THESIS

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BY

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SOME ASPECTS OF THE CHEMISTRY OF α-AMYRIN AND BREIN.

CONTENTS

INTRODUCTION

- THEORETICAL -

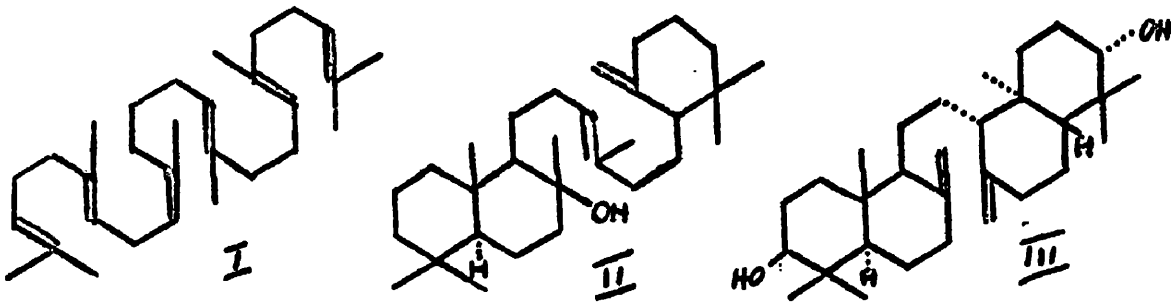
Introduction.

In 1851 an examination [1] of Arbol ∝-brea resin, later shown to be identical with Manila elemi resin from Canarium commune L. [2], led to the isolation of four crystalline compounds, "amyrin" [3], brein, breidin and bryoidin. "Amyrin" was later found to be a mixture of two isomeric alcohols, $C_{30}H_{50}O$, ∝-amyrin and β-amyrin, which can be separated by fractional crystallisation of the derived acetates [4]. Brein was shown to be a dihydric alcohol, $C_{30}H_{50}O_2$, and it was suggested on biogenetic grounds that it might prove to be a hydroxy-amyrin [5]. Breidin has not been since isolated and may have been a mixture. Bryoidin has been shown to be a dihydric, sesquiterpene alcohol, $C_{15}H_{30}O_2$ [6].

∝-Amyrin, β-amyrin and brein, are members of the group of naturally-occurring compounds known as triterpenes, the molecules of which characteristicly contain thirty carbon-atoms. As this thesis is concerned with the chemistry of these three compounds, this introduction takes the form of a description of the triterpene group as a whole.

Classification of the Triterpenes

Apart from squalene (I)[7,8], which is aliphatic, the tricyclic alcohol, ambrein (II)[9-11], and the tetracyclic diol, onocerin (III)[12,13], all known, naturally-occurring triterpenes have pentacyclic or tetracyclic carbon-skeletons, the latter being similar to the carbon-skeleton of the steroids.

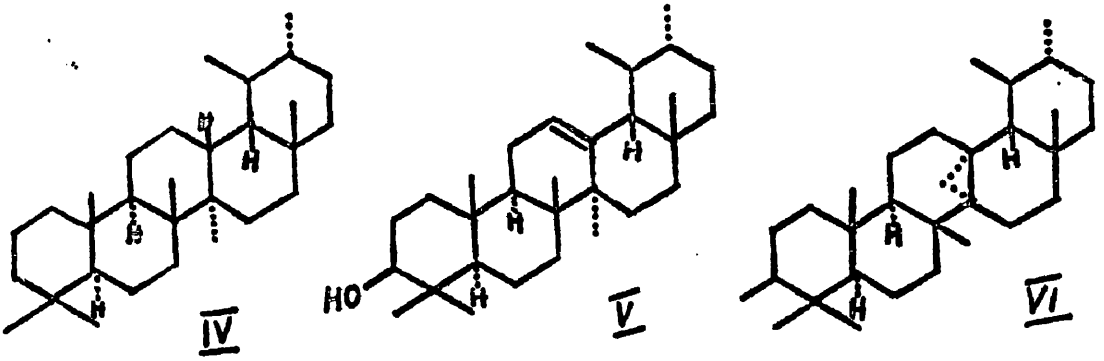


The Pentacyclic Triterpenes

Most naturally-occurring triterpenes are pentacyclic and may be classified into four groups. Members of the same group differ, in general, in the arrangement of functional groups on a common carbon-skeleton, and may be related by standard methods[14-17] to the simple monohydric alcohols on which the classification is based.

