximino-alcohol as in



Oxidation of the oxime to a nitrile and hence carboxylic acid function and elimination of the hydroxyl group would then lead to the system required for cincholic acid, viz.

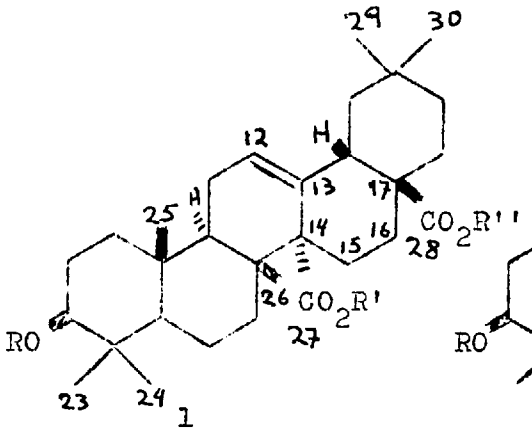


Such a synthetical scheme is aided by the knowledge that oleanolic acid is readily converted by peracids to a hydroxy-lactone[3] (III; R=H), which has a hydroxyl group at the 12 α position, suitably placed for the activation of the C_{14} methyl group.

(ii) Activation of the C_{27} position

The potential of such scheme was readily

realized. Treatment of oleanolic acid acetate[16] (II; R=Ac, R'=H) with peracetic acid afforded 12α -hydroxyoleanolic acetate lactone[3] (III; R=H) which gave 3β -acetoxy-13β -hydroxy-12α -nitrito-28-oleananic (28→13β)-lactone (III; R=No) by treatment with nitrosyl chloride in pyridine. Photolysis[2] of the nitrite ester (III; R=No) in dry benzene as solvent gave a mixture of compounds. Chromatography yielded 12-keto-oleanolic acetate lactone[4] (IV) (13.6%), 12α-hydroxyoleanolic acetate lactone[3] (III; R=H) (16-22%), 3β -acetoxy-12α ,13β -dihydroxy-27-oximino-28-oleananic (28→13β)lactone (V; R=Ac)(40-49%). The nuclear magnetic resonance spectrum showed the aldoxime hydrogen of (V; R=Ac) as a singlet (one H of C<u>H</u>=NOH) at 2.44 τ . In addition there was no signal at 8.85 τ corresponding to a methyl group as in 12 α -hydroxyoleanolic acetate lactone (III; R=H). Finally, there was isolated from the photolysis mixture the, unexpected, 3 β -acetoxy-12 α ,13 β -dihydroxy-27-nitrimino-28-oleananic(28→13β)-lactone (VI; R=Ac)(5%).



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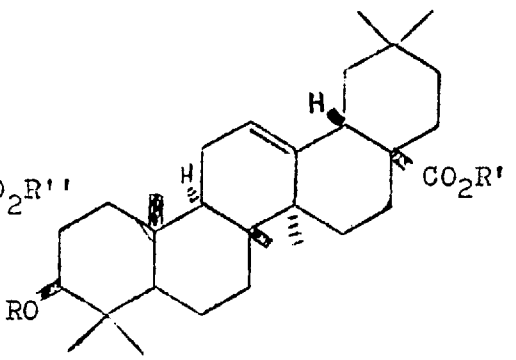

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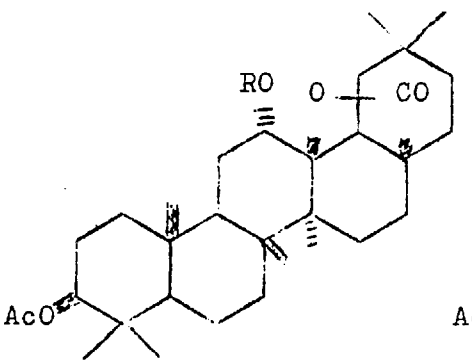

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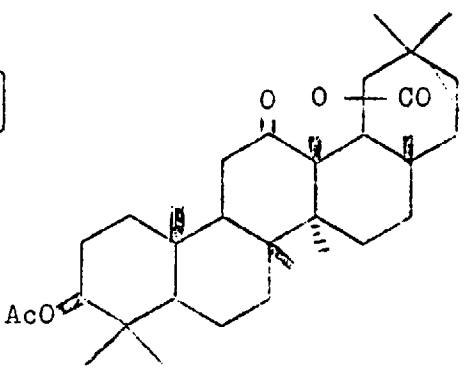
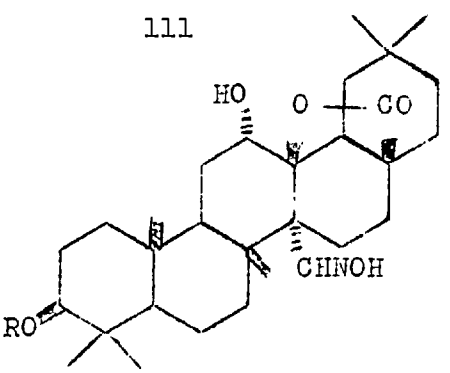


1V



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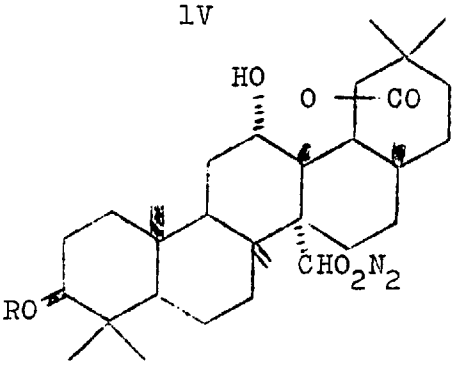


Vl

The next stage in the synthesis involved two problems, the re-introduction of the double bond at C_{12} and the transformation of the oxime function into a carboxyl group.

(iii) Studies on the introduction of the C_{12} double bond.

(a) Wolff-Kishner reduction of ketols. According to the results reported by several workers[5-12] an α ketol such as (VII) produces the olefin (VIII) in Wolff-Kishner reduction, the reaction proceeding according to the mechanism proposed by Barton, Holness and Klyne[13].

It was hoped that this reaction could be used in the introduction of the double bond in the cincholic acid synthesis. As a model compound 3β-acetoxy-13β-hydroxy-12-oxo-28-oleananic(28$\rightarrow$13β)-lactone (IV) prepared by the oxidation of 12α-hydroxyoleanolic acetate lactone (III; R=H) was chosen. This model has a similar environment at C_{12} and C_{13} as was anticipated for the actual synthesis. The 12 keto function in this compound (IV) is rather

hindered so the first conditions used for the reduction were those of the Barton modification[14] of the Wolff-Kishner reaction.

The reaction gave oleananolic acid[15] (IX; R=R'=H)(31.4%) instead of the expected oleanolic acid and a neutral alcohol (47%). The experiment was repeated under nitrogen in the presence of excess hydroquinone in order to avoid any oxygen participation, however the result was the same. Another attempted reduction was made by treating the lactone with base to make the sodium salt before distilling in the anhydrous hydrazine. This procedure only increased the yield of the neutral fraction and gave the same oleananolic acid. To check whether the oleananolic acid containedany oleanolic acid the crude fraction from the reduction was treated with bromine, with which any oleanolic acid[16], if present, would give a bromolactone[17] (XI). In the event no bromolactone could be detected.

The neutral fraction was characterized as a mixture of three compounds, probably 3β-hydroxyoleanane (X), 3β-hydroxydehydro-oleanane

and dehydro-nor-

oleananol by mass spectrometry.

The Huang-Minlon modification[18] of the Wolff-Kishner reduction gave a complex mixture which was not further studied.

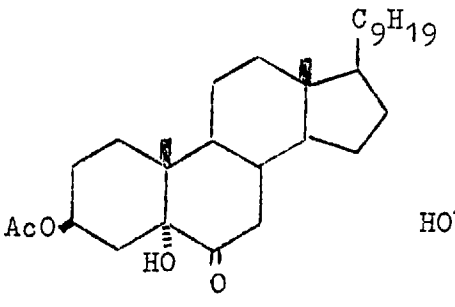
Finally, in a third attempt to obtain oleanolic acid, the ketone (IV) was treated with hydrazine in an attempt to prepare the hydrazone[6] which after treatment with base would afford the desired oleanolic acid (II; R=R'=H). However, the hydrazone could not be isolated from the complex mixture obtained.

The Wolff-Kishner reduction under Barton's conditions[14], was applied to methyl oleanolate acetate (II; R=Ac, R'=CH_3) and to oleanolic acid acetate (II; R=Ac, R'=H) to see whether reduction of the double bond occurred.

In both cases oleanolic acid (II; R=R'=H) was isolated and no oleananolic acid (IX; R=R'=H) could be detected.

With these results in hand this route to the double bond at C-12 was abandoned.

(b) Metal-ammonia reduction.- Metal in liquid ammonia reduction of ester groups, in certain instances leads to alkyl oxygen



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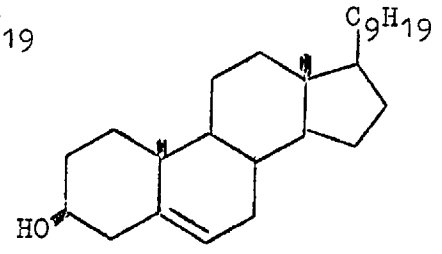

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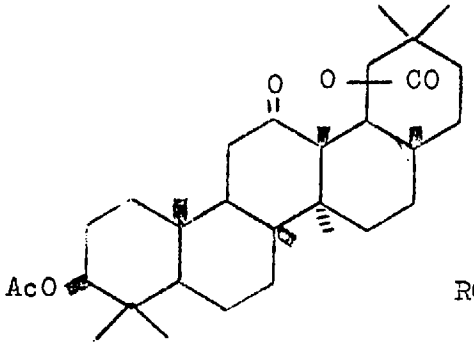
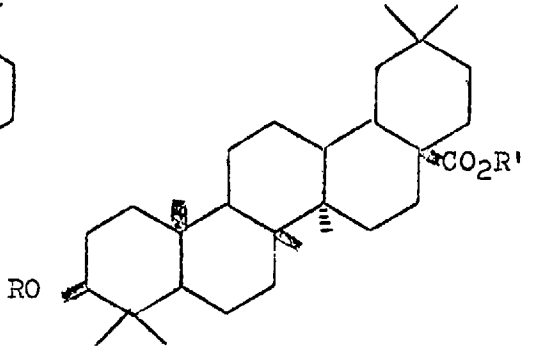
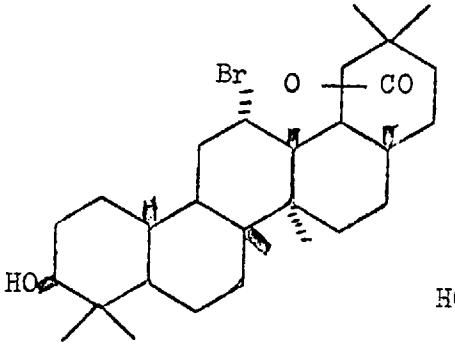


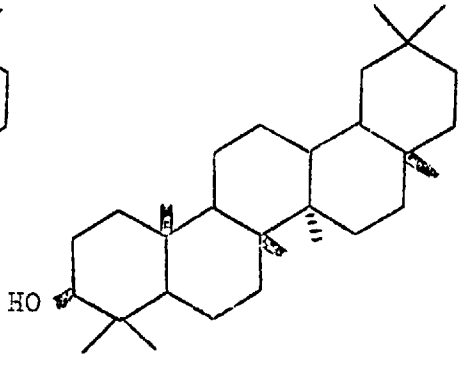
1V



1X

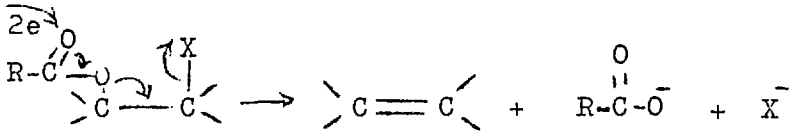


X1



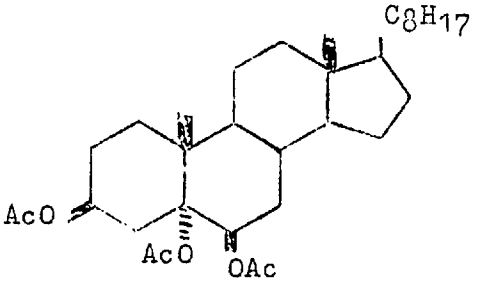
X

fission[19-21]. It was considered that in the presence of a neighbouring leaving group, such a fission could lead to a double bond as

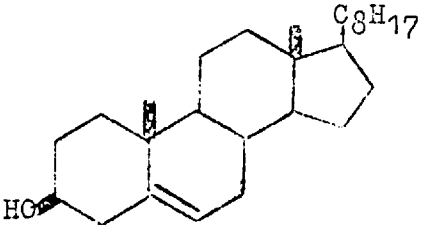


It was found that 3β , 5α , 6β - triacetoxycholestane (XII) (see p. 91) on lithium or calcium-ammonia reduction produced cholesterol (XIII) in good yield (60-74%). When the reaction was applied to the reduction of 3β ,12α -diacetoxy-13β -hydroxy-28-oleonanic (28→13β)-lactone (III; R=Ac) with lithium or calcium oleanolic acid (II; R=R'=H)(17.2-20%) was formed. The corresponding 12α -methane-sulphonate (III; R=SO_2CH_3) under the same conditions again gave oleanolic acid but in slightly lower yield (10%). The scope of this novel reaction was further examined and is detailed on p.91.

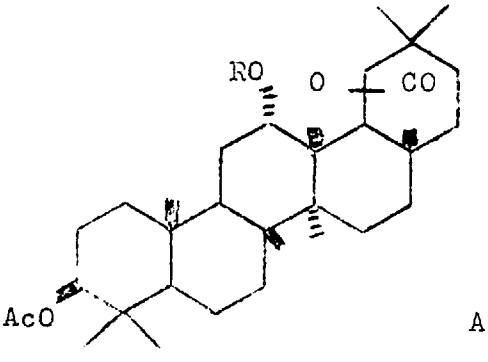
(c) Dehydration.- The third route considered for the introduction of the double bond was by dehydration of methyl 3β -acetoxy-12 ξ -hydroxy-oleananate (XV), prepared by zinc-



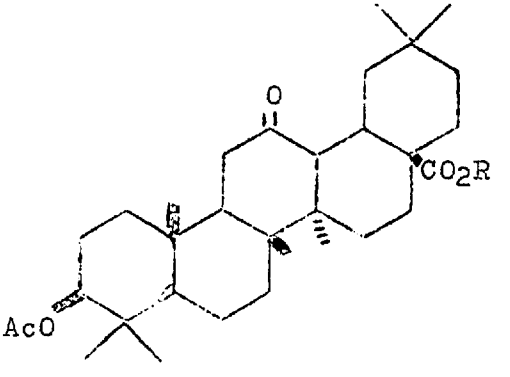
XII



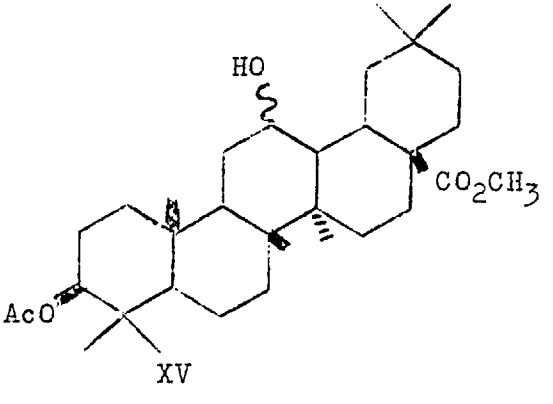
XIII



III



XIV



XV

acetic acid reduction of 3β -acetoxy-13β -hydroxy-12-oxo-28-oleananic (28→13β)-lactone (IV) followed by methylation of the 3β -acetoxy-12-oxo-28-oleananic acid[22] (XIV; R=H) obtained and borohydride reduction of the ketone moiety.

When the resulting alcohol (XV) was treated with methanesulphonyl chloride in pyridine oleanolic acid (II; R=$^{CH_3}_{Ac}$, R'=) formed.

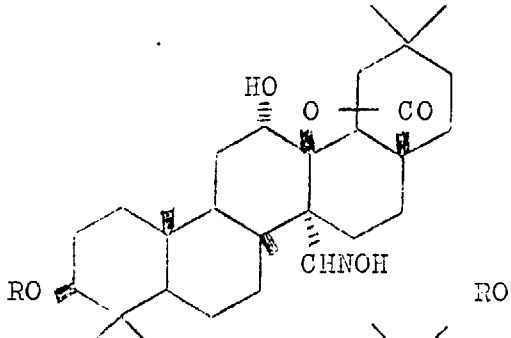
(iv) Formation and reactions of the C_{27} nitrile.-

The 3β -acetoxy-12α ,13β -dihydroxy-27-oximino-28-oleananic (28→13β)-lactone (V; R=Ac) on treatment with p-toluenesulphonyl chloride in pyridine gave 3β -acetoxy-12α ,13β -dihydroxy-27-nitrilo-28-oleananic(28→13β)-lactone (XVI; $^{R'=H}_{R=Ac}$).

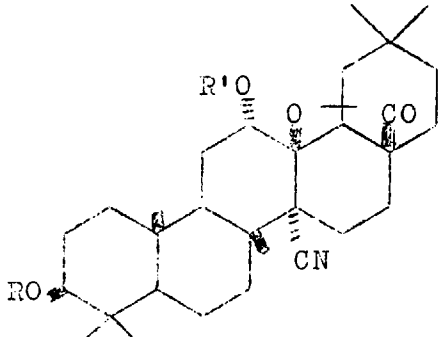
Treatment of the hydroxy-nitrile (XVI; $^{R'=H}_{R=Ac}$) with 4N sodium hydroxide in ethanol[23] failed to bring about hydrolysis of the nitrile function, giving instead the 27-nitrilo-3β ,12α ,13β -trihydroxy-28-oleananic (28→13β -lactone (XVI; R=R'=H). In a second

attempt the hydroxy-nitrile compound (XVI; $^{R'=H}_{R=Ac}$) was treated with sodium in diethylene glycol at 200° for 24 hours. From the complex mixture formed only one compound (10%) could be isolated but this still possessed the nitrile absorption band in its infrared spectrum. A third attempt at hydrolysis, using acid[24], was made but again without any success. Normally nitriles produced by application of the Barton reaction can be readily hydrolysed, however the C-14 methyl group in compounds of the α or β -amyrin series is known to be extremely hindered. This has been shown from structural studies of quinovic acid[25] and cincholic acid[1], to be due mainly to the cis fusion of the rings D and E. For instance dimethyl cincholate (I; R=H, R'=R"=^{CH_3}), on lithium aluminium hydride reduction, gives the diol ester (XVII), with the C-27 ester intact. It was expected, that the attempted basic hydrolysis of the 12α-hydroxy-27α-nitrile (XVI; $^{R'=H}_{R=Ac}$) would be assisted by the axial 12α-hydroxy group. In a normal chair-ring conformation this 1,3 diaxial interaction is known to occur readily, giving

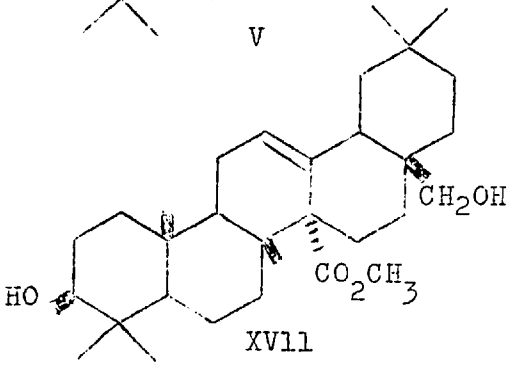
initially, the imino-lactone[26] and hence, via hydrolysis, the lactone. That the hydroxy-nitrile (XVI; $\begin{matrix} R'=H \\ R=Ac \end{matrix}$) did not hydrolyse was probably due to the strain in ring C twisting it from a true chair conformation. A study of a Dreiding model of the hydroxy-nitrile (XVI; $\begin{matrix} R'=H \\ R=Ac \end{matrix}$) showed that the (28→13β)lactone introduces tension into ring C, partially relieved by twisting ring C into a twist-chair conformation in which the 12α-hydroxy group cannot reach the C_{27} nitrile group.



V



XVI



XVII

It was thus considered that hydrolysis of the nitrile function by external agents was being hindered by the non-participating 12α - hydroxy function. Models showed that the steric pressure on the nitrile group was eased in the presence of the C-12 double bond. Therefore attempts to introduce the unsaturation at C-12 were made prior to further hydrolytic experiments.