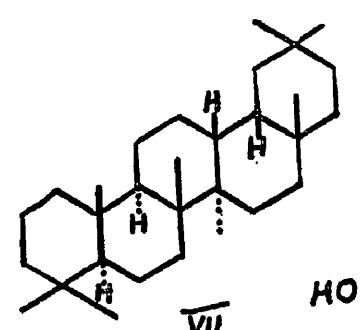
1). The ∝-amyrin group are derivatives of the hydrocarbon, ursane (IV), and include such compounds as ∝-amyrin (urs-12-en-3β-ol)

(V), uvaol, brein, quinovic acid, β-boswellic acid, ursolic acid, and the hexacyclic alcohol, phyllanthol (VI), which is easily isomerised to α-amyrin (V).

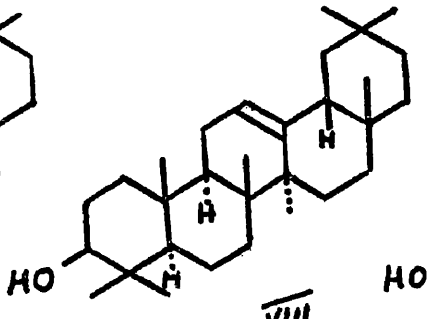


IV V VI

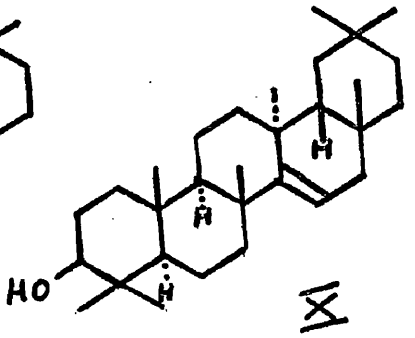
2). The β-amyrin group are derivatives of the hydrocarbon oleanane (VII) and include such compounds as β-amyrin (VIII), α-boswellic acid, oleanolic acid, maniladiol, glycyrrhetic acid, soyasapogenols A, B, and C, and numerous cactus triterpenes. Also included in this group are taraxerol (X), friedelin (XI), cerin (XII) and alnusenone (XIII) which do not possess the oleanane skeleton but are easily converted into oleanane derivatives.



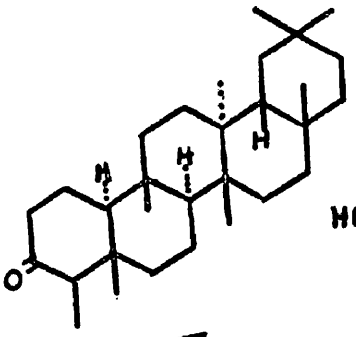
VII



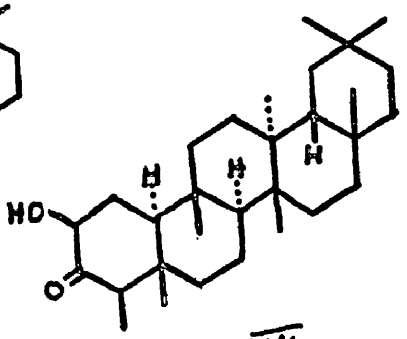
VIII



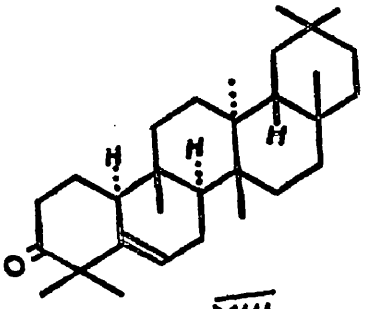
X



XI

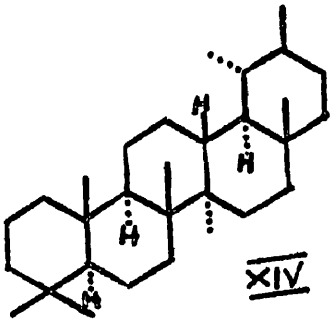


XII

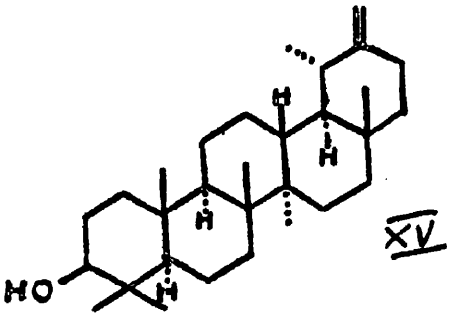


XIII

3). The taraxasterol group are derivatives of the hydrocarbon, taraxastane (XIV) and are represented by the alcohol, taraxasterol (XV).