Reductive elimination methods were originally tried.

The 3β ,12α -diacetoxy-13β -hydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XVI; R=R'=Ac) was obtained by acetylation, at 100°, of the hydroxy lactone (XVI; $\begin{matrix} R'=H \\ R=Ac \end{matrix}$) and also by treatment of the oxime (V; R=Ac) at 100° with acetic anhydride in pyridine.

Lithium or calcium in ammonia reduction of 3β ,12α -diacetoxy-13β -hydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XVI; R=R'=Ac) failed to introduce the double bond (see p.100) giving instead 27-nitrilo-3β ,12α ,13β -trihydroxy-28-oxo-oleanane (28→13β)-hemiacetal (XVIII; R=R'=H). The nuclear magnetic resonance

spectrum of this compound showed a triplet at 6.15 τ (C-12α methine) and the hemiacetal hydrogen at 4.98 τ as a singlet.

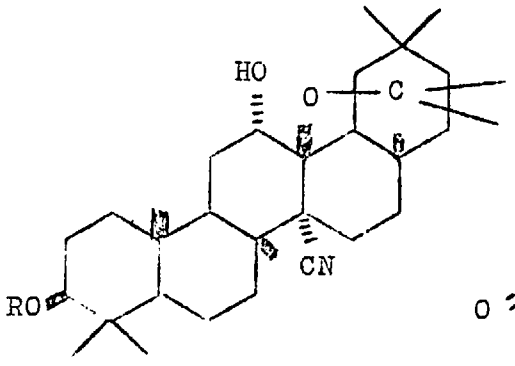
The hemiacetal (XVIII; R=R'=H) on acetylation, at room temperature, gave 3β -acetoxy-12α ,13β -dihydroxy-27-nitrilo-28-oxo-oleanane (28$\rightarrow$13β)-hemiacetal-28-acetate (XVIII; R=R'=Ac).

The nuclear magnetic resonance spectrum of this acetate showed two signals for acetates at 7.96 τ and 7.91 τ , a triplet at 6.18 τ (C-12β methine), a triplet at 5.48 τ (C-3α methine) and a singlet at 4.04 τ (H hemiacetal acetate). The structure of the hemiacetal (XVIII; R=R'=H) was finally confirmed by oxidation, which gave 3,12-dioxo-13β -hydroxy-27-nitrilo-28-oleananic (28$\rightarrow$13β) lactone (XIX), identical with the compound prepared by acid hydrolysis of the nitrile-lactone (XVI; R=Ac,R'=H) followed by oxidation.

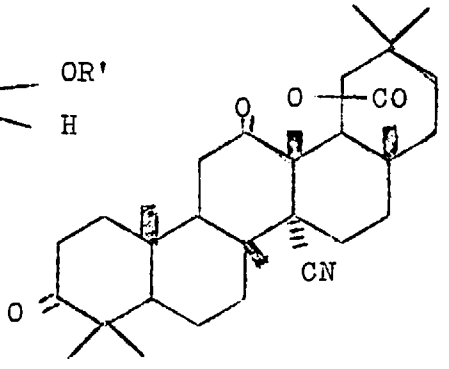
Similarly, attempted formation of the double bond by zinc-acetic acid reduction of the 3β ,12α -diacetoxy-13β -hydroxy-27-nitrilo-28-oleananic(28$\rightarrow$13β) lactone (XVI; R=R'=Ac)

was unsuccessful.

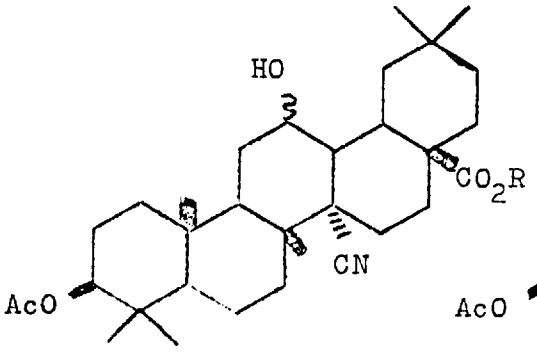
With failure of the reductive elimination methods (see discussion p.100) attention was drawn to the preparation of methyl 12ξ -hydroxy-27-nitrilo-28-oleananic acetate (XX; R=CH_3) in order to obtain the double bond by dehydration. 3β -Acetoxy-13β -hydroxy-27-nitrilo-12-oxo-28-oleananic (28$\rightarrow$13β)-lactone (XXI) was prepared by oxidation of the corresponding alcohol (XVI; R=Ac,R'=H). The keto-nitrile (XXI) on reduction with zinc-acetic acid gave the 3β -acetoxy-27-nitrilo-12-oxo-28-oleananic acid (XXII; R=H) which on methylation afforded the methyl ester (XXII; R=CH_3). The keto-ester (XXII; R=CH_3) was reduced with borohydride to methyl 3β -acetoxy-12ξ -hydroxy-27-nitrilo-28-oleananic (XX; R=CH_3). Dehydration of this compound with methanesulphonyl chloride gave only a very small amount of olefine which was not isolated. Further work along these lines was interrupted by the observation that the oxime (V; R=Ac) could be hydrolysed to the aldehyde (XXIX; $\overset{R'=H}{R=Ac}$) via the nitrimine (VI; R=Ac).



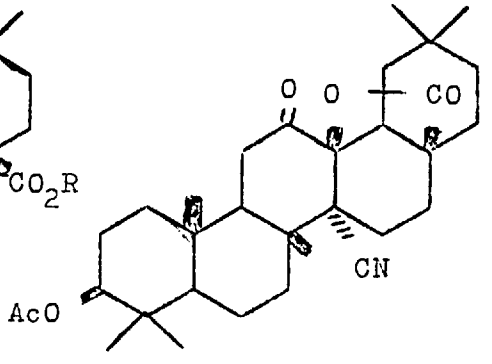
XVIII



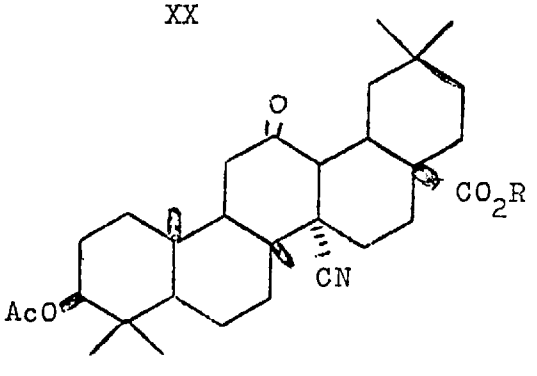
XIX



XX



XXI



XXII

(v) Attempted hydrolysis of the C_{27} oxime (V; R=Ac).-

The 3β -acetoxy-12α ,13β -dihydroxy-27-oximino-28-oleananic (28→13β)-lactone (V; R=Ac) was recovered unchanged after treatment with 2% hydrochloric acid in a solution of acetone:water[26] (5:1) for 21 hours at room temperature and, similarly after being refluxed in this mixture for 70 minutes.

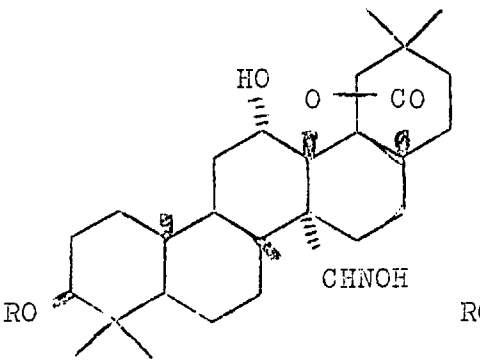
(vi) Nitrimine formation and its reactions.-

The oxime (V; R=Ac), in another attempted hydrolysis with nitrous acid gave 3β -acetoxy-12α ,13β -dihydroxy-27-nitrimino-28-oleananic (28→13β)-lactone (VI; R=Ac) instead of the expected (27→12 α)hemiacetal[27]. This nitrimine was identical with the fourth compound obtained from the photolysis mixture (p.58). This class of aldehyde derivatives has rarely been prepared[28,29] although similar derivatives of ketone are well documented. Brooks[30] et al. have proposed two structures according to the degree of hindrance to the function (XXIII) and

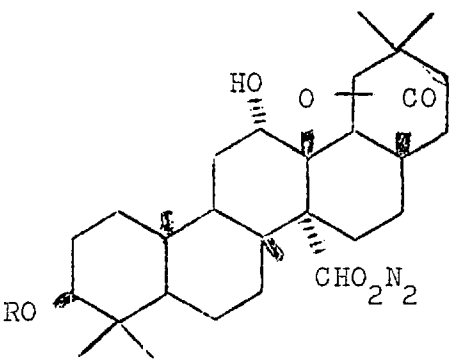
(XXIV), but Freeman[31] has suggested that their structure must be of the formulation (XXV) on the grounds of their spectral and chemical properties.

According to the nuclear magnetic resonance spectrum of 3β-acetoxy-12α,13β-dihydroxy-27-nitrimino-28-oleananic (28$\rightarrow$13β)-lactone the most probable formulation has the mesoionic structure (XXIV). If this nitrimine (VI; R=Ac) had Freeman's formulation the aldoxime hydrogen should show as a singlet at about 2.5 τ (R-C<u>H</u>=N-NO_2) but this singlet was not present.

Attempts to decompose the nitrimine (VI; R=Ac) under reported conditions, either with refluxing aqueous dioxan or with urea in aqueous dioxan[30], were not successful. Nitrimine (VI; R=Ac) was also recovered unchanged by treatment with either 2% or 10% sulphuric acid. Sodium borohydride failed to reduce it. Attempted oxidation gave back the nitrimine. After it was observed that the nitrimine (VI; R=Ac) had a double melting point, this compound was heated at 240° when it gave, almost quantitatively (thin layer chromatography), the previously obtained



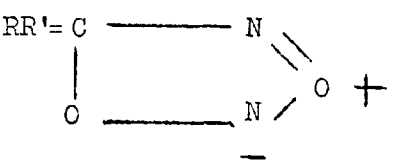
V



VI

RR'C=NONO

XXIII



XXIV

RR'C=N-NO_2

XXV

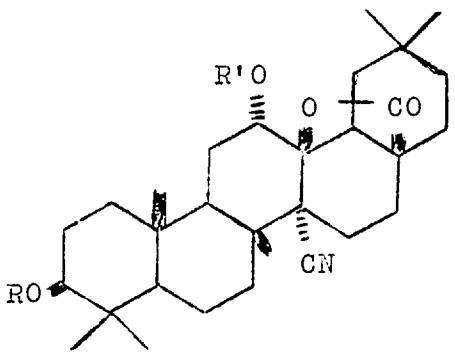
3β-acetoxy-12α,13β-dihydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XVI; R=Ac, R'=H).

Catalytic reduction of the nitrimine (VI; R=Ac) gave 3β-acetoxy-27-amino-12α, 13β-dihydroxy-28-oleananic (28→13β)-lactone (XXVI; R=Ac, R'=H). A variety of hydrogenation conditions were tried in the hope of detecting any intermediate, but in every case only amine (XXVI; R=Ac, R'=H) was obtained - even under such mild reducing conditions as zinc-ammonium chloride in ethanol.

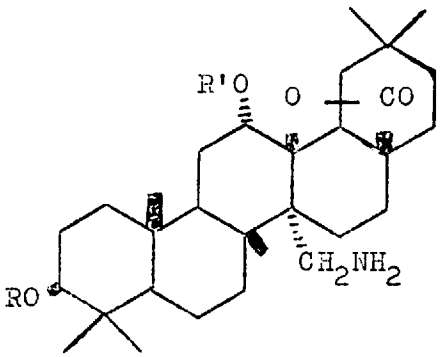
Finally in order to decompose the nitrimine moiety it was treated with acylating agents.

Upon treatment with methanesulphonyl chloride in pyridine the nitrimine (VI; R=Ac) gave two compounds.

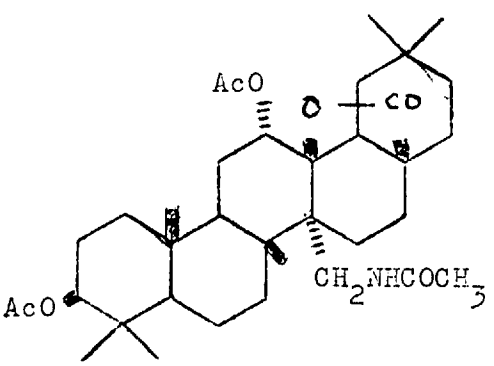
Chromatographic separation gave 3β-acetoxy-12ξ-chloro-13β-hydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXVIII) (19.6%) and 3β-acetoxy-12α,13β-dihydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; $\begin{matrix} R'=H \\ R=Ac \end{matrix}$) (27.4%).



XVl



XXVl



XXVll

Treatment of the nitrimine (VI; R=Ac) with acetic anhydride in pyridine gave 3β-acetoxy-12α,13β-dihydroxy-27-oxo-28-oleonanic (28→13β)-lactone-(27→12α) hemiacetal-27-acetate (XXX). The nuclear magnetic resonance spectrum of this compound had bands at 7.95 τ, and 7.87 τ (acetates), a triplet at 5.78 τ (C-12β methine), a triplet at 5.45 τ (C-3α methine) and a singlet at 3.68 τ (C-27 H hemiacetal acetate). This hemiacetal acetate (XXX) upon selective hydrolysis gave 3β-acetoxy-12α,13β-dihydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=Ac, R'=H), identical with the compound obtained from the methanesulphonyl chloride treatment of the nitrimine (VI; R=Ac), instead of the expected (27→12α)hemiacetal. The hydroxy-aldehyde (XXIX; R=Ac, R'=H) on acetylation gave 3β,12α-diacetoxy-13β-hydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=R'=Ac). The nuclear magnetic resonance spectrum of this compound (XXIX; R=R'=Ac) showed at 7.95 and 7.85 τ acetates, a triplet at 5.50 τ

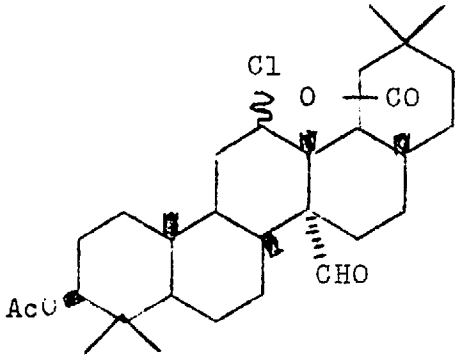
(C-3 α methine), a triplet at 4.75 τ (C-12 α methine) and at -0.22 τ an aldehyde. No hemiacetal acetate could be detected in the acetylation product.

This abnormal behaviour is again probably due to the strain in ring C (see also p.68). Models showed that, because of the (28 $\rightarrow$ 13β) lactone and the cis fusion of ring D and E, ring C is twisted out of the expected chair conformation. In all the previous examples so far known, the formation of hemiacetals, with Barton reaction, readily occurs, as is expected for the 1,3 diaxial interaction between the alcohol and aldehyde functions.

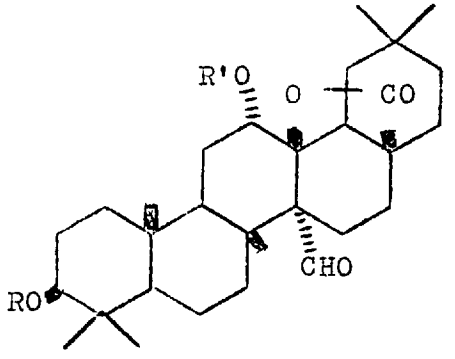
An attempt to reconvert the aldehyde (XXIX; R=Ac, R'=H) into its parent oxime failed. After leaving the aldehyde with hydroxylamine in pyridine for 7 months not a trace of oxime could be detected by thin layer chromatography.

(vii) Planned routes to cincholic acid.-

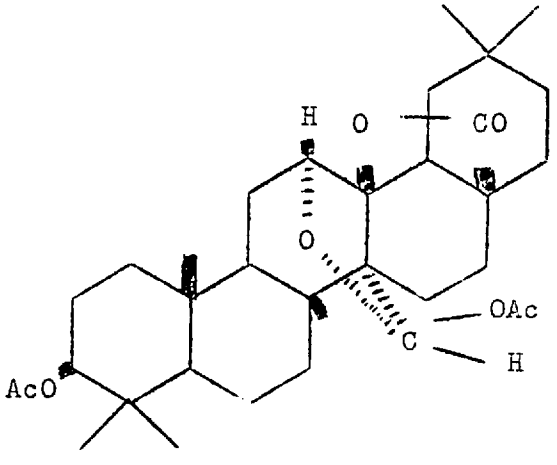
With desired aldehyde (XXIX; R=Ac, R'=H) and its derivatives in hand three possible routes to cincholic acid were envisaged (Scheme I).



XXVlll



XXlX

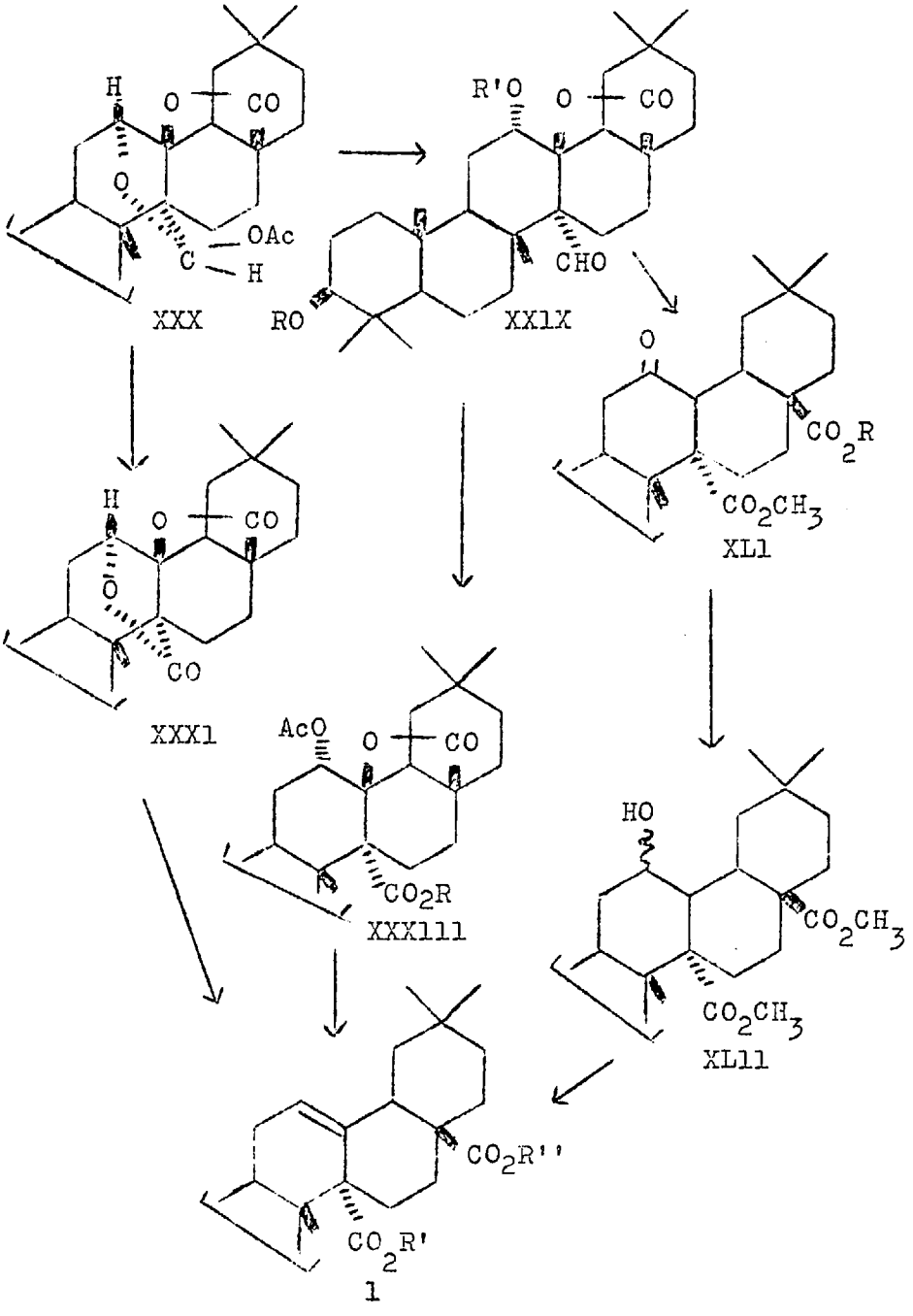


XXX

(a) Firstly, the hemiacetal acetate (XXX), upon oxidation, would give a dilactone (XXXI; R=Ac) which, by reductive elimination with metal in ammonia would give the diacid (I; R=R'=R"=H) with the double bond at C_{12}-C_{13}. (b) The second route involved oxidation of 3β,12α-diacetoxy-13β-hydroxy-27-oxo-28-oleananic (28$\rightarrow$13β)-lactone (XXIX; R=R'=Ac) to the 3β,12α-diacetoxy-13β-hydroxy-27-oleananic acid-28-oic (28$\rightarrow$13β)-lactone (XXXIII; R=H).