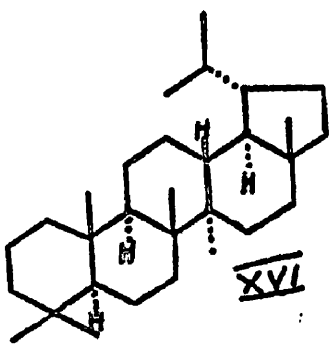
XIV



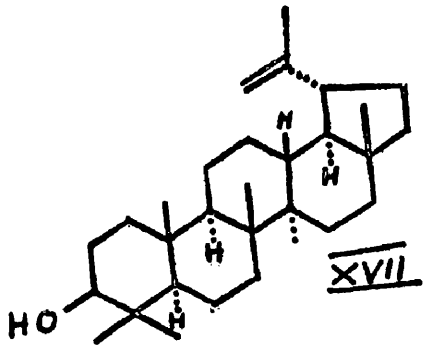
XV

4). The lupeol group are derivatives of the hydrocarbon, lupane (XVI), and apart from

lupeol (XVII) are isolated mainly from cactii .

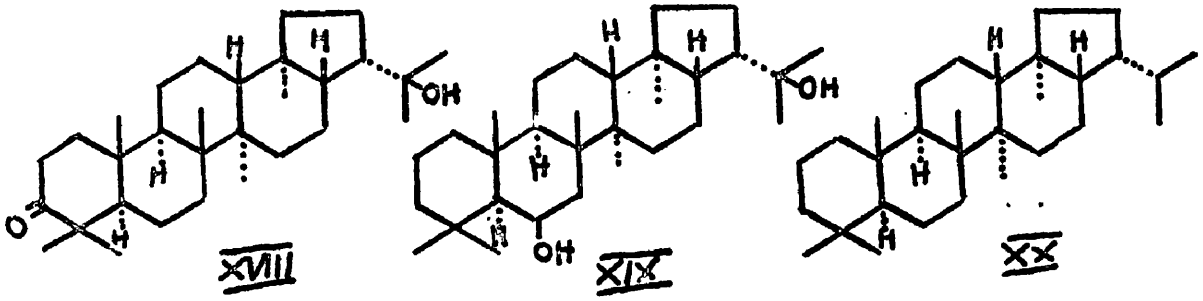


XVI



XVII

Two recently elucidated, naturally-occurring, pentacyclic triterpenes, hydroxyhopanone (XVIII)[18-21] and zeorin (XIX)[22-26] cannot be included in this classification but appear to be members of a fifth group, based on the hydrocarbon, hopane (XX)

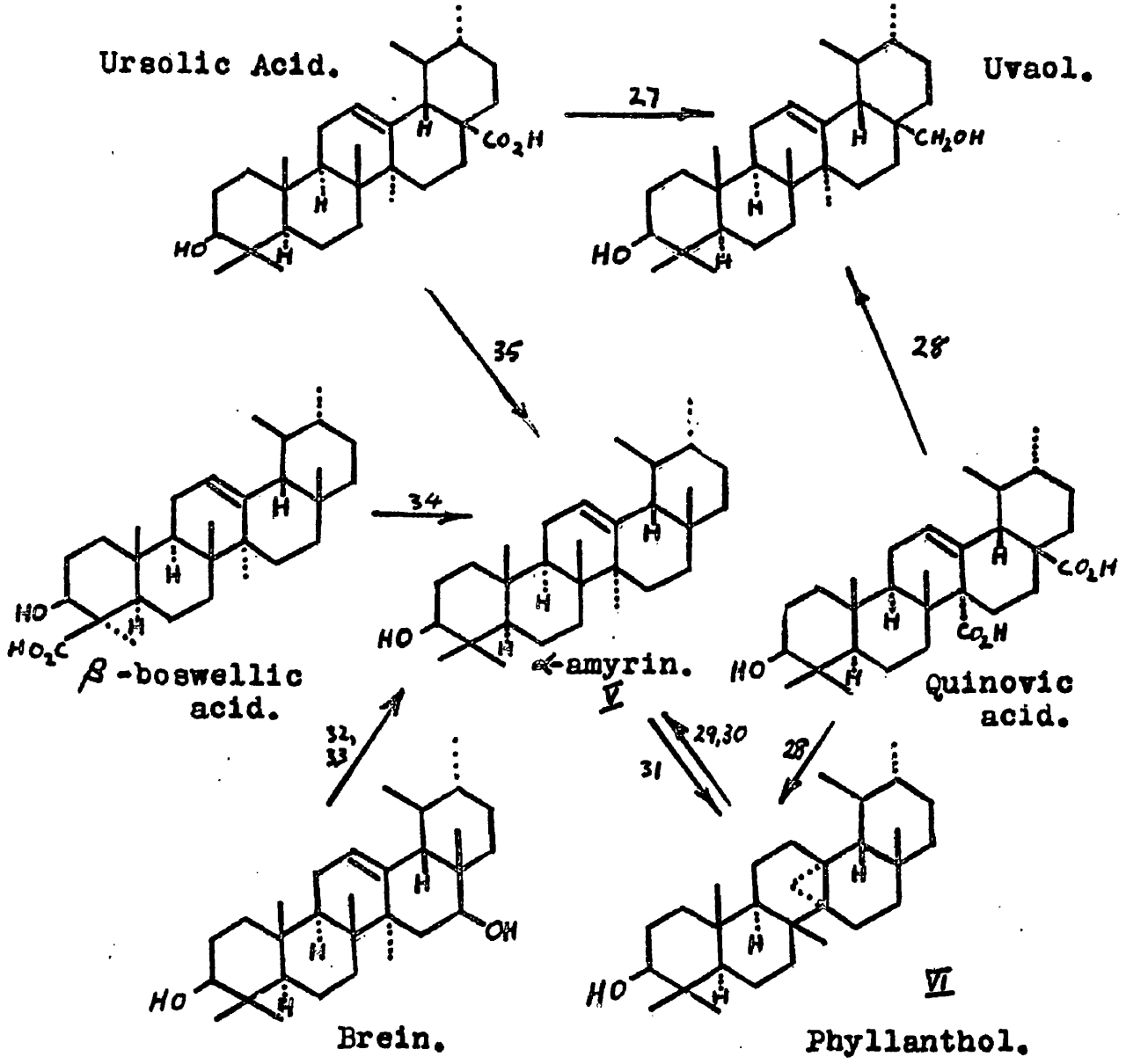


XVIII XIX XX

Numerous interconversions have been achieved between members of the same groups. As an example the conversions within the α-amyrin group are illustrated in table I. In addition inter-relationship between the

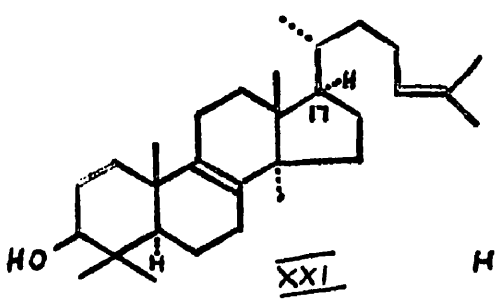
four groups based on ∝-amyrin, β-amyrin, lupeol and taraxasterol, has been achieved and has tended, especially in view of the conception of a common biogenetic precursor to all triterpenes, to make the classification into these groups appear artificial.

TABLE I.

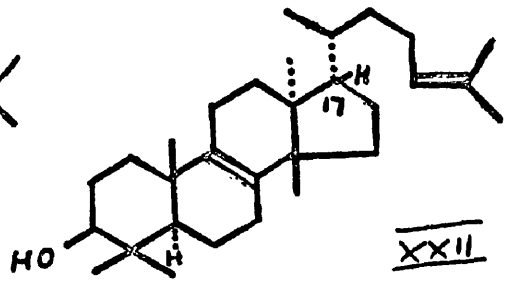


The Tetracyclic Triterpenes.

Unlike the pentacyclic triterpenes which are isolated from vegetable sources only, the tetracyclic triterpenes have been isolated not only from vegetable material but also from fungii and, in one case, from sheep-wool fat. Nevertheless they may be classified into two structural groups which are typified by lanosterol (XXI) and euphol (XXII), and to which allocation is made by standard reactions[36].



XXI



XXII

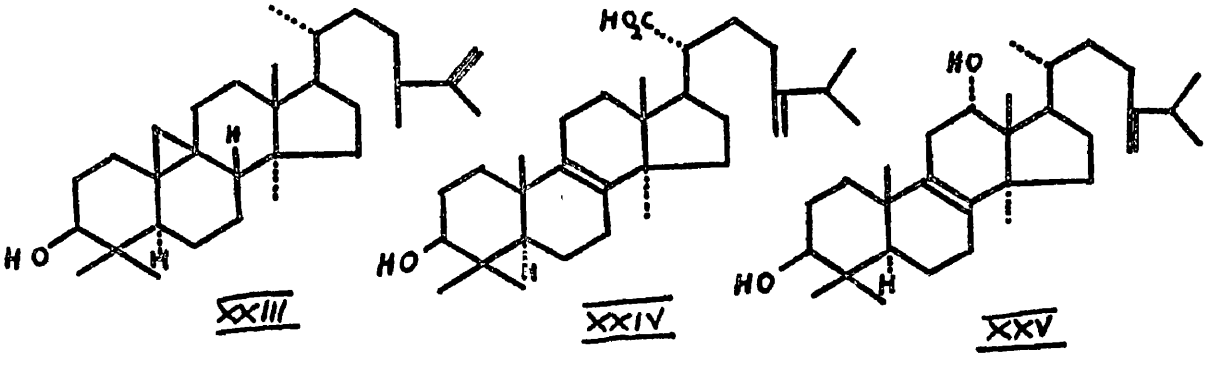
1). The euphol group are isolated from vegetable sources and include such compounds as tirucallol, elemadienolic acid, masticadienonic acid, butyrospermol, and the tetracyclic "dammar" triterpenes.