This compound on metal-ammonia reduction would form the C-12 double bond (thus leading to cincholic acid), as did the model compound, 3β,12α-diacetoxy-13β-hydroxy-28-oleananic (28$\rightarrow$13β)-lactone (III; R=Ac). (c) The third possibility considered was by dehydration of dimethyl 3β-acetoxy-12ξ-hydroxy-oleanan-27,28-dioate (XLII), which could be prepared by oxidation of 3β-acetoxy-12α,13β-dihydroxy-27-oxo-28-oleananic (28$\rightarrow$13β)-lactone (XXIX; R=Ac, R'=H). The acid obtained, after methylation would afford a methyl ester (XL; R=CH_3) which on reduction with zinc-acetic acid would produce the keto-acid (XLI; R=H). Methylation of this

SCHEME 1


H
O
CO
O
OAc
C
H
XXX
R'O
O
CO
CHO
RO
XX1X
O
CO2R
CO2CH3
XL1
H
O
CO
O
CO
XXX1
AcO
O
CO
CO2R
XXX111
HO
CO2CH3
CO2CH3
XL11
CO2R''
CO2R'
1

compound and borohydride reduction would give dimethyl 3β -acetoxy-12ε -hydroxy-oleanan-27, 28-dioate (XLII) and hence, by dehydration, cincholic acid.

Route (a): Via dilactone (XXXI; R=Ac). The hemiacetal acetate (XXX) on oxidation with 8N chromium trioxide in 2N sulphuric acid and acetone gave 3β -acetoxy-12α ,13β -dihydroxy-oleanan-27,28-dioic (27→12 α), (28→13 β)-dilactone (XXXI; R=Ac)(38.8%). The nuclear magnetic resonance spectrum showed at 7.98 τ a singlet, due to an acetyl group, at 5.56 τ a triplet (C-3α methine) and at 5.41 τ a triplet (C-12β methine). Acid hydrolysis of this compound (XXXI; R=Ac) gave 3β ,12 α ,13β -trihydroxy-oleanan-27,28-dioic(27→12 α), (28→13β)-dilactone (XXXI; R=H).

The dilactone (XXXI; R=Ac) on reduction with lithium or calcium gave 3β ,12α ,13β -28-tetrahydroxy-27-oleananic (27→12 α)-lactone (XXXII; R=R'=H) which on acetylation afforded 3β ,28-diacetoxy-12α ,13β -dihydroxy-27-oleananic (27→12 α)-lactone (XXXII; R=R'=Ac).

The infrared spectrum of this compound showed, hydroxyl, lactone and acetate absorption and the nuclear magnetic resonance spectrum had signals at 7.98 τ and 7.92 τ (acetates), 5.78 τ a singlet (C-28 methylene), 5.70 τ a triplet (C-12 β methine) and at 5.56 τ a triplet (C-3 α methine).

This compound, upon oxidation, was recovered unchanged.

According to these results it can be concluded that the (28 $\rightarrow$ 13β)-lactone function was opened. If the (27 $\rightarrow$ 12α)-lactone had been reduced it should show the C 12β proton adjacent to the α-hydroxyl group at 6.15 τ in its n.m.r. spectrum and this secondary hydroxyl group should give the ketone on oxidation.

The reduction result (see discussion p.102) showed that this route to cincholic acid was closed.

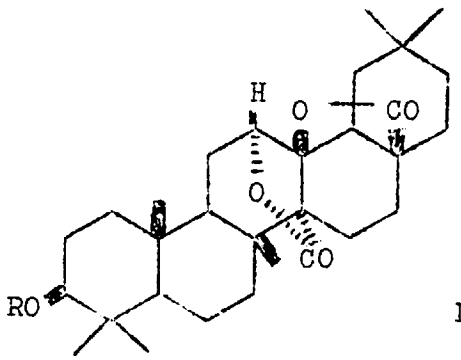
Route (b): Via the acetate lactone (XXXIII; R=CH_3). The diacetate (XXIX; R=R'=Ac) on chromic oxidation, gave the acid, 3β , 12α -diacetoxy-13β -hydroxy-27-oleananic acid-28-oic (28 $\rightarrow$13β)

lactone (XXXIII; R=H)(33%). This compound was characterized as its methyl ester (XXXIII; R=CH_3). The nuclear magnetic resonance spectrum of the ester showed at 7.95 τ and 7.88 τ (acetates), 6.37 τ (methyl ester), 5.56 τ a triplet (C-3 α methine) and at 4.90 τ a triplet (C_{12} β methine).

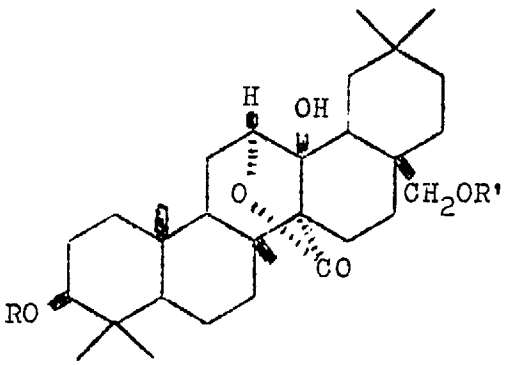
The acetate lactone (XXXIII; R=CH_3) on reduction with lithium or calcium in liquid ammonia gave the desired cincholic acid (I; R=R'=R"=H) in a 22% yield, which was separated from the reaction mixture as its dimethyl ester. The nuclear magnetic resonance spectrum of the diester (I; R=H, R'=R"=CH_3) had signals at 6.35 τ (methyl ester), 6.31 τ (methyl ester) and at 4.25 τ a triplet (C-12 methine).

The dimethyl ester (I; R=H, R'=R"=CH_3) was characterized by comparison on thin layer chromatography and mixed m.p. with an authentic specimen[32], also by its optical rotation.

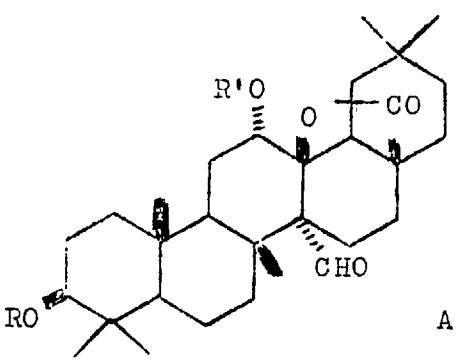
Dimethyl cincholate acetate (I; R=Ac, R'=R"=CH_3) was obtained by acetylation at room temperature of the ester (I; R=H, R'=R"=CH_3). The nuclear magnetic resonance spectrum of this compound showed at 7.95 τ (acetate), 6.35 τ and



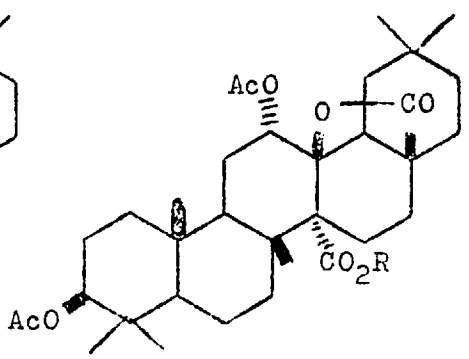
XXX1



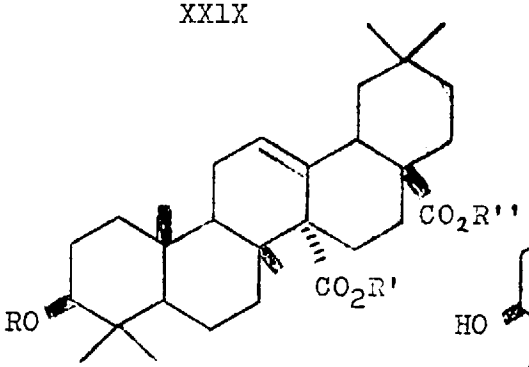
XXX11



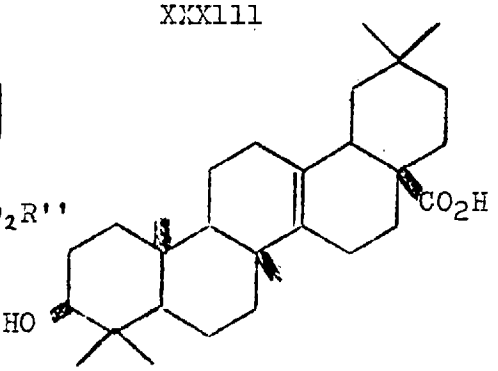
XX1X



XXX111



1



XXX1V

6.31 τ (methyl esters), a triplet at 5.50 τ (C-3 α methine) and at 4.27 τ a triplet (C-12 vinyl proton). The mixed m.p. of this compound with an authentic specimen[32] showed no depression and was identical by direct comparison on thin layer chromatography and its physical constants. The mass spectra of this compound and the authentic material[32] were identical.

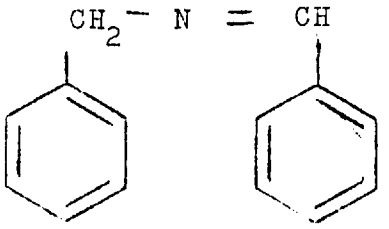
Treatment of the dimethyl ester (I; R=H, R'=R"=CH_3) with lithium iodide in anhydrous collidine[33] gave cincholic acid and, probably, pyrocincholic acid (XXXIV). The mixture was treated with bromine to form the bromolactone[1] of the pyrocincholic acid. From the remaining acidic fraction was obtained the pure cincholic acid, which was characterized by thin layer chromatography and further characterized by formation of its methyl ester (I; R=H, R'=R"=CH_3), which again showed identical properties with an authentic sample.

Route (c): Via dehydration of the 12ϵ alcohol (XLII).

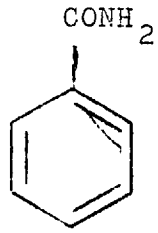
Attempts to oxidise the aldehyde (XXIX; R=Ac,R'=H) with silver oxide[34] failed to give the

27-acid.

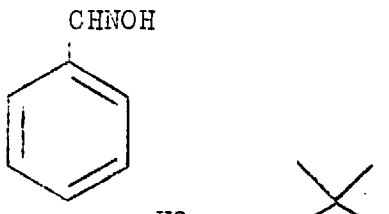
Attempted autoxidation of the aldehyde. This experiment was carried out after it was found that benzaldoxine (XXXVII) could be autoxidised to benzoic acid (XXXVIII) in 66% yield. 3β -Acetoxy-12α ,13β -dihydroxy-27-oximino-28-oleananic (28→13β)-lactone (V; R=Ac) was dissolved in a solution of dimethyl= sulphoxide and t-butanol (4:1) in the presence



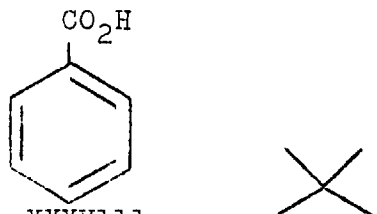
XXXV



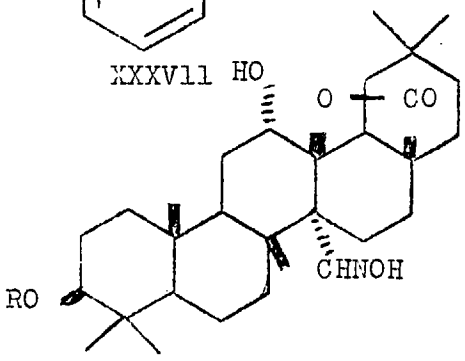
XXXVI



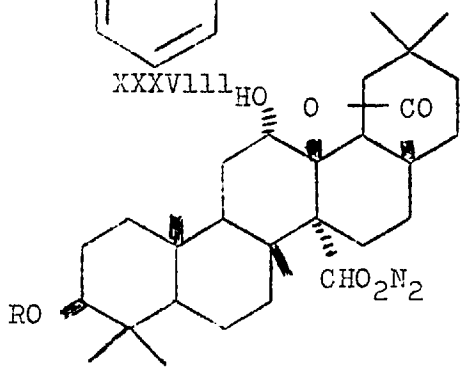
XXXVII



XXXVIII



V



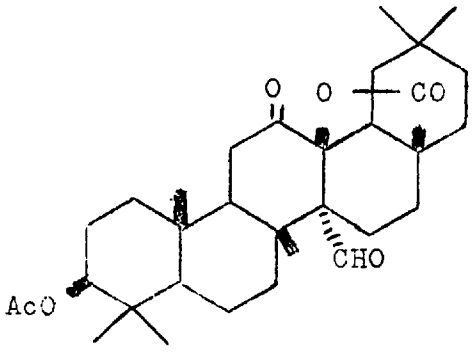
VI

of a trace of water containing potassium t-butoxide before shaking under oxygen. Products isolated were 27-oximino-3β,12α,13β-trihydroxy-28-oleananic (28→13β)-lactone (V; R=H) (48%) and a compound (24% possessing strong band at 2320 cm^{-1} in its infrared spectrum, no acid could be detected. When the nitrimine (VI; R=Ac) was similarly treated only starting material was recovered.

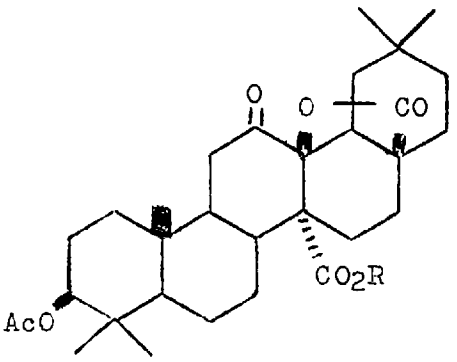
Chromic oxidation of 3β-acetoxy-12α, 13β-dihydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=Ac, R'=H) gave 3β acetoxy-13β-hydroxy-12-oxo-27-oleananic acid-28-oic(28→13β)-lactone (XL; R=H). This acid was characterized as its methyl ester (XL; R=CH_3) which showed, in its nuclear magnetic resonance spectrum signals at 7.93τ (acetate), 6.30τ (methyl ester) and 5.56τ a triplet (C-3α methine).

Methyl 3β-acetoxy-13β-hydroxy-12-oxo-27-oleananate-28-oic (28→13β)-lactone (XL; R=CH_3) on reduction with the zinc-acetic acid and treatment with diazomethane gave dimethyl 3β-acetoxy-12-oxo-oleanan-27,28-dioate (XLI; R=CH_3). Sodium borohydride reduction of the keto-ester (XLI; R=CH_3) gave the corresponding alcohol (XLII) which on treatment with methanesulphonyl

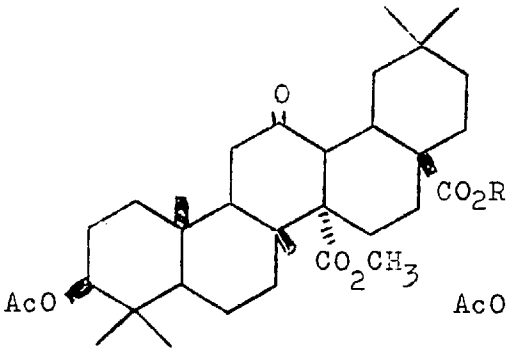
chloride in pyridine on the steam bath gave dimethyl cincholate acetate (I; R=Ac, R'=R"=CH_3) characterized by its physical constants, thin layer chromatography and mixed m.p. with an authentic specimen[32].



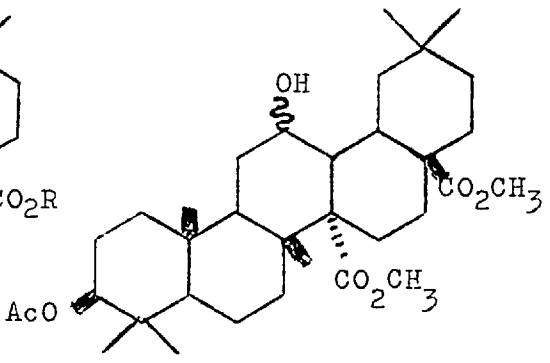
1XL



XL



XL1



XL11

(b) Metal-liquid ammonia reductions

Reduction of esters by metal in liquid ammonia can, in certain circumstances[19-21] lead to alkyl-oxygen fission and formation of the hydrocarbon. Earlier it has been mentioned (p. 64) that lithium or calcium in liquid ammonia reduced 3β,12α-diacetoxy-13β-hydroxy-28-oleananic (28→13β)-lactone (III; R=Ac) and the corresponding 12α-mesylate (III; R=SO_2CH_3) with the introduction of a double bond.

In order to explore the scope of this reaction it was applied to a series of simple model compounds.

The model used, and the results of the reaction are tabulated in Table I.

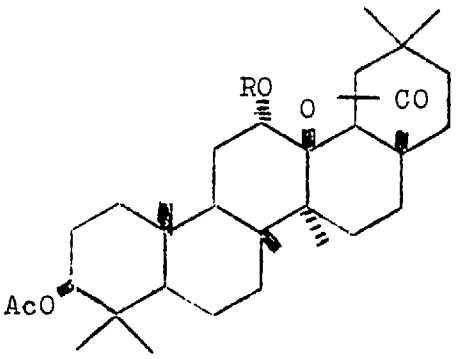
Variations employed included the use of either lithium or calcium as reducing metal and of the use of tetrahydrofuran or ether as solvent.

Both 3β,5α,6β[38] and 3β,5α,6α-triacetoxycholestane (XLIII; R'=R"=Ac-XLV; R=R'=R"=Ac) were prepared. The former (XLIII; R=R'=R"=Ac), trans-diaxial ester,

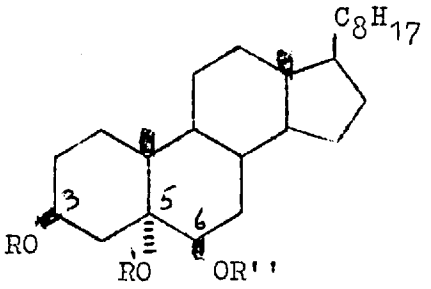
Table I

	Olefine % Li	Olefine % Ca	Alcohol % Li	Alcohol % Ca
Cholestane Series				
3β,5α,6β-Triacetate (XLIII;R=R'=R"=Ac)	72-74[e]	61[t]		35[t]
3β,5α,6α-Triacetate (XLV;R=R'=R"=Ac)	52[t]		48[t]	
2α,3α-Diacetate (LIV;R=R'=Ac)			90[t]	
2α,3β-Diacetate (LVI;R=R'=Ac)			92[t]	
2β,3α-Diacetate (LVII;R=R'=Ac)	8.9[t]		79[t]	
Sugar Series				
α-methyl glucoside tetra-acetate (XLVII)			96[t]	
α-methyl glucoside 2,3-diacetate (XLVIII;R=R'=Ac)			88[t]	
Mannitol hexa-acetate (IL)			95[t]	
Oleanane Series				
lactone-acetate (III;R=Ac)	17-20[e,t]	20[t]		
lactone-mesylate (III;R=SO_2CH_3)	10[t]	10.5[t]		
lactone-acetate-27-ester (XXXIII;R=CH_3)	22[t]	22[t]		
lactone-acetate-27-nitrile (XVI;R=R'=Ac)			69[e]	64[e]
dilactone (XXXI)			72[e,t]	79[t]

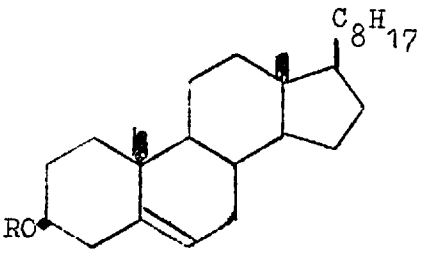
Solvents:
Ether = e
Tetrahydrofuran = t



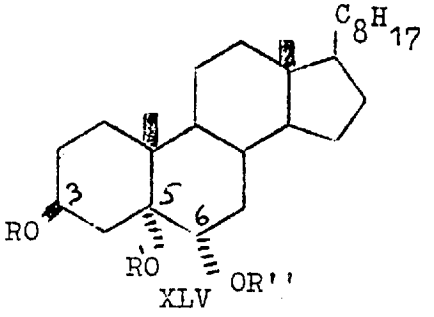
111



XL111



XL1V



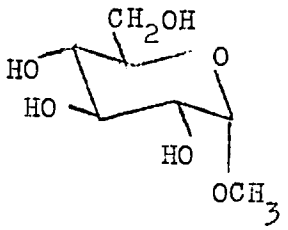
XLV

upon reduction with lithium in liquid ammonia, gave a 73% yield of cholesterol, the only other product detected being the corresponding triol (XLIII; R=R'=R"=H). Use of calcium also gave cholesterol but in slightly lower yield, a trend repeatedly observed (see Table I).