2). The lanosterol group includes agnosterol, parkeol, dihydrolanosterol, dihydroagnosterol, pinacolic acid A, and the pentacyclic compounds, cycloartenol and cyclooresterol, which contain

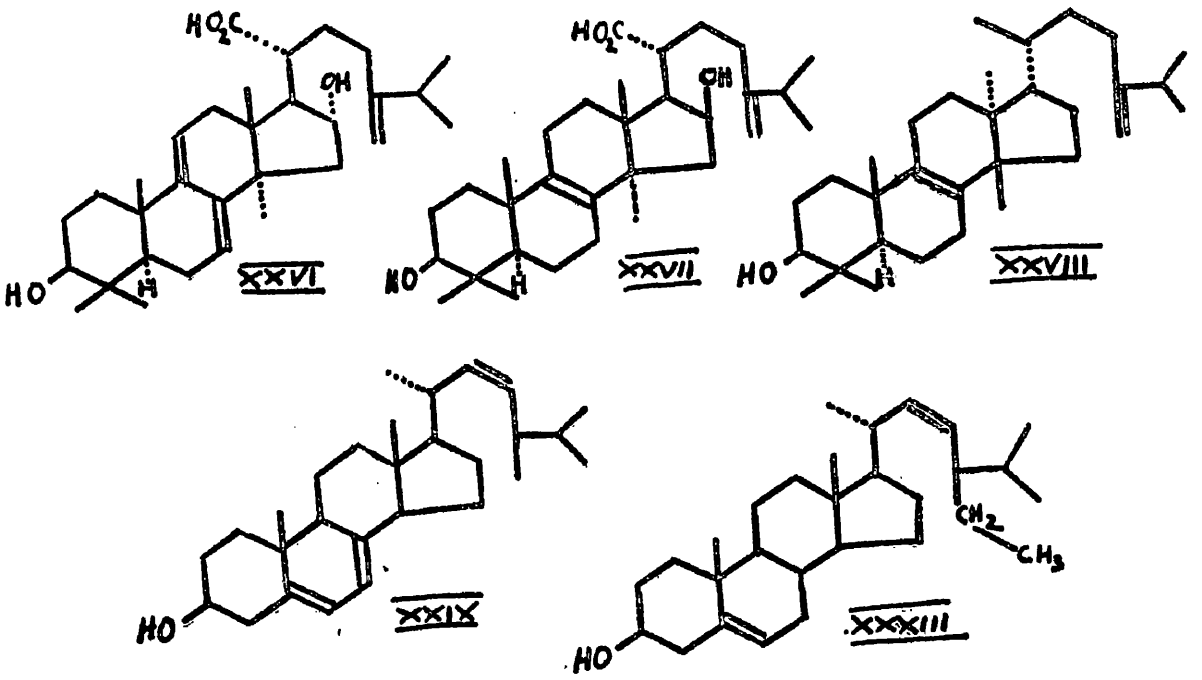
cyclopropane rings.

C(31)-Triterpenes.

A number of compounds have been isolated, from similar sources as the tetracyclic triterpenes, which contain thirty-one carbon atoms per molecule and yet show structural similarity to the triterpenes. These compounds, to which the term "triterpenoid" may be applied, include such compounds as cyclolaudenol (XXIII), eburicoic acid (XXIV), polyporenic acid A (XXV), polyporenic acid C (XXVI) and tumolosic acid (XXVII), which are related to lanosterol (XXI), and euphorbol (XXVIII) which is related to euphol (XXII). These C(31)-triterpenoids differ from the normal C(30)-triterpenes in the constitution of the side-chain attached to $C_{(17)}$, which in the C(31)-compounds is similar to that found in ergosterol (XXIX).