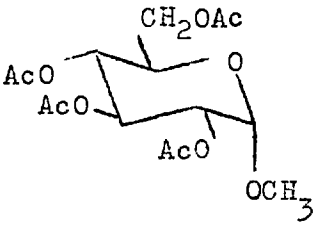
The 3β,5α,6α-triacetate (XLV; R=R'=R"=Ac) (cis, axial-equatorial) on reduction gave a lower yield of cholesterol (48%) with more of the corresponding triol (XLV; R=R'=R"=H).

Application of the reaction to the sugar derivatives described in Table I did not give any olefine. In all these models the acetate groups are primary or secondary, also for α-methyl glucoside tetra-acetate (XLVII) and 2,3-diacetyl-4,6-benzylidene α-methyl glucoside[42] (XLVIII; R=R'=Ac) the acetate groups are equatorial as well.

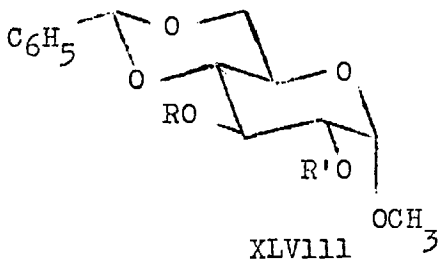
To investigate steric and conformational effects on the reaction, three of the possible isomers of 2,3-diacetoxycholestane were prepared.



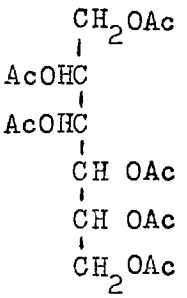
XLV1



XLV11



XLV111



1L

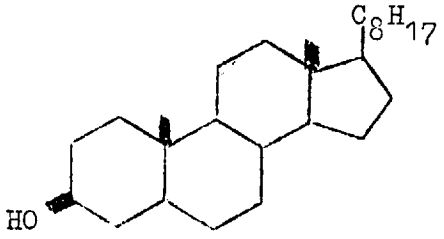
2α,3α-Diacetoxycholestane[47-48] (LIV; R=R'=Ac) did not give any olefine. The acetates are held cis, axial-equatorial.

2α,3β-Diacetoxycholestane[49] (LVI; R=R'=Ac) having the acetate groups in trans, diequatorial positions, upon reaction did not produce olefine.

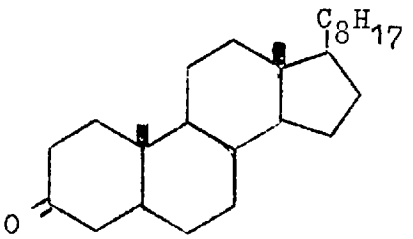
Finally, the trans, diaxial 2β,3α-diacetoxycholestane[50] gave a very low yield of the corresponding olefine (8%).

According to these results the optimum conditions required for the reaction to produce olefine are firstly, that one ester group should be tertiary - as indicated in Table I - and secondly, better yields are obtained if the ester groups are attached in a diaxial manner. Relatively little olefine is produced if ester functions are diequatorial. It is to be noted that the diacetate (LVII; R=R'=Ac) in which neither function is tertiary did give some of the desired cholest-2-ene (LIII), although only in low yield.

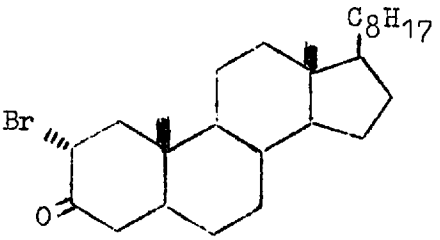
These optimum conditions hold for the acetate lactone of oleanolic acid (III; R=Ac),



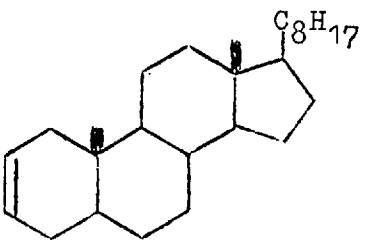
L



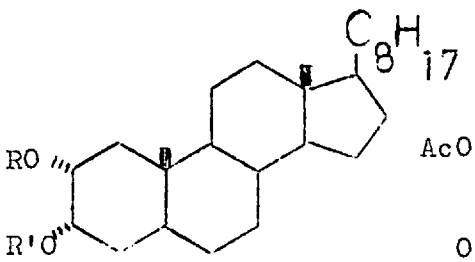
Ll



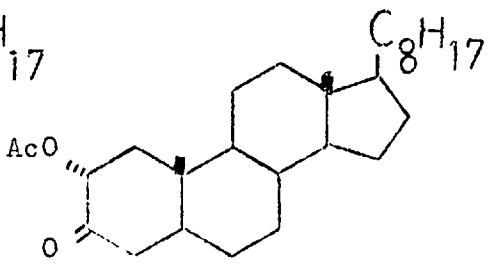
Lll



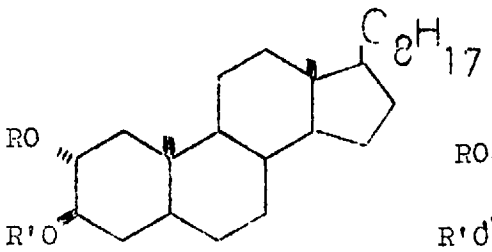
Llll



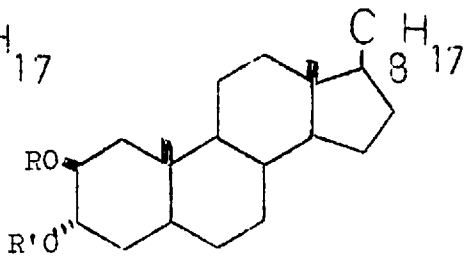
LIV



LV



LVI



LVII

except that one of the ester groups has been replaced by a lactone moiety.

3β ,12α -Diacetoxy-13β -hydroxy-28-oleananic (28→13β)-lactone (III; R=Ac) gave olefine in 17.2% yield with lithium and in 20% yield with calcium.

In 3 β -acetoxy-13β -hydroxy-12 α -methanesulphonyloxy-28-oleananic (28→13β)-lactone (III; R=SO_2CH_3) one of the ester functions taking part in the reaction, is present as a mesylate. Reduction of this compound produced oleanolic acid (olefine) in 10% yield.

The related methyl 3β ,12α -diacetoxy-13β -hydroxy-27-oleananate 28-oic (28→13 β) lactone (XXXIII; R=CH_3) reacted similarly to give cincholic acid (olefine) in 22% yield.

It can be concluded, therefore, that the electronic displacement which produces the reductive elimination must start at the secondary ester, the tertiary ester acting as the leaving group.

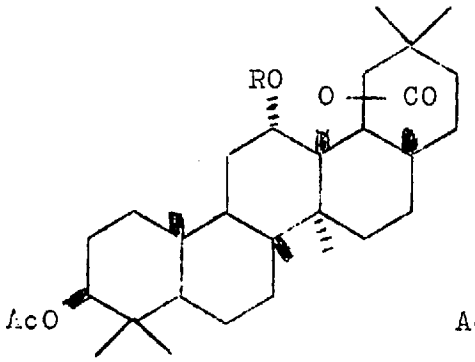
If the reaction occurred in the opposite direction, that is by initial attack at the

tertiary lactone function, the degree of steric pressure on the secondary ester group showed its ease of departure. However, the olefine has been obtained in lower yields in the oleananic series (III; R=Ac-XXXIII; R=CH_3), in which the position 12 is highly hindered, than in the cholestane esters (XLIII; R=R'=R"= Ac-XLV; R=R'=R"=Ac) where steric hindrance is far less.

This mode of reaction is further substantiated by the low yield of olefine produced by reduction of the mesylate (III; R=SO_2CH_3) which, if acting as a leaving group, would have given a better, instead of poorer, yield of olefine.

It remains to rationalize the 'abnormal' result of the reduction reaction on the nitrile (XVI; R=R'=Ac) and the dilactone (XXXI; R=Ac).

The nitrile (XVI; R=R'=Ac) on reduction gave as product the hemiacetal (XVIII; R=R'=H) (see p. 71). Presumably the environmental change near the reaction site, in that the C-27 methyl group has been replaced by the nitrile function, affects the course of reduction.



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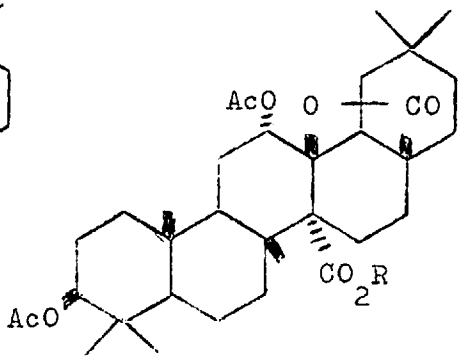
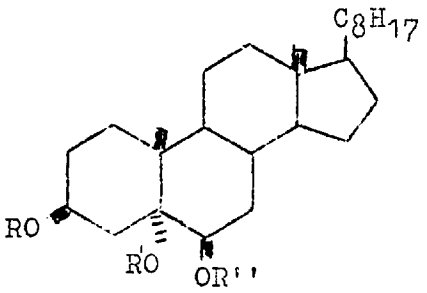
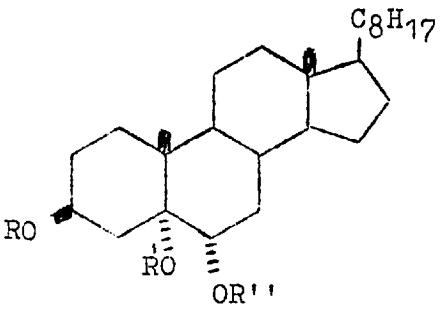


XXX111



XL111

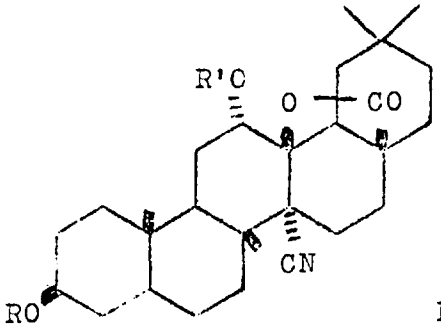


XLV

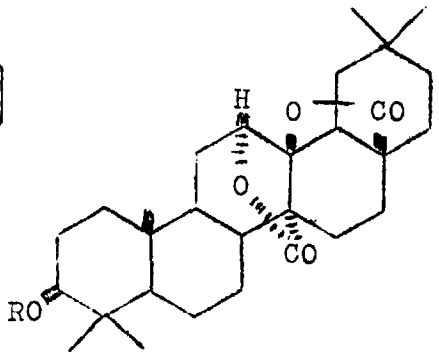
This result is not likely to be due to steric reasons but probably reflects the repulsion of electrons from the α face of the alicyclic system near the polar nitrile group.

The dilactone (XXXI; R=Ac) under the reduction conditions gave a compound having one lactone group remaining and the other reduced to the diol (XXXII; R=R'=H). For this compound reductive attack, to give olefine, should commence at the C-27 carboxyl function. It is known, however, that this position in the molecule is extremely unreactive and hence attack preferentially occurs at the C-28 carboxyl function. That the elimination reaction did not occur via opening of the (C-27 $\rightarrow$ 12 α)-lactone function again indicates the necessity for a tertiary centre as the leaving group. In contrast to the nitrile (XVI; R=R'=Ac) reduction of the dilactone does not stop at the hemiacetal stage. Models showed that the (C27 $\rightarrow$ 12α)-lactone introduces strain into the hemiacetal function; which then opens to the hydroxy

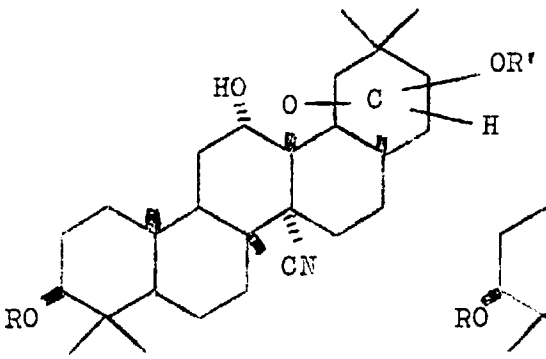
aldehyde and hence, via further reduction, to the diol (XXXII; R=R'=H).



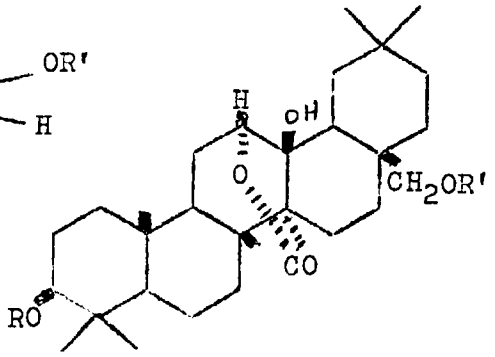
XVI



XXXI



XVIII



XXXII

(C) Pyrethrol

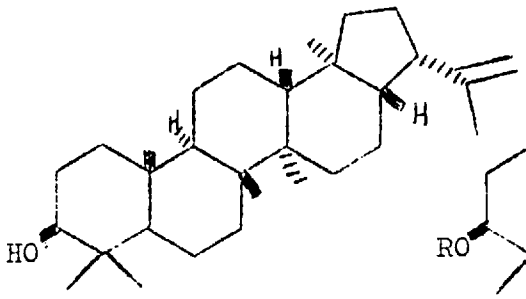
Pyrethrol, a triterpenic alcohol, has been reported several times in the literature.

In 1909 Fujitani[51] isolated the alcohol out of an extract from pyrethrum flowers and claimed that an ester of pyrethrol was responsible for the insecticidal properties. After this Yamamoto[52] isolated two higher alcohols from pyrethrum extract, but later still Staudinger and Ruzicka[53] isolated pyrethrol from the distillation residue of pyrethrum extract, which had no insecticidal properties. Hamilton[54] and Swanback[55] reiterated that pyrethrol is of value as an insecticide. In 1953 Fukushi[56] characterized pyrethrol, incorrectly, as β-amyrin. Recently Elliott[57] again reported the presence of a triterpenic alcohol, called pyrethrol, in the pyrethrum extract.

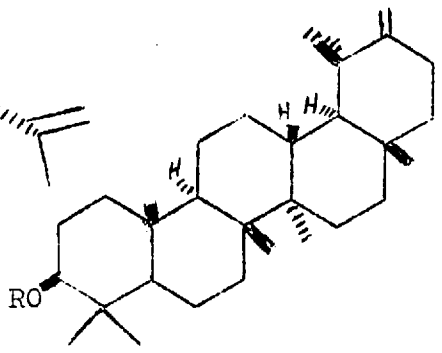
This present study was made in the hope of characterising this material. At the outset of this work it was known that the mixed m.p. of pyrethrol with α-amyrin, or

β-amyrin, was depressed and that pyrethrol, in its infrared spectrum, was reported to have an absorption at 885 cm^{-1}[58], characteristic of a methylene group.

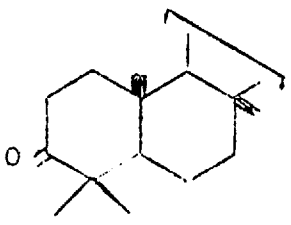
The sample of pyrethrol used in this investigation[59] showed in the nuclear magnetic resonance spectrum of its acetate (LVIII; R=Ac) a multiplet at 5.37 τ and 5.34 τ (exocyclic methylene) in agreement with the infrared assignment. At this stage it was assumed that pyrethrol was possibly 3-hydroxy-diploptene (LIX). The hydroxyl group was placed at C-3 because the product after oxidation to the ketone (LX) gave a positive Zimmermann test[60]. Pyrethrol formed a mono-acetate (LVIII; R=Ac) and a dihydromonoacetate (LXI; R=Ac) with disappearance of the infrared absorption at 885 cm^{-1}. The selenium dioxide oxidation product of the acetate (LVIII; R=Ac) was an α, β-unsaturated aldehyde (LXII) having ultraviolet absorption at 233 mμ (ε 12,174[63]) and infrared absorption at 2750, 1670 and 1648 cm^{-1} with the acetate at 1740 and 1250 cm^{-1}, and no absorption at 885 cm^{-1}.



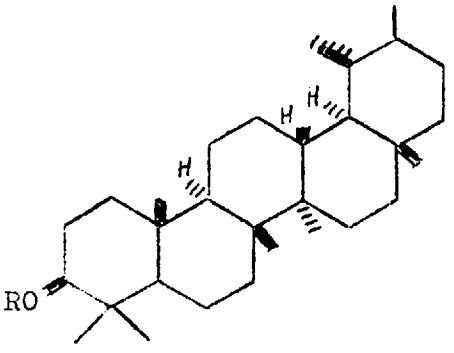
LlX



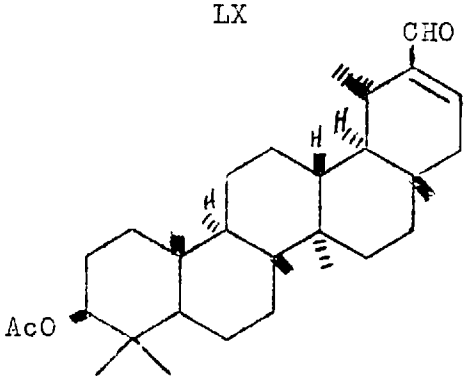
LVlll



LX



LXl

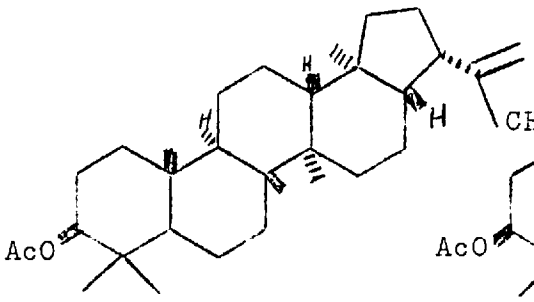


LXll

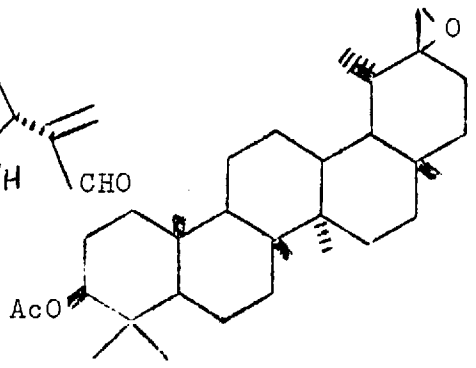
The nuclear magnetic resonance spectrum showed a singlet at 7.94 τ (acetate), a triplet 5.42 τ (C-3 α methine), a triplet at 3.20 τ (J=4.5 cps.; C-21 methine) and at 0.52 τ (aldehyde). This n.m.r. data did not support the diploptene structure considered, which should give the aldehyde (LXIII). In order to confirm, this result the epoxide (LXIV) was prepared of the methylene group. Treatment with lithium aluminium hydride gave a diol (LXV), which, on mild oxidation, produced a ketol (LXVI).

If the pyrethrol was a hydroxy diploptene (LIX) this compound must be the known hydroxy hopanone[61] (LXVII). Comparison of the physical constants of the ketol (LXVI) with those of hydroxy hopanone (LXVII) showed clearly that they were different compounds.