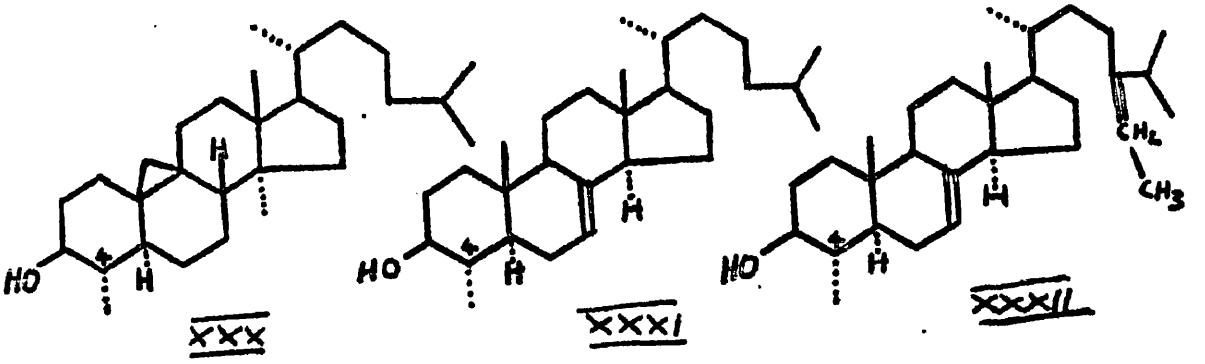


XXIII XXIV XXV



4α-Methyl-steroids

Recently a number of naturally-occurring compounds have come to light which are intermediate in structure to the steroids, in which there is no substitution at $C_{(4)}$, and the triterpenes, which have a gem-dimethyl group at this centre. These compounds which contain a single methyl substituent at $C_{(4)}$, are cycloeucalenol (XXX), from the heartwood of eucalyptus microcorys[37], and lophenol (XXXI) from the giant cactus[38]. Citrostadienol[39] (XXXII), from grapefruit, is a methyl derivative of stigmasterol (XXXIII), and although a C(30)-compound is not structurally a triterpene.