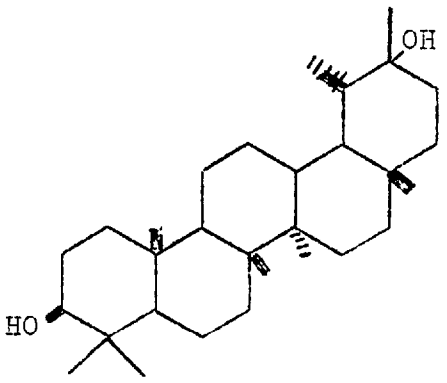
At this stage the observed physical constants of the derivatives prepared from pyrethrol showed very similar resemblances to those prepared from taraxasterol[62]. The mixed m.p. of pyrethrol and taraxasterol acetates showed no depression. Confirmation



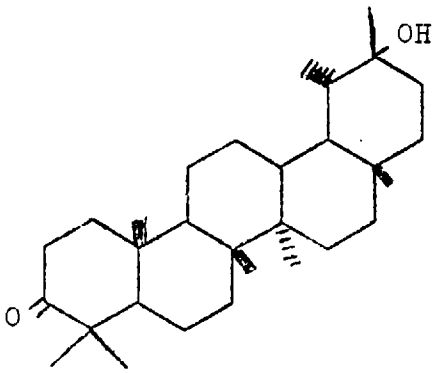
LXIII



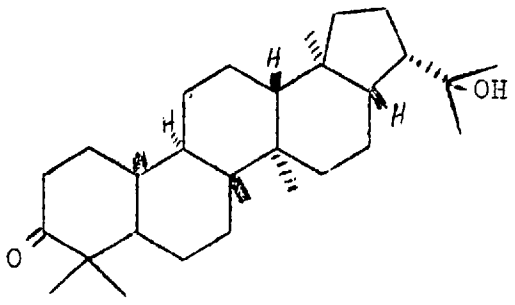
LXIV



LXV



LXVI



LXVII

of this result was obtained by taking the mixed m.p. of dihydropyrethrol acetate (LXI; R=Ac) and dihydrotaraxasterol acetate when there was again no depression (see Table II). Thus pyrethrol is identical to taraxasterol.

Table II

	Taraxasterol m.p.	$[\alpha]_D$	Pyrethrol m.p.	$[\alpha]_D$
	226-227°	+ 77°	218-219°	+ 88°
Acetate	242-243°	+100°	233-234°	+ 95.3°
Benzoate	242-245°	+105°	235-240°	+ 89°
Dihydroacetate	264-265°	+ 25°	243-244°	+ 29.5°
Epoxide acetate	208-210°		211-212°	+105°
3 Ketoaldehyde	λ_{max} 230 mμ (ε13900)			
3 acetate aldehyde			λ_{max} 233mμ (ε12174)	

SECTION III

Experimental

Melting points (uncorrected) were determined on a Kofler block. Rotations were measured at 20-25° in chloroform unless otherwise stated.

Ultraviolet spectra were recorded on solutions in absolute ethanol on a Unicam SP 700 Spectrophotometer. Infrared spectra were recorded either on a Perkin Elmer 137 or a Unicam SP 200 instrument. Nuclear magnetic resonance spectra were determined by Mrs. A.I. Boston at Imperial College on a Varian A-60 spectrometer using deutero-chloroform as solvent and tetramethylsilane as an internal reference. Mass spectra were performed with an AEI MS9, double focussing, mass spectrometer.

Petroleum ether refers to the fraction of b.p. 60-80°.

The course of reactions and of column chromatography was followed by thin layer chromatography on silica gel G.

Solutions obtained after extractions

were washed with water before drying over anhydrous sodium sulphate and working up.

Microanalyses were performed by the Staff of the Microanalytical Laboratory of Imperial College.

12α -Hydroxyoleanolic acetate lactone[3] (III; R=H).-

Oleanolic acid was acetylated with pyridine-acetic anhydride overnight at room temperature to give the oleanolic acid acetate[16] (II; R=Ac,R'=H), m.p. 259-260°, $[\alpha]_D$ +75.2°(c 0.76).

The acetate (II; R=Ac, R'=H) (28 g.), in chloroform (224 ml.) was treated with peracetic acid in acetic acid (2.7 N; 56 ml.) at room temperature for 48 hours. Working up afforded, after crystallization from methanol-chloroform 3β -acetoxy-12α ,13β -dihydroxy-28-oleananic (28→13 β)-lactone[3] (III; R=H) (21.2g., 75.7%), m.p. 294-296°, $[\alpha]_D$+49°(c 0.54) $\nu_{max.}$ Nujol 3550(OH), 1725(lactone and acetate), 1250(acetate) cm^{-1}, ν_{max} chloroform 1760(lactone), 1720 and 1250(acetate) cm^{-1},

τ =8.85(singlet; methyl), 7.95(acetate), 6.09(triplet, J=2.5 cps., C-12β methine) and 5.50(triplet, J=7.5 cps., C-3α methine).

3β -Acetoxy-13β -hydroxy-12-oxo-28-oleananic (28→13β)lactone[4](IV).-

12α -Hydroxyoleanolic acetate lactone (III; R=H) in acetone was treated at room temperature with 8N chromium trioxide in aqueous (2N) sulphuric acid. After 10 minutes excess methanol and water were added and the product was isolated by extraction with methylene chloride. The ketone[4](IV) obtained had m.p. 284-286°, $[\alpha]_D$+8.3°(c 0.84).

3β ,12α -Diacetoxy-13β -hydroxy-28-oleananic (28→13β)lactone[3](III; R=Ac).-

Treatment of the lactone acetate (III; R=H) with pyridine-acetic anhydride, on the steam bath for 3 hours, gave the diacetate[3] (III; R=Ac) m.p. 285°, $[\alpha]_D$+64.5°(c 0.62) ν_{max} Nujol 1765(lactone), 1740-1720 and 1245(acetates) cm^{-1}.

3β -Acetoxy-13β -hydroxy-12α -methanesulphonyloxy-28-oleananic (28→13β) lactone (III; R=SO_2CH_3).-

The 12-hydroxy compound (III; R=H) (542 mg.) in dry pyridine (12 ml.) was treated overnight with methanesulphonyl chloride (5 ml.) at room temperature. The mixture was poured into ice-water and extracted with methylene chloride to give, after chromatography over alumina (Grade III) and elution with benzene, the mesylate (III; R=SO_2CH_3) (324 mg.), m.p. (from petroleum ether) 260° dec., $[\alpha]_D$+233°(c 1.2), ν_{max} chloroform 1770(lactone), 1720 and 1275(acetate) cm^{-1}, τ =7.95(acetate), 6.93(methanesulphonate), 5.55(triplet, J=7.5 cps.; C-3 α methine) and 5.17 (broad triplet; C-12 β methine).

3β -Acetoxy-13β -hydroxy-12α -nitrito-28-oleananic (28→13β)-lactone (III; R=NO).-

The lactone acetate (III; R=H) (687 mg.) in dry pyridine (15 ml.) at -20° was treated with excess nitrosyl chloride for 20 minutes. The mixture was poured into ice-water and extracted with ether. The dry ethereal solution was evaporated to dryness at room

temperature in vacuo to give, after crystallization from methanol-methylene chloride, the nitrite ester (III; R=NO) (578 mg.; 80-90%), m.p. 271-274°, $[\alpha]_D$+75.3(c 0.73), ν_{max} chloroform 1760(lactone), 1720 and 1270(acetate) and 1665(nitrite) cm^{-1}.

Photolysis of the nitrite ester (III; R=NO).-

The apparatus consisted of a pyrex vessel with a gas inlet tube at the bottom, a water-cooled pyrex immersion well and a 125 watt Philips high pressure mercury arc lamp. While a slow stream of pure dry nitrogen was passed into the vessel, a solution of the nitrite (III; R=NO) (200 mg.) in dry benzene (50 ml.) was irradiated at 12°. The reaction was followed by taking the infrared spectra of aliquots. The characteristic nitrite absorption band disappeared after 45 minutes. The photolysis was repeated on further portions (2 X 200 mg.). Chromatography of the combined products on alumina (Grade III; 30 g.) gave four products. The

less polar fractions, eluted with methylene chloride, gave 3β-acetoxy-13β-hydroxy-12-oxo-28-oleananic (28→13β)-lactone[4] (IV) (81 mg.; 13.6%), needles from chloroform-methanol m.p. 284-286°, mixed m.p. with an authentic specimen (m.p. 281-286°) was 283°. A second, more polar product, eluted with methylene chloride, was crystallized from methanol-chloroform to give the 3β-acetoxy-12α,13β-dihydroxy-28-oleananic (28→13β)-lactone[3] (III; R=H) (99 mg.; 16-22%) m.p. 292-296°. Mixed m.p. with an authentic specimen showed no depression. Further elution with ethyl acetate: methanol (8:2) gave another two compounds. The third compound was crystallized from methanol to give 3β-acetoxy-12α,13β-dihydroxy-27-oximino-28-oleananic (28→13β)-lactone (V; R=Ac) (297 mg.; 40-49%), m.p. 292-295°, $[\alpha]_D$+24.5°(c 0.75), ν_{max} chloroform 3550 and 3300(OH), 1770(lactone), 1725 and 1270(acetate) and 1635, weak (oxime) cm^{-1}, τ =7.92(acetate), 6.16(broad triplet; C-12β methine), 5.48(broad triplet; C-3α methine)

and 2.44(singlet; C-27 methine). The methyl at τ =8.85 present in the 3β -acetoxy-12α 13β -dihydroxy-28-oleananic (28$\rightarrow$13β) lactone (III; R=H) had disappeared. (Found: C, 70.96; H, 9.15; N, 2.59. $C_{32}H_{49}O_6N$ requires C, 70.68; H, 9.08; N, 2.58%). The fourth compound was characterized as <u>3β -acetoxy-12α ,13β -dihydroxy-27-nitrimino-28-oleananic (28$\rightarrow$13β) lactone</u> (VI; R=Ac) (30 mg.; 5%) and crystallized from chloroform-methanol, had m.p. 224° and 305-308°, $[\alpha]_D$+3.8°(c 0.78), λ_{max} 206 mμ (ε 5000), ν_{max} chloroform 3450(OH), 1770(lactone), 1720 and 1270(acetate) and 1615-1580 and 1305(nitrimine)[30] cm^{-1}. (Found: N,4.98. $C_{32}H_{48}O_7N_2$ requires N, 4.89%).

<u>Wolff-Kishner reduction of 3β -acetoxy-13β -hydroxy-12-oxo-28-oleananic (28$\rightarrow$13β)-lactone</u> (IV).-

a) <u>Barton's conditions</u>[14].

To redistilled diethylene glycol (20 ml.) was added sodium (415 mg.) followed by hydroquinone (564 mg.; 5m. equiv.). The clear

solution was heated to 180° under purified nitrogen before distilling in anhydrous hydrazine and until the mixture refluxed freely at 180°. The solution was cooled, the keto-lactone (IV) (554 mg.; 1 m. equiv.) quickly added and the solution then heated to reflux for 16 hours 30 minutes. The temperature was then raised to 210° by distilling some hydrazine back into the hydrazine generator and the solution refluxed at this temperature for a further 24 hours. After cooling, dilution with water and extraction with ether gave an acidic fraction (160 mg.; 31.4%), characterized by methylation as methyl oleananolate[15] (IX; R=H,R'=CH_3) m.p. 202°, $[\alpha]_D$+15.2°(c 0.39). The nuclear magnetic resonance spectrum of this compound did not show any olefinic protons. (Found: C, 78.38; H, 11.34. Calc. for $C_{31}H_{52}O_3$ requires C, 78.76; H, 11.09%). The acid from the Wolff-Kishner reduction, on treatment with bromine in carbon tetrachloride did not yield any bromolactone[17]. The methyl ester acetate (IX; R=Ac,R'=CH_3)

prepared by acetylation of the methyl ester (IX; R=H,R'=CH_3), had m.p. 195-196°, $[\alpha]_D$+ 20°(c 0.6). These compounds gave no end absorption in their ultraviolet spectra and a negative tetranitromethane test.

A neutral fraction (208 mg.; 47%) was also obtained, m.p. 170-180°,$[\alpha]_D$+57.4°(c 0.94) λ_{max} 206 mμ 6718, ν_{max} chloroform 3500(OH) cm^{-1}. Its mass spectrum indicated this to be a mixture of oleananol (X) parent ion M/e 428), together with dehydro-oleananol (M/e 426) and a dehydro-nor-oleananol(M/e 410).

b) Huang-Minlon conditions[18].-

This attempt was unsuccessful even with different variations of the time of reaction. It was only possible to obtain a 25% yield of a mixture of acids, which could not be separated.

Attempted preparation of the hydrazone[6] of 3β-acetoxy-13β-hydroxy-12-oxo-28-oleananic (28→13β)-lactone (IV).-

The keto-lactone (IV)(142 mg.) in a solution of absolute ethanol (10 ml.) and hydrazine hydrate 100% (1 ml.) was refluxed

for 22 hours. The product was a complex mixture of acid and neutral material. Infrared spectra showed that in both fractions the lactone ring had been cleaved.

Modified Wolff-Kishner reduction[14] of methyl oleanolate acetate (II; R=Ac, R'=CH_3).-

This experiment was carried out under the conditions described above but without hydroquinone and not using nitrogen.

Oleanolic acid[16] (II; R=R'=H) was isolated and characterized as its methyl ester (II; R=H, R'=CH_3)(75%) m.p. 198°, $[\alpha]_D$+ 69.4°(c 0.72). This methyl ester (II; R=H, R'=CH_3) gave an acetate (II; R=Ac, R'=CH_3) m.p. 218°$[\alpha]_D$+74°(c 0.62). The mixed m.p. of the methyl ester showed no depression with authentic methyl oleanolate.

Modified Wolff-Kishner reduction[14] of oleanolic acid acetate (II: R=Ac, R'=H).-

This experiment was carried out under the conditions described above. Oleanolic acid was isolated and characterized as its methyl ester[16] (II; R=H, R'=CH_3)(87%), m.p.193°,

$[\alpha]_D$+70° (c 0.74) and methyl ester acetate (II; R=Ac,R'=CH_3) m.p.217-219°,$[\alpha]_D$+74° (c 0.82). The mixed m.p. of the methyl ester (II; R=H,R'=CH_3) and methyl oleanolate showed no depression.

Metal-liquid ammonia reduction of 3β-12α-diacetoxy-13β-hydroxy-28-oleananic (28→13β)lactone (III; R=R'=Ac).-

a) Lithium. The diacetate (III; R=Ac)(528 mg.) in dry ether (50 ml.) was added to a solution of metallic lithium (435 mg.) in liquid ammonia (50 ml.) with vigorous stirring. The mixture was stirred for 30 minutes whilst cooling in a cardice-acetone bath before destroying the excess lithium with n-propanol (10 ml.). The mixture was left at room temperature until all the ammonia was evaporated and then acidified. After extraction with ether, the acidic product was treated with diazomethane. Evaporation afforded a methyl ester (II; R=H,R'=CH_3)(68 mg.; 17.2-20%) m.p. 198°,$[\alpha]_D$+75.2°(c 1.1). The mixed m.p. with authentic methyl oleanolate[16] showed no

depression. Treatment of this methyl ester (II; R=H,R'=CH_3) with pyridine-acetic anhydride gave methyl oleanolate acetate[16] (II; R=Ac,R'=CH_3) m.p. 218-220°,$[\alpha]_D$+74°(c 0.73) Repetition of this reduction experiment, using dry tetrahydrofuran instead of ether, gave a similar result.

b) Calcium.- The diacetate (III;R=Ac) (209 mg.) in dry, freshly distilled tetrahydrofuran (40 ml.) was added to a solution of metallic calcium (600 mg.) in liquid ammonia (40 ml.) with stirring.

The mixture was stirred for 30 minutes at -24° before destroying the excess calcium by addition of ammonium chloride. The mixture was left at room temperature until all the ammonia had evaporated and then acidified. The acidic product, after methylation with diazomethane, was characterized as methyl oleanolate[16] (II,R=H,R'=CH_3)(32 mg.; 20%) by mixed m.p. and thin layer chromatography.

Metal-liquid ammonia reduction of 3β-acetoxy-13β-hydroxy-12α-methanesulphonyloxy-28-oleananic (28→13β)lactone (III;R=SO_2CH_3).

a) Lithium.- Treatment of the mesylate (III; R=SO_2CH_3) (231 mg.) to the conditions described above yielded oleanolic acid[16], characterized as its methyl ester (II; R=Ac,R'=CH_3)(20 mg.; 10%) by mixed m.p. and thin layer chromatography.

b) Calcium.- The mesylate (III; R=SO_2CH_3)(324 mg.) was reduced with calcium-ammonia under the conditions described above. The acidic fraction was characterized as methyl oleanolate[16] (II; R=H,R'=CH_3)(26 mg.; 10.5%) m.p. 192-196°, $[\alpha]_D$+70.7°(c 0.82).

Mixed m.p. with an authentic specimen showed no depression.

Methyl 3β-acetoxy-12-oxo-28-oleananate (XIV; R=CH_3).-

A mixture of 3β-acetoxy-13β-hydroxy-12-oxo-28-oleananic (28→13β)-lactone (IV) (200 mg.), zinc dust (800 mg.) and acetic acid (20 ml.) was refluxed for 2 hours and filtered.

The filtrate was poured into water and extracted. Two fractions were obtained. The neutral fraction was characterized as the starting keto-lactone (IV)(95 mg., 43.5%). The mixed m.p. of this compound with an authentic specimen showed no depression. The

acidic fraction was characterized as 3β - acetoxy-12-oxo-28-oleananic acid[22] (XIV; F H) (104 mg.; 56%) m.p. 288-295°, $[\alpha]_D$-16.7°(c 0.54) ν_{max} Nujol 3300 and 1675(acid), 1725(acetate-ketone) and 1250(acetate)cm^{-1}. This acid on treatment with diazomethane, gave the methyl ester[22](XIV; R=CH_3) m.p. 193-195°,$[\alpha]_D$-13.4° (c 0.53). The mixed m.p. of this compound with an authentic specimen showed no depression.

Methyl oleanolate acetate (II; R=Ac R'=CH_3).-

Methyl 3β -acetoxy-12-oxo-28-oleananate (XIV; R=CH_3)(.7 mg.) was treated with sodium borohydride (40 mg.) in ethanol (20 ml.) at room temperature for 1 hour. After chromatography over alumina (Grade III; 5g.) and elution with benzene, yielded starting material and the corresponding alcohol (XV)(21 mg.;44%) m.p. 236-243°, $[\alpha]_D$+8.8°(c 0.45), ν_{max} Nujol 3550(OH), 1720 and 1250(acetate) and 1700 (ester)cm^{-1}. The latter alcohol (XV)(13 mg.) in pyridine (2 ml.) and methanesulphonyl chloride (0.5 ml.) was heated on the steam bath for 3 hours. The product, after extraction with methylene chloride, crystallized from

methanol to give methyl oleanolate acetate (II; R=Ac,R'=CH_3) m.p. 218°, λ_{max} 206 mμ (ε 4900); the infrared spectrum was identical with that of an authentic sample. The mixed m.p. with an authentic specimen showed no depression.

3β -Acetoxy-12α ,13β -dihydroxy-27-nitrilo-28-oleananic (28→ 13β)lactone (XVI; R=Ac,R'=H).-

The 3β -acetoxy-12α ,13β -dihydroxy-27-oximino-28-oleananic (28→13β)-lactone (V; R=Ac)(129 mg.) in dry pyridine (5 ml.) was treated at room temperature for 16 hours with p-toluenesulphonyl chloride (280 mg.; recrystallized). The mixture was poured into water, acidified and extracted with methylene chloride. Crystallization from chloroform-methanol gave 3β -acetoxy-12α ,13β -dihydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XVI; R=Ac, R'=H)(80 mg.; 64%) m.p. 305-308°, $[\alpha]_D$+30.3° (c 0.66), ν_{max} chloroform 3500(OH), 2260 (nitrile),1770(lactone) and 1720-1270(acetate) cm^{-1}. (Found: C, 72.98; H, 8.82. $C_{32}H_{47}O_5N$

requires C, 73.11; H, 9.01%).

Attempted hydrolysis of the nitrile (XVI; R=Ac,R'=H).-

a) 4N Sodium hydroxide[23].- 3β-acetoxy-12α,13β-dihydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XVI; R=Ac, R'=H)(67 mg.) was treated with 4N sodium hydroxide in 80% ethanol (15 ml.) on the steam bath for 24 hours. The complex mixture of products gave a neutral fraction. Chromatography over alumina (Grade III; 3g.) with benzene: methylene chloride (1:1) gave a nitrile compound believed to be 27-nitrilo-3β,12α,13β-trihydroxy-28-oleananic ((28→13β)-lactone (XVI; R=R'=H), ν_{max} chloroform 3500(OH), 2260(nitrile) and 1760 (lactone) cm^{-1}.

b) Sodium in diethylene glycol.- The nitrile (XVI; R=Ac,R'=H)(57 mg.) in diethylene glycol (12 ml.), in which sodium (355 mg.) had been dissolved, was heated under nitrogen at 200°. Drops of dioxan were added until the solution refluxed freely. The solution was refluxed for 24 hours, before pouring into

water, acidifying and extracting with methylene chloride. The product was a complex mixture which gave, after chromatography over alumina (Grade III; 3g.) with benzene, only one crystalline product (6 mg.) which possessed nitrile absorption in its infrared spectrum but no lactone peak.

c) Sulphuric acid[24].- The nitrile (XVI; R=Ac,R'=H)(57 mg.) was dissolved in a solution of concentrated sulphuric acid (6 ml.) and water (4 ml.) and heated for 2 hours on the steam bath. The solution was poured into water and extracted with methylene chloride. The product proved to be a complex mixture that was not further studied.