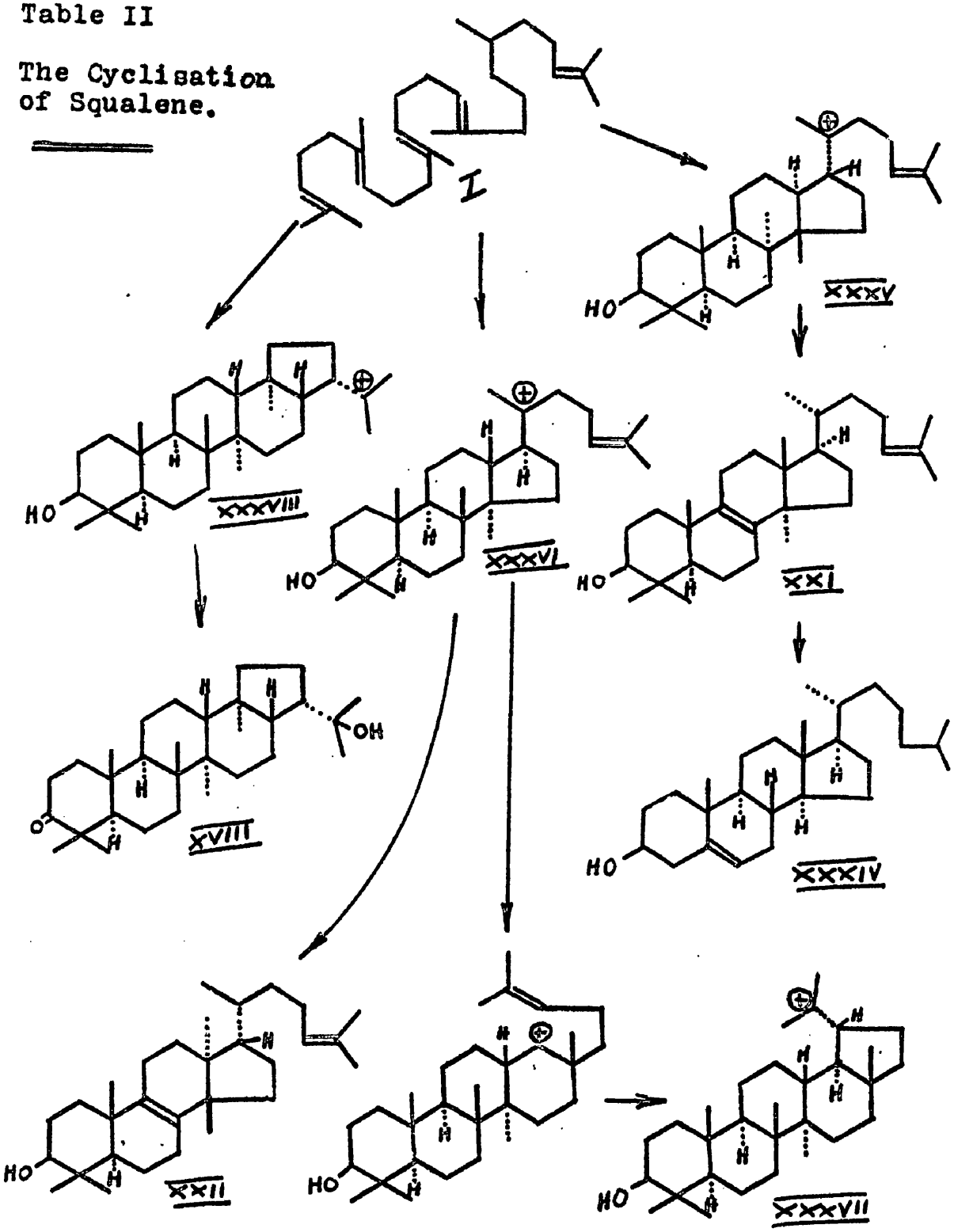
The Biogenesis of Triterpenes

It has been established that in certain animal homogenates squalene (I) cyclises to lanosterol (XXI) which is then oxidatively demethylated to cholesterol (XXXIV)[40-46]. It has been suggested that squalene (I) may be an intermediate common to the biosynthesis of all triterpenes[47-53].

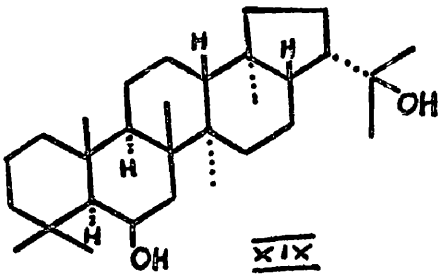
Squalene (1) was first isolated from shark-liver oil by Tsujimoto[54] and has since been shown to be widely distributed in both animal and vegetable kingdoms[55-62]. Squalene exists naturally in the all-*trans* configuration[63] necessary to give the known stereochemistry of the steroids and triterpenes on cyclisation[64]. It has been suggested that this cyclisation leads to two cations, one of which (XXXV) gives lanosterol (XXI) by a series of tertiary group

Table II

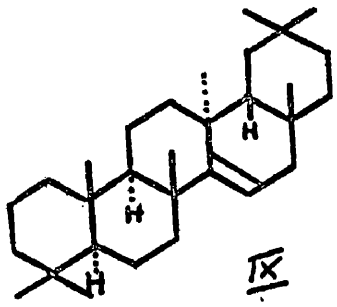
The Cyclisation of Squalene.



migrations and loss of a proton at $C_{(9)}$, while the second (XXXVI) leads to the euphol (XXII) group by a similar mechanism, or undergoes Wagner-Meerwein type ring enlargement to give the lupanyl cation (XXXVII), the precursor of the pentacyclic triterpenes of the α-amyrin, β-amyrin, lupeol and taraxasterol groups. A further mode of cyclisation has been postulated to account for the formation of hydroxyhopanone (XVIII) and this involves the cation (XXXVIII) which can be stabilised by the addition of an hydroxy-(OH^-)-anion. This mode of cyclisation could equally well be applied to the biogenesis of zeorin (XIX). [49,52,53,18,cf.22.]