3β,12α-Diacetoxy-13β-hydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XVI; R=R'=Ac).-

The 3β-acetoxy-12α,13β-dihydroxy-27-oximino-28-oleananic (28→13β)-lactone (V; R=Ac)(392 mg.) in dry pyridine (5 ml.) was treated with acetic anhydride (5 ml.) on the steam bath for 3 hours and then at room temperature overnight. The solution was

poured into water, acidified, and extracted with methylene chloride. Crystallization from methanol afforded 3β,12α -diacetoxy-13β -hydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XVI; R=R'=Ac)(341 mg; 83%) m.p. 193°, which after further crystallizations gave m.p. 200-202°, $[\alpha]_D$+45.7°(c 0.83), ν_{max} chloroform 2260(nitrile), 1780(lactone), 1745, 1725 and 1265(acetates) cm^{-1}, τ =7.95 acetate 7.81(acetate), 5.50(broad triplet; C=3α methine) and 4.95(broad triplet; C-12β methine). (Found: C, 72.01; H, 8.44. $C_{34}H_{49}O_6N$ requires C, 71.92; H, 8.70%).

Lithium-liquid ammonia reduction of 3β , 12α -diacetoxy-13β -hydroxy-27-nitrile-28-oleananic (28→13β)-lactone (XVI; R=R'=Ac).-

The diacetate (XVI; R=R'=Ac)(108 mg.) in dry ether was added to a mixture of metallic lithium (121 mg.) in liquid ammonia (20 ml.) with stirring. The mixture was stirred for a further 45 minutes whilst cooling with a cardice-acetone bath. The excess lithium was destroyed by n- propanol (10 ml.). The mixture was left at room temperature until the liquid

ammonia had evaporated and then acidified. The mixture, on working up afforded a small amount (3% by weight) of acidic material, not further investigated, and a neutral fraction which was chromatographed over alumina (Grade III; 3g.) eluting with methylene chloride. The main product was eluted with ethyl acetate and characterized as 27-nitrilo-28-oxo-3β ,12α ,13β -trihydroxy-oleanane (28→13β)-hemiacetal (XVIII; R=R'=H)(64 mg.; 69%), m.p. 275-278,$[\alpha]_D$+ 45°(c 0.4), ν_{max} chloroform 3600-3450(OH) and 2250(nitrile) cm^{-1}, τ =6.15(broad triplet; C-12β methine) and 4.98(singlet; C-28 H hemiacetal). A similar result was obtained using calcium-liquid ammonia. Acetylation of the hemiacetal (XVIII; R=R'=H) at room temperature, overnight, with pyridine-acetic anhydride gave 3β -acetoxy-12α ,13β-dihydroxy-27-nitrilo-28-oxo-oleanane (28→13β)-hemiacetal-28-acetate (XVIII; R=R'=Ac) m.p. (from methanol) 292-294°, $[\alpha]_D$+78.9(c 0.2), ν_{max} Nujol 3550(OH), 2260(nitrile) and 1725-1250(acetate), τ =7.96(acetate), 7.91(acetate),

6.18(broad triplet; C-12 β methine), 5.48 (broad triplet; C-3 α methine) and 4.04(singlet; C-28 H hemiacetal acetate). (Found: C, 71.93; H, 9.12. $C_{34}H_{51}O_6N$ requires C, 71.67; H, 9.02%).

Final proof of this compound was obtained by oxidation of the hemiacetal (XVIII; R=R'=H)(10 mg.) in acetone with 8N chromium trioxide (3 equiv.) in aqueous (2N) sulphuric acid.

After 10 minutes excess aqueous methanol was added and the product was isolated by extraction with methylene chloride. The 3,12-dioxo-13β -hydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XIX)(see below) had m.p. 282-287°, ν_{max} chloroform 2260(nitrile), 1785(lactone) and 1715-1700 (ketones)cm^{-1}. The mixed m.p. with an authentic specimen showed no depression and their infrared spectra were identical.

Preparation of 3,12-dioxo-13β -hydroxy-27-nitrilo-28-oleananic (28→13 β)-lactone (XIX).-

3β -Acetoxy-12α,13β -dihydroxy-27-nitrilo-28-oleananic (28→13β)-lactone

(XVI; R=Ac,R'=H)(160 mg.) was refluxed 2 hours in ethanolic hydrochloric acid (2N)(45 ml.). The mixture was poured into water and extracted with methylene chloride to give after crystallization from benzene, the 27-nitrilo-3β, 12α,13β-trihydroxy-28-oleananic (28→13β)-lactone (XVI; R=R'=H)(120 mg.) m.p. 304-309°, $[\alpha]_D$+22.8°(c 1.2), ν_{max} chloroform 3600-3470 (OH), 2260(nitrile) and 1768(lactone) cm^{-1}. (Found: C, 74.71; H, 9.23. $C_{30}H_{45}O_4N$ requires C, 74.49; H, 9.38%).

Oxidation of this compound in acetone with 8N chromium trioxide (3 equiv.) in aqueous (2N) sulphuric acid gave, after extraction with methylene chloride and crystallization from methanol, 3,12-dioxo-13β-hydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XIX) m.p. 285-288°$[\alpha]_D$+20.9(c 0.81), ν_{max} chloroform 2260(nitrile), 1780(lactone) and 1715-1700(ketones) cm^{-1}. (Found: C, 75.05; H, 8.80. $C_{30}H_{41}O_4N$ requires C, 75.12; H, 8.62%).

Attempted zinc-acetic acid reduction of 3β,12α-diacetoxy-13β-hydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XVI; R=R'=Ac).-

A mixture of the diacetate (XVI; R=R'=Ac) (25 mg.) zinc dust (104 mg.) and acetic acid (5 ml.) was refluxed for 15 hours and then filtered. The filtrate was poured into water and extracted with methylene chloride to give, after crystallization from methanol, the unchanged starting material (23 mg.).

3β -Acetoxy-13β -hydroxy-27-nitrilo-12-oxo-28-oleananic (28→13 β)-lactone (XXI).- 3β -Acetoxy-12α ,13β -dihydroxy-27-nitrilo-28-oleananic (28→13 β)-lactone (XVI; R=Ac, R'=H) (27 mg.) in acetone (5 ml.) was treated at room temperature with 8N chromium trioxide (3 equiv.) in aqueous (2N) sulphuric acid. After 10 minutes excess aqueous methanol was added and the product isolated by extraction with methylene chloride.

The 3β -acetoxy-13β -hydroxy-27-nitrilo-12-oxo-28-oleananic (28→13 β)-lactone (XXI) (21 mg.; 80%) crystallized from chloroform-methanol and showed m.p. 291-292°, $[\alpha]_D$ -3.3° (c 0.56), ν_{max} chloroform 2260(nitrile), 1780 (lactone), 1720(acetate-ketone) and 1270(acetate)

cm^{-1}. τ =7.98(acetate) 5.50(broad triplet; C-3α methine). (Found: C,72.52; H, 8.84. $C_{32}H_{45}O_5N_{\frac{1}{2}}$ H_2O requires C, 72.14; H, 8.70%).

Methyl 3β-acetoxy-27-nitrilo-12-oxo-28-oleananate (XXII; R=CH_3)

A mixture of 3β-acetoxy-13β-hydroxy-27-nitrilo-12-oxo-28-oleananic (28$\rightarrow$13β)-lactone (XXI)(204 mg.), zinc dust (821 mg.) and acetic acid (20 ml.) was refluxed for 24 hours and then filtered. The filtrate was poured into water and extracted with ether. The product was treated with diazomethane in ether and then filtered through alumina (Grade III; 3g.) with benzene to give, after crystallization from methanol, methyl 3β-acetoxy-27-nitrilo-12-oxo-28-oleananate (XXII; R=CH_3) (145 mg.; 69%) m.p. 285-287° $[\alpha]_D$-16.2° (c 0.74), ν_{max} chloroform 2260(nitrile), 1720 broad(acetate, ester and ketone) and 1260 (acetate) cm^{-1}. (Found: C, 73.89; H, 9.32. $C_{33}H_{49}O_5N$ requires C, 73.43; H, 9.15%).

Methyl 3β-acetoxy-12ϵ-hydroxy-27-nitrilo-28-oleananate (XX; R=CH_3).-

The keto-ester (XXII; R=CH_3)(39 mg.) was treated with sodium borohydride (38 mg.) in ethanol (20 ml.), with stirring at room temperature, for 1 hour. After chromatography over alumina, crystallization gave methyl 3β-acetoxy-12ε-hydroxy-27-nitrilo-28-oleananate (XX; R=CH_3)(20 mg.) m.p. 294-295° $[\alpha]_D$+1° (c 0.93), ν_{max} chloroform 3500(OH), 2260 (nitrile) and 1720-1270(ester and acetate) cm^{-1} τ =7.98(acetate) and 6.31(methyl ester). (Found: C,73.36; H, 9.20. $C_{33}H_{51}O_5N$ requires C, 73.16; H, 9.49%).

Attempted hydrolysis of 3β-acetoxy-12α,13β-dihydroxy-27-oximino-28-oleananic (28→13β)-lactone[26] (V; R=Ac).-

The oxime (V; R=Ac)(27 mg.) in a solution of acetone:water (5:1) with 2% concentrated hydrochloric acid(10 ml.) was treated at room temperature for 21 hours and then on the steam bath for 70 minutes. The mixture was poured into water and the product extracted with methylene chloride. The starting oxime (V; R=Ac)(20 mg.; 74%) m.p. 290-292° was obtained and characterized by thin layer

chromatography. The mixed m.p. with an authentic oxime showed no depression.

3β -Acetoxy-12α ,13β -dihydroxy-27-nitrimino-28-oleananic (28 →13 β)-lactone (VI; R=Ac).-

The oxime (V; R=Ac)(120 mg.) in dioxan (3 ml.) and acetic acid (8 ml.) was treated with a solution of sodium nitrite 5% (6 ml.) at room temperature with stirring. A compound started to precipitate out after 15 minutes. The precipitate was filtered off after 24 hours to give the crystalline 3 β -acetoxy-12α ,13 β -dihydroxy-27-nitrimino-28-oleananic (28→13β)-lactone (VI; R=Ac)(106 mg.; 84%) m.p. 222-224° and 305-308°.

After several crystallizations from chloroform-methanol the m.p's. were 224° and 305-308°, $[\alpha]_D$+4°(c 1). This compound was shown to be identical with the nitrimine obtained from the photolysis by thin layer chromatography and their infrared spectra. The mixed m.p. showed no depression. Neither the expected (27 →12 α) hemiacetal[27] nor the

corresponding 27-aldehyde could be obtained from this reaction.

Attempts to decompose the nitrimine (VI; R=Ac).-

a) Heating[30].- The nitrimine (VI; R=Ac)(40 mg.) was heated in dioxan (10 ml.) and water (5 ml.) for 5.5 hours on the steam bath and then evaporated under reduced pressure to small bulk. Methanol (20 ml.) was added before re-evaporating under reduced pressure. Only starting material (30 mg.; 75%) was recovered (confirmed by mixed m.p. and infra-red spectrum 1615-1580 and 1305(nitrimine)[30] cm^{-1}).

b) Urea treatment[30].- The nitrimine (VI; R=Ac)(143 mg.) in dioxan (5 ml.) and water (2.5 ml.) was treated with urea (137 mg.) under reflux for 51 hours. This mixture was poured into water and extracted with methylene chloride to give the unchanged nitrimine (VI; R=Ac)(110 mg.; 78.2%).

Treatment of 3β-acetoxy-12α,13β-dihydroxy-27-nitrimino-28-oleananic (28→13β)-lactone (VI; R=Ac) with sulphuric acid.-

The nitrimine (VI; R=Ac)(176 mg.) was refluxed in a solution of acetone:water (5:1) containing 2% v/v) of concentrated sulphuric acid(15 ml.) for 27 hours. Extraction with methylene chloride gave a product believed to be the 27-nitrimino-3β,12α,13β-trihydroxy-28-oleananic (28→13β)-lactone (VI; R=H), since the carbonyl absorption band in its infrared spectrum due to the acetate at 1725 cm^{-1} had disappeared but still possessed the infrared bands due to nitrimine (1615-1580 and 1305 cm^{-1}). This compound (VI; R=H) was refluxed in a solution of acetone:water (5:1) containing 10% (v/v) of concentrated sulphuric acid (15 ml.) for 19 hours. After extraction the nitrimine (140 mg.; 86.5%) was recovered unchanged. The recovered nitrimine (VI; R=H)(30 mg.) was refluxed in a solution of dioxan:water with 10% of concentrated sulphuric acid (9 ml.) for 22 hours. The nitrimine (VI; R=H) was again found to

be unchanged (ν_{max} chloroform 3500(OH), 1770(lactone) and 1615-1850-1305(nitrimine)[30] cm^{-1}).

Attempted reduction of the nitrimine (VI; R=Ac).-

The nitrimine (VI; R=Ac)(30 mg.) in absolute ethanol (20 ml.) was treated at room temperature with sodium borohydride (30 mg.) for 1 hour. The product was found to be the unchanged starting material. The nitrimine (VI; R=Ac) was also recovered unchanged after 21 hours under the same conditions.

Attempted oxidation of the nitrimine (VI; R=Ac).-

The nitrimine (5 mg.) in acetone was treated at room temperature with 8N chromium trioxide (3 equiv.) in aqueous (2N) sulphuric acid.

After 10 minutes, excess methanol and water were added and the product isolated by extraction into methylene chloride. The product, believed to be 3β-acetoxy-13β-hydroxy-27-nitrimino-12-oxo-28-oleananic

(28→13β)-lactone, possessed the infrared bands due to nitrimine (1615-1580 and 1305 cm^{-1}).

Pyrolysis of the nitrimine (VI; R=Ac).-

The nitrimine (VI; R=Ac)(20 mg.) on heating at 240° gave 3β-acetoxy-12α,13β-dihydroxy-27-nitrilo-28-oleananic (28→13β)-lactone (XVI; R=Ac, R'=H)(13 mg.; 72%) m.p. (from chloroform-methanol) 305-308°, infrared spectrum superimposable with that of an authentic specimen. A mixed m.p. with authentic material showed no depression.

3β-Acetoxy-27-amino-12α,13β-dihydroxy-28-oleananic.(28→13β)-lactone (XXVI; R=Ac,R'=H).-

The nitrimine (VI; R=Ac)(109 mg.) in ethyl acetate (7 ml.) was hydrogenated, over platinum oxide (126 mg.) as catalyst, until uptake of hydrogen was complete. The compound consumed 4 ml. of hydrogen. The product was characterized as 3β-acetoxy-27-amino-12α-13β-dihydroxy-28-oleananic (28→13β)-lactone (XXVI; R=Ac,R'=H)(80 mg.; 80%) m.p. (from chloroform-methanol) 267-270°, $[\alpha]_D$+

36.9° (c 0.71), ν_{max} chloroform 3500(OH), 1760(lactone, 1720-1265(acetate) and 1635 (amine) cm^{-1}, τ=7.98(acetate), 6.23(broad triplet; C-12β methine) and 5.50(broad triplet; C-3α methine). (Found: C, 72.78; H, 9.32. $C_{32}H_{51}O_5N$ requires C, 72.55; H, 9.70%). This compound was not soluble in dilute hydrochloric acid at room temperature.

The amine (XXVI; R=Ac,R'=H)(110 mg.) was refluxed in an ethanolic solution (2N) of hydrochloric acid (12 ml.). After 19 hours the mixture was poured into water and extracted with ether to give 27-amino-3β, 12α,13β-trihydroxy-28-oleananic (28→13β)-lactone (XXVI; R=R'=H)(47 mg.; 51.5%) m.p. (from methanol-chloroform) 231-234°, $[\alpha]_D^+$ 36.6° (c 0.73), ν_{max} chloroform 3500(OH), 1760(lactone) and 1640(amine) cm^{-1}. The fraction soluble in acid after treatment with sodium hydroxide was extracted with ether to yield a substance (41 mg.; 45%) identical with the first compound (XXVI; R=R'=H) isolated from this reaction.

The amine (XXVI; R=Ac,R'=H)(100 mg.) was treated with pyridine-acetic anhydride at room temperature overnight but gave the starting material unchanged.

Treatment of the amine (XXVI; R=Ac, R'=H)(80 mg.) with pyridine (2 ml.)- acetic anhydride (5 ml.) at reflux temperature for 3 hours gave a compound in small yield which had ν_{max} chloroform 1760(lactone), 1740-1720 and 1270(acetates) and 1680(amide) cm^{-1} (XXVII). The major product was 27-amino-3β, 12α-diacetoxy-13β-hydroxy-28-oleananic (28$\rightarrow$13β)-lactone (XXVI; R=R'=Ac)(36 mg.; 49%) m.p. (from methanol) 198-203°, $[\alpha]_D$+39.6° (c 1), ν_{max} chloroform 1765(lactone), 1740-1720 and 1270(acetates) and 1635(amine) cm^{-1}.

Hydrogenation of the nitrimine (VI; R=Ac) was also carried out with palladium in charcoal (10%; 5%; 1%). In each case only the amine (XXVI; R=Ac,R'=H) was recovered. Partial reduction reactions, carried out stopping the reaction after the uptake of 1,2 and 3 moles of hydrogen, gave, in every case only starting material and the amino

compound.

Zinc-ammonium chloride reduction of the nitrimine (VI; R=Ac).-

The nitrimine (VI; R=Ac)(182 mg.) was dissolved in a solution of ethanol (18 ml.), water (1.8 ml.) containing ammonium chloride (400 mg.) and treated with zinc dust (2.7 g.) under reflux for 2 hours. After extraction with methylene chloride 3β -acetoxy-27-amino-12α ,13β -dihydroxy-28-oleananic (28$\rightarrow$13β)-lactone (XXVI; R=Ac; R'=H)(120 mg.;71.5%) was obtained m.p. 269-275°, $[\alpha]_D$+37.8°(c 0.83). The infrared spectrum was identical with the product of the hydrogenation of the nitrimine, and mixed m.p. showed no depression.

Treatment of the nitrimine (VI; R=Ac) with methanesulphonyl chloride.-

A solution of the nitrimine (VI; R=Ac) (889 mg.) in dry pyridine (13.3 ml.) containing methanesulphonyl chloride (3.35 ml.) was heated for 3 hours on the steam bath. The resulting brown solution was poured into water, acidified and extracted with methylene

chloride.

The mixture obtained was chromatographed on alumina (Grade III; 40 g.). Elution with benzene gave 3β -acetoxy-12ε -chloro-13β -hydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXVIII)(160 mg.; 19.6%) which, after crystallization from chloroform-methanol, had m.p. 305-307°, $[\alpha]_D$+27.3°(c 1.4), ν_{max} chloroform 2750(aldehyde), 1770(lactone), 1720(acetate-aldehyde) and 1270(acetate) cm^{-1}, τ =7.93 (acetate), 5.55(broad triplet; C-3α methine) and 0.13(aldehyde). This compound, according to the mass spectrum had its parent ion peak at m/e 546. (Found: C, 70.30; H, 8.70; Cl,6.58. $C_{32}H_{47}O_5Cl$ requires C, 70.23; H, 8.65; Cl, 6.48).

Elution of the column with benzene: ethyl acetate (95:5) afforded another compound identified as (XXIX; R=Ac,R=H)(224 mg., 27.4%). This compound upon recrystallization gave needles of 3β -acetoxy-12α,13β -dihydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=Ac,R'=H), m.p. 264-269°, $[\alpha]_D$+31.2°(c 0.8), ν_{max} chloroform 3500(OH) 2750(aldehyde), 1765(lactone),1720(acetate-

aldehyde) and 1275(acetate) cm^{-1}, τ =7.95 (acetate), 6.03(broad triplet; C-12β methine) 5.56(broad triplet; C-3α methine) and -0.13 (aldehyde). (Found: C, 72.59; H, 9.09. $C_{32}H_{48}O_6$ requires C, 72.69; H, 9.15%).

Treatment of 3β-acetoxy-12α,13β-dihydroxy-27-nitrimino-28-oleananic (28→13β)-lactone (VI; R=Ac) with acetic anhydride in pyridine.-

The nitrimine (VI; R=Ac)(526 mg.) in dry pyridine (5 ml.) was treated on the steam bath for 3 hours with acetic anhydride (10 ml.) and then poured into water and extracted into methylene chloride. Crystallization of the product from chloroform-methanol gave 3β-acetoxy-12α,13β-dihydroxy-27-oxo-28-oleananic (27→12α)-hemiacetal-(28→13β)-lactone-27-acetate (XXX)(41 mg.; 79%), m.p. 306-308°, $[\alpha]_D$-13.6°(c 1.2), ν_{max} Nujol 1775 (lactone), 1748(hemiacetal acetate) and 1730-1245(acetates) cm^{-1}, τ =7.95 (acetate), 7.87 (acetate), 5.78(broad triplet; C-12β methine), 5.45(broad triplet; C-3α methine) and 3.68 (singlet; C-27 H hemiacetal acetate). (Found:

C, 71.15; H, 9.16. $C_{34}H_{50}O_7$ requires C, 71.55; H, 8.83%).

The nitrimine (VI; R=Ac) after refluxing 3 hours in pyridine was recovered unchanged.

Selective hydrolysis of the hemiacetal acetate (XXX).-

3β-Acetoxy-12α,13β-dihydroxy-27-oxo-28-oleananic (27→12α)-hemiacetal (28→13β)-lactone 27-acetate (XXX)(1.014 mg.) was dissolved in benzene : methylene chloride (9:1) before pouring very slowly onto an alumina (Grade III; 50 g.) column and leaving overnight.

After 16 hours careful elution gave 3β-acetoxy-12α,13β-dihydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=Ac, R'=H)(837 mg.; 89%) identical with the product isolated from the treatment of the nitrimine (VI, R=Ac) with methanesulphonyl chloride in pyridine.

27-oxo-3β,12α,13β-trihydroxy-28-oleanic (28→13β)-lactone(XXIX;R=R'=H).-

The hemiacetal acetate lactone (XXX) (49 mg.) in tetrahydrofuran (2 ml.) was hydrolysed with 6N hydrochloric acid (8 ml.) in ethanol (16 ml.) under reflux for 2 hours. The product was 27-oxo-3β,12α,13β-trihydroxy-28-oleananic (28→13β)-lactone (XXIX, R=R'=H) which crystallized from methanol had m.p. 270-274°, $[\alpha]_D$+29.3° (c 0.92) ν_{max} Nujol 3500(OH), 1765(lactone) and 1700 (aldehyde) cm^{-1}, τ =6.78(broad triplet; C-3α methine), 6.04(broad triplet; C-12β methine) and -0.04(aldehyde).

3β,12α-Diacetoxy-13β-hydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=R'=Ac).-

The 3β-acetoxy-12α,13β-dihydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=Ac,R'=H) (62 mg.) was treated with dry pyridine (1 ml.) and acetic anhydride (1.5 ml.) on the steam bath for 3 hours to give 3β,12α-diacetoxy-13β-hydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=R'=Ac)(49 mg.), m.p. (from methanol) 271-274°, $[\alpha]_D$+ 36.1° (c 0.9), ν_{max} Nujol 1780(lactone), 1740-

1260-1240(acetates) and 1707(aldehyde)cm^{-1}, τ =7.95(acetate), 7.85(acetate), 5.50(broad triplet; C-3 α methine), 4.75(broad triplet; C-12 β- methine) and -0.22(aldehyde). (Found: C, 71.90; H, 8.92. $C_{34}H_{50}O_7$ requires C,71.55; H, 8.83%). The same diacetate (XXIX; R=R'=Ac) was obtained by acetylation on the steam bath of 27-oxo-3 β ,12α ,13β -trihydroxy-28-oleananic (28$\rightarrow$13β)-lactone (XXIX; R=R'=H).

Attempted preparation of 3β -acetoxy 12 α,13 β -dihydroxy-27-oximino-28-oleananic (28$\rightarrow$13 β)-lactone (V; R=Ac).-

a) The aldehyde (XXIX; R=Ac, R'=H) (24 mg.) in ethanol (10 ml.) was treated with hydroxylamine hydrochloride (674 mg.) in water (3 ml.) and sodium acetate (1370 mg.) in water (3 ml.) under reflux for 16 hours. The starting material was recovered unchanged.

b) The aldehyde (XXIX; R=Ac,R'=H)(34 mg.) in pyridine (2 ml.) was treated with hydroxylamine hydrochloride (108 mg.) and ethanol (2 ml.) on the steam bath during 5 hours and left at room temperature for 7 months.

The starting material was recovered unchanged.

3β-Acetoxy-12α,13β-dihydroxy-oleanan-27,28-dioic (27→12α), (28→13β)-dilactone (XXXI; R=Ac).-

3β-Acetoxy-12α,13β-dihydroxy-27-oxo-oleananic (27→12α)-hemiacetal-(28→13β)-lactone-27-acetate (XXX) (1030 mg.) in acetone (400 ml.)[1] was treated at room temperature, with 8N chromium trioxide in aqueous (2N) sulphuric acid (40 ml.) and the mixture stirred for 15 hours. After this time excess methanol and water were added and the products isolated by extraction with methylene chloride. Chromatography over alumina (Grade III; 40g.) gave, on elution with benzene and crystallization from chloroform-methanol, the dilactone acetate (XXXI; R=Ac)(381 mg.; 38.8%), together with 3β-acetoxy-12α,13β-dihydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=Ac, R'=H), due to the hydrolysis on the alumina column of the unoxidised hemiacetal acetate (XXX) and a small amount of the starting hemiacetal acetate.

The dilactone acetate (XXXI; R=Ac) had m.p. over 345°, $[\alpha]_D$+5.2° (c 0.96), ν_{max}

chloroform 1780(lactone),1720-1275(acetate) cm^{-1}, τ =7.98(acetate), 5.56(broad triplet; C-3 α methine) and 5.41(broad triplet; C-12β methine). (Found: C, 72.87; H, 8.43. $C_{32}H_{46}O_6$ requires C, 72.97; H, 8.80%).

3β,12α,13β-Trihydroxy-oleanan-27, 28-dioic (27→12 α),(28→13 β)-dilactone (XXXI; R=H).

The dilactone acetate (XXI; R=Ac)(61 mg.) was refluxed in ethanolic hydrochloric acid (2N, 30 ml.; from 10 ml. 6N hydrochloric acid).

After 3 hours the dilactone (XXXI; R=H) formed was crystallized from methanol to give m.p. >345° $[\alpha]_D$-1.2° (c 0.77), ν_{max} chloroform 3500(OH) and 1770(lactone) cm^{-1} This compound, on acetylation at room temperature gave back the starting dilactone acetate (XXXI; R=Ac).

Lithium-liquid ammonia reduction of 3β-acetoxy-12α,13β-dihydroxy-oleanan-27,28-dioic (27→12α), (28→13β)-dilactone (XXXI; R=Ac).-

The dilactone acetate (XXXI; R=Ac)(95 mg.) in dry ether (40 ml.) was treated in the usual

way with lithium (123 mg.) in liquid ammonia (40 ml.) for 40 minutes. The product was 3β,12α,13β-28-tetrahydroxy-27-oleananic-(27→12α)-lactone (XXXII; R=R'=H)(63.4 mg.; 72.5%) m.p. (from methanol) 325-327°, $[\alpha]_D$+3.1° (c 0.63), ν_{max} chloroform 3500(OH) and 1760(lactone) cm^{-1}. (Found: C, 73.81; H, 10.07. $C_{30}H_{48}O_5$ requires C, 73.73; H, 9.90%).

The same result was repeated using tetrahydrofuran as solvent instead of ether.

Treatment of this compound (XXXII; R=R'=H) (32 mg.) with pyridine (2 ml.) and acetic anhydride (2 ml.) on the steam bath for 3 hours gave, from methanol, the diacetate (XXXII; R=R'=Ac), m.p. 314-318°, $[\alpha]_D$+1.6° (c 0.61), ν_{max} Nujol 3500(OH), 1760(lactone) and 1718-1265 and 1245(acetates) cm^{-1}, τ=7.98(acetate), 7.92(acetate), 5.78 (singlet; C-28 methylene), 5.70(broad triplet; C-12β methine) and 5.56(broad triplet; C-3α methine). (Found: C, 71.12; H, 8.97. $C_{34}H_{52}O_7$ requires C, 71.29; H, 9.15%).

This diacetate (XXXII; R=R'=Ac) was recovered unchanged after oxidation with 8N

chromium trioxide (3 equiv.) in aqueous (2N) sulphuric acid in acetone for 15 minutes at room temperature.

Calcium-liquid ammonia reduction of 3β-acetoxy-12α,13β-dihydroxy-oleanan-27,28-dioic (27→12α),(28→13β)-dilactone (XXXI; R=Ac).-

The dilactone acetate (XXXI; R=Ac), upon calcium-liquid ammonia reduction gave the same hydroxy-lactone (XXXII; R=R'=H)(79%) as was obtained using lithium-ammonia.

Attempted zinc-acetic acid reduction of the dilactone acetate (XXXI; R=Ac).-

The dilactone acetate (XXXI; R=Ac) (48 mg.) was recovered unchanged after treatment with zinc dust (236 mg.) in refluxing acetic acid (20 ml.) for 52 hours.

Methyl 3β,12α-diacetoxy-13β-hydroxy-27-oleananate-28-oic(28→13β)-lactone (XXXIII; R=CH_3).

3β-12α-Diacetoxy-13β-hydroxy-27-oxo-28-oleananic-(28→13β)-lactone (XXIX; R=R'=Ac)(107 mg.) in acetone (20 ml.) was

treated with 8N chromium trioxide in aqueous (2N) sulphuric acid (4 ml.). After 5 hours a mixture of the 27-acid and the starting material was obtained.

The mixture, in ether, was treated with diazomethane and chromatographed over alumina (Grade III; 25g.). Elution with benzene:ethyl acetate (98:2) gave the starting material and methyl 3β,12α-diacetoxy-13β-hydroxy-27-oleananate-28-oic (28→13β)-lactone (XXXIII; R=CH_3)(35 ml.; 33%), m.p. (from methanol) 331-333°, $[\alpha]_D$+32.7° (c 1.17), ν_{max} chloroform 1770(lactone), 1720(ester and acetate) and 1275(acetate) cm.$^{-1}$, τ 7.95(acetate), 7.88(acetate), 6.37(methyl ester), 5.56 (broad triplet; C-3 α methine) and 4.90 (broad triplet; C-12β methine). (Found: C, 69.65; H, 8.86. $C_{35}H_{52}O_8$ requires C, 69.97; H, 8.72%).

Dimethyl cincholate (I: R=H,R'=R"=CH_3).- methyl 3β,12α-diacetoxy-13β-hydroxy-27-oleananate-28-oic(28→13β)-lactone (XXXIII; R=CH_3)(180 mg.) in dry tetrahydrofuran (40 ml.) was added to a solution of metallic lithium (295 mg.) in liquid ammonia (40 ml.) at -60° and stirred for 30 minutes. The

product was treated with diazomethane and chromatographed over alumina (Grade III; 15 g.). Elution with benzene:ethyl acetate (97:3) gave dimethyl cincholate (I; R=H,R'=R"=CH_3) 46 mg.; 22%). Crystallization from methanol afforded needles m.p. 214-216° $[\alpha]_D$+ 114.5° (c 1.2), ν_{max} chloroform 3550(OH) and 1710(ester) cm^{-1}, τ =6.35(methyl ester), 6.31(methyl ester) and 4.25(broad triplet; C-12 methine). The mixed m.p. with authentic specimen[32] showed no depression. The same yield of dimethyl cincholate was obtained using calcium, instead of lithium, in the reduction.

Dimethyl cincholate acetate (I; R=Ac, R'=R"=CH_3).

Dimethyl cincholate (I; R=H,R'=R"=CH_3) on acetylation with pyridine-acetic anhydride, at room temperature, gave dimethyl cincholate acetate(I; R=Ac,R'=R"=CH_3). Crystallized from methanol had m.p. 249-254°,$[\alpha]_D$+ 110°(c 1.05), $[\alpha]_D$+ 96°(pyridine c 0.9), ν_{max} chloroform 1710 and 1270(ester and acetate) cm^{-1}, τ =7.95 (acetate), 6.35(methyl ester), 6.31(methyl ester), 5.50(broad triplet; C-3 α methine)

4.27(C-12 vinyl proton). The mass spectra of this compound and the natural material[32] were identical. The mixed m.p. with an authentic specimen[32] showed no depression.

Cincholic acid (I; R=R'=R"= H).-

Dimethyl cincholate (I; R=H, R'=R"=CH_3) (46 mg.) in dry, freshly distilled collidine (50 ml.) was refluxed 2 hours with dry lithium iodide[33] (1 g.). The product was shown, by thin layer chromatography (disopropyl-ether: acetone (5:2) to be a mixture of cincholic acid (I; R=R'=R"=H) and, probably, pyrocincholic acid. The mixture in methanol was treated dropwise with a solution of bromine in carbon tetrachloride, until the bromine colour just persisted, in order to convert the pyrocincholic acid (XXXIV) to its neutral bromolactone.

The acid present in the acidic fraction was identical with an authentic specimen of cincholic acid[32] by comparison on thin layer chromatography. This acid was characterized as its methyl ester by mixed m.p. and comparison of the infrared spectra.

Attempted oxidation of 3β -acetoxy-12α ,13 β-dihydroxy-27-oxo-28-oleananic (28→13 β)-lactone (XXIX; R=Ac, R'=H).-

a) The aldehyde (XXIX; R=Ac,R'=H) (30 mg.) in dioxan (3 ml.) was treated with silver nitrate[34] (112 mg.) in water (1.5 ml.) and 4N sodium hydroxide (1 ml.), with stirring. After 1 hour at room temperature the aldehyde was recovered unchanged.

b) The aldehyde (XXIX; R=Ac,R'=H) treated with the same mixture for 1 hour on the steam bath was recovered unchanged after acetylation.

c) The aldehyde (XXIX; R=Ac,R'=H) (33 mg.) in dry tetrahydrofuran (5 ml.) was treated with freshly prepared silver oxide (110 mg.) in the dark at room temperature with stirring for 12 days.

After acetylation was found the starting material unchanged.

Autoxidation experiments[35]

Benzylamine-N-benzylidene[36](XXXV).-

Benzaldehyde (10 ml.) in ethanol (5 ml.)

was added to benzylamine (10 ml.) in water (50 ml.). The heavy oil which separated from the exothermic reaction was collected and distilled to give the imine[36] (XXXV) b.p. 141-142° (3 mm.), ν_{max} film 1635 (C=N) and 1605-1588(benzene) cm^{-1}.

Autoxidation of benzylamine-N-benzylidene (XXXV).-

Potassium t-butoxide (610 mg.) was dissolved in dry t-butanol (8 ml.) and water (0.05 ml.) was added. After saturation of this solution with oxygen the benzylamine-N-benzylidine (XXXV)(339 mg.) was added. The solution was shaken until the uptake of oxygen was 1.1 mol. and then poured into water containing excess acetic acid.

The product was extracted with chloroform to give, after crystallization, benzamide (XXXVI)(125 mg.; 59.5%) m.p. 128°, ν_{max} Nujol 3400-3200-1660 and 1632(amide) and 1582 (benzene) cm^{-1}. The mixed m.p. with an authentic specimen showed no depression. A second compound, m.p. 230° ν_{max} Nujol 3300, 1650, 1605 and 1583 cm^{-1}, was also isolated

from this reaction. This compound was not further studied.

α-Benzaldoxime[37] (XXXVII).-

Hydroxylamine hydrochloride (3.5 g.) in water (10 ml.) was added to a solution of benzaldehyde (6.3 g.) in ethanol (20 ml.) and the mixture treated with sodium acetate (7 g.) in water (10 ml.). The solution was refluxed for 2 hours, poured into water and extracted with chloroform.

An oily mixture of α and β -benzaldoxime was obtained. The oil was distilled at 88° (2 mm.) to give the α -benzaldoxime[37] m.p. 32°, ν_{max} film 3350-1632(oxime) and 1605-1582(benzene) cm^{-1}.

Autoxidation of α -benzaldoxime (XXXVII).-

Potassium t-butoxide (7.2 g.) was dissolved in a mixture of dry dimethylsulphoxide (48 ml.), dry t-butanol (12 ml.) and water (0.3 ml.).

The α-benzaldoxime was dissolved in this mixture and shaken with oxygen for 24 hours. The oxygen uptake was 500 ml. The

mixture was poured into water containing excess acetic acid. The acidic fraction was shown to be benzoic acid m.p. 122° (XXXVIII) (1.12 g.; 65.5%). The infrared spectrum was superimposable with that of an authentic sample and its mixed m.p. showed no depression. The neutral fraction was not examined.

Attempted autoxidation of 3β -acetoxy-12 α,13β -dihydroxy-27-oximino-28-oleananic (28 →13β)-lactone (V; R=Ac).-

The oxime (V; R=Ac)(169 mg.) was dissolved in a solution of dry dimethyl sulphoxide (4 ml.), dry t-butanol (1 ml.) and water (0.05 ml.) containing potassium t-butoxide (528 mg.). The mixture was shaken under oxygen for 19 hours, then poured into water containing excess acetic acid. The products were 27-oximino-3β , 12 α ,13β -trihydroxy-28-oleananic-(28 —> 13β)lactone (V; R=H)(75 mg.; 48%) and a compound (24%) which had a strong band at 2320 cm^{-1} but no lactone in its infrared spectrum. The expected 27-acid could not be detected.

Attempted autoxidation of 3β -acetoxy-12α,13β -dihydroxy-27-nitrimino-28-oleananic (28→13β)-lactone (VI; Ac).-

The nitrimine (VI; R=Ac)(42 mg.) was added to a mixture of dry dimethylsulphoxide (8 ml.), dry t-butanol (2 ml.) and water (0.05 ml.). The mixture was shaken under oxygen for 3 hours 30 minutes when 2.29 ml. of oxygen was absorbed.

The mixture was poured into water containing excess acetic acid. The product was 27-nitrimino-3β,12α,13β -trihydroxy-28-oleananic (28→13β)-lactone (VI; R=H).

Methyl 3β -acetoxy-13β -hydroxy-12-oxo-27-oleananate-28-oic(28→13β)-lactone (XL; R=CH_3).-

3β -Acetoxy-12α,13β -dihydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=Ac,R'=H)(224 mg.) in acetone (40 ml.) was treated at room temperature with 8N chromium trioxide in aqueous (2N) sulphuric acid (5 ml.). After 3 hours excess aqueous methanol was added and the products isolated by extraction

with methylene chloride and then treatment with an ethereal solution of diazomethane.

After chromatography over alumina (Grade III; 90 g.) and elution with benzene, 3β -acetoxy-12,27-dioxo-13β -hydroxy-28-oleananic (28→13β)-lactone (IXL) and methyl 3β -acetoxy-13β -hydroxy-12-oxo-27-olecananate-28-oic(28→13β)-lactone (XL; R=CH_3)(71 mg.; 31%) were obtained. The methyl ester (XL; R=CH_3), crystallized from chloroform-methanol had m.p. 244-248°, $[\alpha]_D$ -8.7° (c 1.1), ν_{max} chloroform 1780(lactone), 1720(ester and acetate) and 1280(acetate) cm^{-1} τ =7.93(acetate), 6.30(methyl ester) and 5.56(broad triplet; C-3α methine). (Found: C, 71.42; H, 8.55. $C_{33}H_{48}O_7$ requires C, 71.19; H, 8.69%).

Dimethyl 3β -acetoxy-12-oxo-oleanan-27, 28-dioate (XLI; R=CH_3).-

3β -Acetoxy-12α ,13β -dihydroxy-27-oxo-28-oleananic (28→13β)-lactone (XXIX; R=Ac,R'=H)(834 mg.) was oxidised in acetone (100 ml.) with 8N chromium trioxide in aqueous (2N) sulphuric acid (10 ml.) during 24 hours

at room temperature whilst stirring. The resultant product was methylated with diazomethane and treated with zinc dust (2g.) in refluxing acetic acid (40 ml.) for 30 hours.

The mixture was re-treated with diazomethane and chromatographed over alumina (Grade III; 60g.) eluting with benzene to give dimethyl 3 β-acetoxy-12-oxo-oleanan-27, 28-dioate (XLI; R=CH_3) (364 mg.; 40.5%). Crystallized from methanol, this had m.p. 235-237°, $[\alpha]_D$-21.2° (c 0.8), ν_{max} chloroform 1718 (acetate, ester and ketone) and 1275(acetate) cm^{-1}, τ=7.98(acetate), 6.34 (methyl ester), 6.32(methyl ester) and 5.56 (broad triplet; C-3 α methine). (Found: C, 71.32; H, 9.14. $C_{34}H_{52}O_7$ requires C, 71.29; H, 9.15%).

Dimethyl cincholate acetate (I; R=Ac, R'=R"=CH_3).-

Dimethyl 3 β -acetoxy-12-oxo-oleanan-27, 28-dioate (XLI; R=CH_3) (90 mg.) was treated with sodium borohydride (100 mg.) in ethanol (25 ml.) at room temperature for 28 hours. The

mixture obtained was treated in pyridine (25 ml.) with methanesulphonyl chloride (1 ml.) on the steam bath for 1 hour. The product, after acidification and extraction with methylene chloride, was chromatographed over alumina (Grade III; 6g.) to give, on elution with benzene, dimethyl cholestane acetate (I; R=Ac, R'=R"=CH_3) (12 mg.) m.p. (from methanol) 248-252°. $[\alpha]_D$ + 94.7° (pyridine c 0.8), characterized by thin layer chromatography and mixed m.p. with an authentic specimen[32].

3β,5α,6β-Triacetoxycholestane[38] (XLIII; R=R'=R"=Ac).-

Cholesterol acetate (XLIV; R=Ac)(490 mg.) was treated on the steam bath with a mixture of acetic acid (25 ml.) and 30% hydrogen peroxide (5 ml.) for 3 hours. The product of this reaction was acetylated overnight with pyridine-acetic anhydride at room temperature. 3β,6β-Diacetoxycholestan-5α-ol[39] (XLIII; R=R"=Ac,R'=H) (310 mg.; 55%) was obtained, m.p. 164° $[\alpha]_D$ - 41° (c 0.89) ν_{max} Nujol 3500(OH), 1730-1710 and

1270-1250(acetates) cm^{-1}. This compound (XLIII; R=R'=Ac,R'=H) (2 g.) was acetylated with excess acetic anhydride (100 ml.) and p-toluenesulphonic acid (440 mg.) on the steam bath for 30 minutes. 3β,5α,6β-Triacetoxycholestane[38] (XLIII; R=R'=R"=Ac) (1.34 g.; 62%) crystallized from aqueous methanol had m.p. 148-149°, $[\alpha]_D$-37.4° (c 0.85), τ=8.00(acetate), 7.92(two acetates), 5.32(broad triplet; C-3 α methine) and 4.11 (broad triplet; C-6 α methine).

Lithium-liquid ammonia reduction of 3β-5α,6β-triacetoxycholestane (XLIII; R=R'=R"=Ac).-

The triacetate (XLIII; R=R'=R"=Ac)(100 mg.) in dry ether (10 ml.) was added to a solution of metallic lithium (200 mg.) in liquid ammonia (60 ml.) with vigorous stirring. The mixture was stirred for 45 minutes in an acetone-cardice bath and then the excess of lithium was destroyed by n-propanol. The main product was found to be cholesterol (XLIV; R=H)(54 mg.; 72-74%, m.p. 148-149°,

$[\alpha]_D$-37.8°(c 0.94), characterized by thin layer chromatography and mixed m.p. with an authentic specimen. The acetate (XLIV; R=Ac) had m.p. 115°, $[\alpha]_D$-44.6(c 1.5).

Calcium-liquid ammonia reduction of 3β,5α,6β-triacetoxycholestane (XLIII; R=R'=R"=Ac).-

The triacetate (XLIII; R=R'=R"=Ac) (184 mg.) in dry tetrahydrofuran (40 ml.) was treated in the usual way with calcium (600 mg.) in liquid ammonia (40 ml.). The products were cholesterol (XLIV; R=H)(84 mg.; 61%) and 3β, 5α,6β-trihydroxycholestan[39] (XLIII; R=R'=R"=H) (50 mg.; 35%), m.p. 231-233°, $[\alpha]_D$+3.5°(dioxan c 0.85).

Lithium-liquid ammonia reduction of 3β, 6β-diacetoxycholestan-5α-ol(XLIII; R=R"=Ac,R'=H).-

The diacetate (XLIII; R=R"=Ac,R'=H) (215 mg.) in dry tetrahydrofuran (35 ml.) was treated by the usual way with metallic lithium (331 mg.) in liquid ammonia (40 ml.). The product was 3β,5α, 6β-trihydroxycholestane (XLIII; R=R'=R"=H)(160 mg.; 89%

3β,5α,6α-Triacetoxycholestane (XLV;R=R'=R"=Ac).-

Cholesterol was oxidised with potassium permanganate[40] to give 3β,5α,6α-trihydroxycholestane[40-41](XLV;R=R'=H R"=H) m.p. 235-238°, $[\alpha]_D$+26.5° (dioxan c 0.64).

The trihydroxy compound (XLV; R=R'=R"=H) on treatment with pyridine-acetic anhydride gave 3β,6α-diacetoxycholestan-5α-ol[40-41] (XLV; R=R"=Ac,R'=H) m.p. 188-189°,$[\alpha]_D$+19.6° (c 0.61). The diacetate (XLV; R=R"=Ac,R'=H) (338 mg.) was further acetylated with acetic anhydride (37 ml.) and p-toluenesulphonicacid (130 mg.) on the steam bath for 105 minutes. 3β,5α,6α-Triacetoxycholestane (XLV; R=R'=R"=Ac)(90 mg.; 34%) was isolated after chromatography through alumina (Grade III; 6g.) eluting with petroleum ether. Crystallized from petroleum ether (b.p. 30-40°) at -60° it had m.p. 112-114°,$[\alpha]_D$+13.8°(c 0.69), τ =8.00(acetate), 7.95(acetate), 7.91(acetate), 5.30(broad triplet; C-3 α methine) and 4.94 (broad triplet; C-6β methine). (Found: C, 72.51; H, 9.84. $C_{33}H_{54}O_6$ requires C, 72.49; H, 9.96%).

Lithium-liquid ammonia reduction of 3β,5α,6α-triacetoxycholestane (XLV; R=R'=R"=Ac).-

The triacetate (XLV; R=R'=R"=Ac)(56 mg.)

in dry tetrahydrofuran (30 ml.) was treated as usual with lithium (237 mg.) in liquid ammonia (30 ml.). Chromatography over alumina (Grade III; 2g.) with benzene gave cholesterol (XLIV; R=H)(21 mg.; 48%). The cholesterol was identified by thin layer chromatography and mixed m.p. Elution with ethyl acetate:methanol (80:20) gave 3β,5α, 6α-trihydroxycholestane (XLV; R=R'=R"=H) (25 mg.; 52%) m.p. 235-236°, identified by thin layer chromatography and mixed m.p.

α-Methyl glucoside tetra-acetate (XLVII).-

α-Methyl glucoside (XLVI) was acetylated at room temperature overnight with pyridine-acetic anhydride to give the tetra-acetate (XLVII) m.p. 101-102°, $[\alpha]_D$+134(c 2.5).

Lithium-liquid ammonia reduction of α-methyl glucoside tetra-acetate (XLVII).-

The tetra-acetate (XLVII)(1.12 g.) in dry tetrahydrofuran (20 ml.) was treated in the usual way with metallic lithium (992 mg.) in liquid ammonia (40 ml.). The product was

acetylated to give the tetra-acetate (XLVII) (1.090 g.; 96%) m.p. 99-101° identified by thin layer chromatography and mixed m.p.

2,3-Diacetyl-4,6-benzylidene α-methyl glucoside[42] (XLVIII; R=R'=Ac).-

α-Methyl glucoside (XLVI)(14 g.) was shaken with anhydrous zinc chloride (10 g.) in benzaldehyde (35 ml.) for 3 hours after slight warming. The 4,6-benzylidene α-methyl glucoside[43] (XLVIII; R=R'=H) (12.4 g.) obtained was acetylated with pyridine-acetic anhydride at room temperature overnight to give 2,3-diacetyl-4,6-benzylidene α-methyl glucoside[42] (XLVIII; R=R'=Ac). Crystallized from methanol this had m.p. 108-110°, $[\alpha]_D$+ 77.5(c 1.3).

Lithium-liquid ammonia reduction of 2, 3-diacetyl-4,6-benzylidene α-methyl glucoside (XLVIII; R=R'=Ac).-

The diacetate (XLVIII; R=R'=Ac)(1g.) in dry tetrahydrofuran (40 ml.) was treated by the usual way with metallic lithium (390 mg.) in liquid ammonia (40 ml.). The product was acetylated at room temperature overnight with

pyridine-acetic anhydride to give α-methyl glucoside tetra-acetate (XLVII) (877 mg.; 88%) m.p. 101°, characterized by thin layer chromatography and mixed m.p.

Hexa-acetyl mannitol (IL).-

Mannitol on acetylation gave hexa-acetyl mannitol m.p. 121-123°, $[\alpha]_D$+26.3°(c 1.4).

Lithium-liquid ammonia reduction of hexa-acetyl mannitol (IL).

Hexa-acetyl mannitol (IL)(1g.) in dry tetrahydrofuran (40 ml.) was treated in the usual way with metallic lithium (515 mg.) in liquid ammonia (40 ml.). The product was acetylated and characterized as starting material (IL)(951 mg.; 95%) m.p. 119-120° by thin layer chromatography and mixed m.p.

Cholestanol[44](L).-

A solution of recrystallized cholesterol (16 g.) in ethyl acetate (220 ml.) at 30-40° was hydrogenated with the aid of platinum oxide (300 mg.) and perchloric acid 60% (o.1 ml.). The hydrogenation was complete in 18 hours to give cholestanol[44] (L)(10 g.; 62%) m.p. 138°, $[\alpha]_D$+25° (c 0.91).

Cholestan-3-one[45] (LI).-

A hot solution of sodium dichromate dihydrate (9 g.) in acetic acid (56 ml.) was

added to a suspension of cholestanol (9 g.) in acetic acid (51 ml.) and the mixture was heated on the steam bath for few minutes to effect complete solution and then let stand overnight. The resulting mixture was treated with water and the precipitate was collected, washed well with water and crystallized from acetone-ethanol to give cholestanone[45] (LI) (5.5 g.; 62-75%) m.p. 130-131°, $[\alpha]_D$+43° (c 1.1).

2 α-Bromo-cholestan-3-one[45] (LII).-

Bromination was conducted by slow addition (10 minutes) of a solution of bromine (2.3 g.) and hydrobromic acid (3 drops) in acetic acid (10 ml.) to a stirred solution of cholestanone (LI) (5 g.) in acetic acid (150 ml.) at 25°. The bromoketone, which crystallized from the reaction mixture, was collected and washed. After crystallization the bromoketone[45] (LII) (4 g.; 67%) had m.p. 169-171°, $[\alpha]_D$+44°(c 1.4).

Cholest-2-ene[46] (LIII).-

The 2 α-bromo cholestan-3-one (LII)

(1.6 g.) was treated with sodium borohydride (160 mg.) in absolute ethanol (75 ml.) at room temperature with stirring for 28 hours during which time the ketone slowly dissolved.

The total product was dissolved in dry pyridine (20 ml.) and treated with p-toluene-sulphonyl chloride (2 g.) overnight at room temperature. The product, in acetic acid (60 ml.) was heated to reflux whilst adding zinc dust (2 g.) portionwise, over 1 hour.

Filtration through alumina (Grade III; 25 g.) in petroleum ether and crystallization from methylene chloride-methanol gave pure cholest-2-ene[46] (LIII)(704 mg.; 54%) m.p. 74°, $[\alpha]_D$+67(c 1).

2α,3α-Diacetoxy cholestane[47-48] (LIV; R=R'=Ac).-

A mixture of cholest-2-ene (LIII)(1.2 g.) in dry benzene (50 ml.) and osmium tetraoxide (1 g.) in dry pyridine (50 ml.) was kept at room temperature for 96 hours. The solvent was evaporated under reduced pressure and the residue was heated under reflux for 4 hours in a mixture of mannitol (7.5 g.), potassium

hydroxide (7.5 g.), ethanol (75 ml.) and water (15 ml.). The product was isolated with benzene and crystallized from methanol to give the diol[47] (LIV; R=R'=H)(1.18 g.; 90%) m.p. 218°, $[\alpha]_D$+34°(c 0.69).

The diol (LIV; R=R'=H) was acetylated with pyridine-acetic anhydride at room temperature to give, after crystallization from methanol, the diacetate[48] (LIV; R=R'=Ac) m.p. 130-133°, $[\alpha]_D$+34.5° (c 0.84).

Lithium-liquid ammonia reduction of 2α,3α-diacetoxy cholestane (LIV; R=R'=Ac).-

The diacetate (LIV; R=R'=Ac)(50 mg.) in dry tetrahydrofuran (20 ml.) was treated in the usual way with metallic lithium (160 mg.) in liquid ammonia (30 ml.). The product was 2α,3α-dihydroxy cholestane (LIV; R=R'=H) (41 mg.; 90%) m.p. 213-215° characterised by thin layer chromatography and mixed m.p. Cholest-2-ene (LIII) was not detected even by thin layer chromatography.

2α,3β-Diacetoxy cholestane[49] (LVI; R=R'=Ac).-

A solution of cholestan-3-one (LI)(4.7 g.)

and lead tetra-acetate (6.3 g.) in acetic acid (220 ml.) containing boron trifluoride ether complex (9.6 ml.) was stirred at 25° under nitrogen. After 145 minutes the mixture was pouredinto water and extracted with methylene chloride. The methylene chloride solution was washed with a solution of sodium bicarbonate and water. After chromatography over alumina (Grade III; 130 g.) eluting with petroleum ether, 2α -acetoxy-cholestan-3-one (LV) was isolated.

Treatment of the keto-acetate (LV) with lithium aluminium hydride in ether for 20 minutes gave 2α,3β -dihydroxy cholestane (LVI; R=R'=H) m.p. 208-209°, $[\alpha]_D$+19°(c 1).

The diol (LVI; R=R'=H), on acetylation, gave 2α,3β -diacetoxycholestane[49](LVI; R=R'=Ac) m.p. 110-112°, $[\alpha]_D$-12°(c 1.2).

Lithium-liquid ammonia reduction of 2α, 3β -diacetoxy cholestane (LVI; R=R'=Ac).

The diacetate (LVI; R=R'=Ac)(208 mg.) in dry tetrahydrofuran (40 ml.) was treated in the usual way with metallic lithium (293 mg.) in liquid ammonia (40 ml.) The product 2α,3β -dihydroxy cholestane (LVI; R=R'=H)(173 mg.; 92%) m.p. 205° was characterized by thin layer chromatography and mixed m.p. Cholest-2-ene (LIII) was not detected.

2β -3α -Diacetoxy cholestane[58] (LVII; R=R'=Ac).-

To a solution of cholest-2-ene (LIII) (1g.) dissolved in acetic acid (25 ml.) on the steam bath was added 30% hydrogen peroxide (1.7 ml.) over a period of 1 hour with stirring. Water and methylene chloride were added and the acetic acid removed by shaking with sodium bicarbonate. The methylene chloride was evaporated and the residue refluxed for 1 hour with excess of ethanolic potassium hydroxide solution. The product, extracted with methylene chloride and crystallized from methanol gave the diol (LVII; R=R'=H)(600 mg.; 63%) m.p. 202-204°, $[\alpha]_D$+37.6°(c 0.93).

The diol (LVII; R=R'=H) was acetylated with pyridine-acetic anhydride at room temperature overnight to give 2β ,3α -diacetoxy cholestane[50] (LVII; R=R'=Ac) m.p. 132-135°, $[\alpha]_D$+57.5°(c 0.96).

Lithium-liquid ammonia reduction of 2β, 3α -diacetoxy cholestane (LVII; R=R'=Ac).

The diacetate (LVII; R=R'=Ac)(118 mg.) in dry tetrahydrofuran (30 ml.) was treated

in the usual way with metallic lithium (276 mg.) in liquid ammonia (30 ml.). Chromatography over alumina (Grade III; 10 g.) gave cholest-2-ene (LIII)(8 mg.; 8.9%) m.p. 66°, identified by thin layer chromatography and mixed m.p. together with 2β ,3α -dihydroxy cholestane (LVII; R=R'=H)(77 mg.; 79%) m.p. 201-203°, identified by thin layer chromatography and mixed m.p.

Extraction of pyrethrol (LVIII; R=H).- Saponification of pyrethrum waxes[59] (24 g.) gave a mixture of neutral products and a very small amount of an acidic fraction. The neutral fraction (6.33 g.), which crystallized from benzene, was chromatographed over alumina (Grade III; 180 g.). By elution with petroleum ether a crude crystalline fraction (3.2 g.; 13.3%) was obtained m.p. 192-193°. Upon recrystallization this fraction gave pyrethrol (LVIII; R=H) m.p. 218-219°, $[\alpha]_D$+88°(c 0.87), the ultraviolet spectrum showed only end absorption, ν_{max} Nujol 3400(OH), 1640(c=c) and 885(methylene) cm^{-1}.

This compound on chromic oxidation gave a product having a positive Zimmermann test[60].

Pyrethrol acetate (LVIII; R=Ac).-

Pyrethrol, on acetylation with pyridine-acetic anhydride, at room temperature overnight, gave an acetate m.p. 233-234° $[\alpha]_D$+ 95.3°,(c 0.43), ν_{max} Nujol 1720-1245(acetate) 1640(c=c) and 890(methylene) cm^{-1}, τ =7.94 (acetate), 5.45(broad triplet; C-3α methine) and 5.37-5.34(multiplet; C-30 exocyclic methylene).

Pyrethrol benzoate (LVIII; R=Bz).-

The pyrethrol, on treatment with benzoyl chloride in pyridine overnight at room temperature gave a benzoate (LVIII; R=Bz) m.p. 235-240°,$[\alpha]_D$+89(c 0.7).

Dihydropyrethrol acetate(LXI; R=Ac).-

A solution of pyrethrol acetate (in ethyl acetate was hydrogenated, using 10% palladium-charcoal as catalyst.

The compound took up 1 equivalent of hydrogen. The dihydropyrethrol acetate had

m.p. 243-244°, $[\alpha]_D$+29.5°(c 0.44), ν_{max} Nujol 1730 and 1245(acetate). (Found: C,81.96; H, 11.31. Calc. for $C_{32}H_{54}O_2$ C, 81.64; H, 11.56%).

Selenium dioxide oxidation of pyrethrol acetate (LVIII; R=Ac).-

Pyrethrol acetate was oxidised with selenium dioxide in refluxing acetic acid for 6 hours to give an aldehyde (LXII) m.p. 250-251°, $[\alpha]_D$+104.6°(c 0.32), λ_{max} 233.6 mμ (ε 12174[63]), ν_{max} Nujol 2750-1670(aldehyde), 1730-1250(acetate) and 1648(c=c) cm^{-1}, τ =7.94 (acetate), 5.42(broad triplet; C3α methine), 3.20(triplet J=4.5 cps.; C-21 methine) and 0.52 (aldehyde at C-20). (Found: C, 79.75; H, 10.45. $C_{32}H_{50}O_3$ requires C, 79.62; H, 10.44%).

Treatment of pyrethrol acetate (LVIII) with monoperphthalic acid.-

Pyrethrol acetate in chloroform was treated with an ethereal solution of monoperphthalic acid (0.92 N) overnight at 5°.

The product was an epoxide (LXIV) m.p. 211-212°, $[\alpha]_D$+105.2°(c 0.38), ν_{max} Nujol 1720

and 1240(acetate) cm^{-1}.

Lithium aluminium hydride reduction of the epoxide of pyrethrol acetate (LXIV).-

Reduction of the epoxide (LXIV) with excess lithium aluminium hydride in ether, overnight gave a diol (LXV) m.p. 238-239°, $[\alpha]_D$ 0° (c 0.29), ν_{max} chloroform 3500(OH) cm^{-1}.

Chromic oxidation of the diol (LXV).-

The diol (LXV), in acetone, was treated with 8N chromium trioxide (3 equiv.) in aqueous (2N) sulphuric acid. After 10 minutes excess aqueous methanol was added. Extraction with methylene chloride gave a mixture which was chromatographed on alumina (Grade III). By elution with benzene a ketol (LXVI) was obtained.

This ketol had m.p. (from chloroform-methanol) 248-252°, $[\alpha]_D$ +31.1° (c 0.45), ν_{max} Nujol 3500(OH) and 1695(ketone) cm^{-1}.

The physical constants of pyrethrol and its derivatives were found to be very similar to taraxasterol[62]. The mixed m.p. of pyrethrol acetate and taraxasterol acetate showed no depression. Further confirmation

that pyrethrol and taraxasterol were actually the same compound was obtained by taking the mixed m.p. of dihydro pyrethrol acetate and dihydrotaraxasterol acetate, when there was no depression.

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