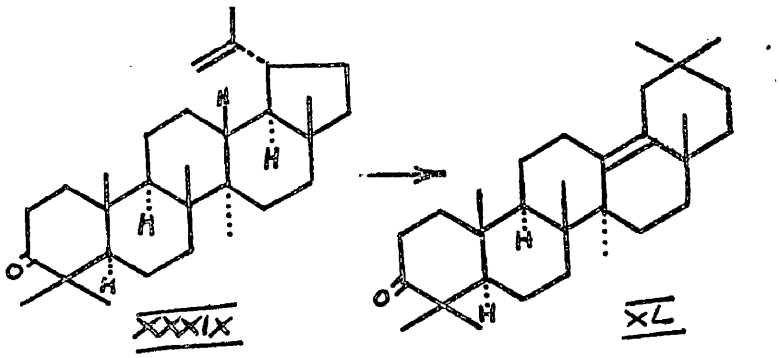
XIX

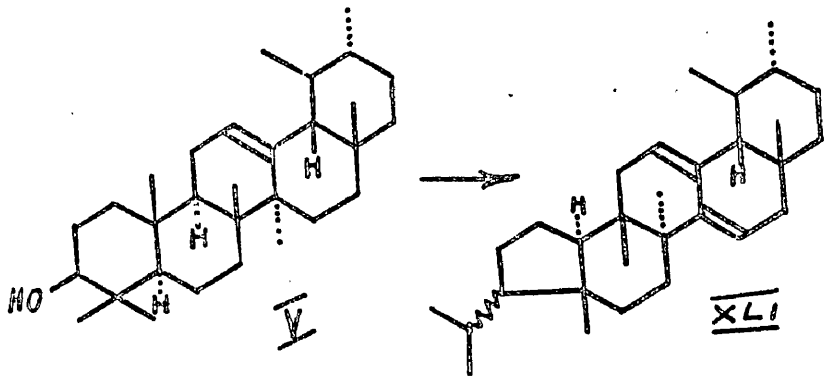


IX

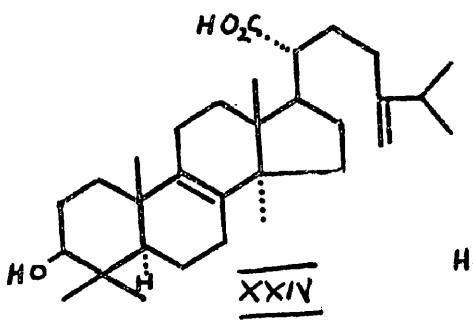
The cyclisation of squalene to lanosterol (XXI) is an aerobic process[65] which is probably induced by the attack of the cation OH^+, derived from molecular oxygen, on the double-bond at one end of the squalene molecule (I), and it proceeds to completion without the formation of stable intermediates[65]. A similar mechanism is probable for the biogenesis of all triterpenes in

which the initiating OH^+ cation appears as the ubiquitous 3-oxygen substituent. The two known, naturally-occurring, pentacyclic triterpenes in which $C_{(3)}$ is not oxygenated, zeorin (XIX) and taraxerene (IX), may be products of enzymic reduction after cyclisation has been completed. Migration of the cationic centre in the lupanyl cation (XXXVII) could lead to the numerous modifications in structure shown by the pentacyclic group of triterpenes. Such ring enlargements and tertiary group migrations have been amply demonstrated in, say, the isomerisation of lupenone (XXXIX) to δ-amyrenone (XL)[69], or the dehydration of α-amyrin (V) to 1-α-amyradiene (XLI)[70,71].

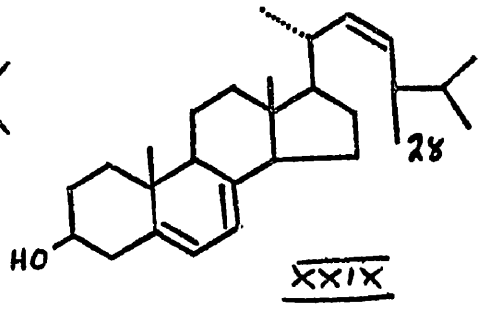




The C(31)-triterpenoids may also be mainly derived from squalene (I). It has been shown in experiments[72,73] using ^{14}C-labelled acetic acid that the molecule of eburicoic acid (XXIV) is derived mainly from acetic acid and conforms in the distribution of isotopic ^{14}carbon to the requirements of the squalene hypothesis. The additional carbon atom of the side chain is derived from formic acid[74]. This is analogous to the biosynthesis of ergosterol (XXIX) in which $C_{(28)}$ is derived from formic acid[75] and is introduced by direct transfer of the methyl group in methionine into the ergosterol molecule[76,77]. A biosynthetic mechanism involving the cyclisation of squalene (I) and subsequent methylation of the side chain could be suggested for all the C(31)-triterpenoids.



XXIV



XXIX

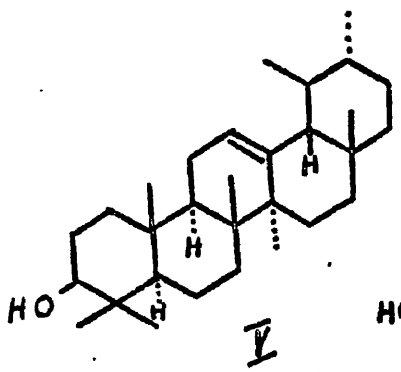
It has recently been shown that the concentrations of radio-carbon are approximately equal in the sitosterol and the soyasapogenols produced by a soya bean plant which has been fed on ^{14}C-labelled substrates,

i.e. ^{14}C-methyl- and ^{14}C-carboxyl-acetic acid, and 2-^{14}C-mevalonalactone.[78] This might be taken to indicate a common mode of biogenesis for triterpenes and sterols. Moreover, the distribution of radio-carbon in a sample of soyasapogenol A, a derivative of β-amyrin (VIII), produced in such a test, is in agreement with the distribution forecast by the "squalene hypothesis". This lends support to the extension of the "squalene hypothesis" to the biogenesis of pentacyclic triterpenes although further experimental evidence is required before the hypothesis can be considered to be established.[78]

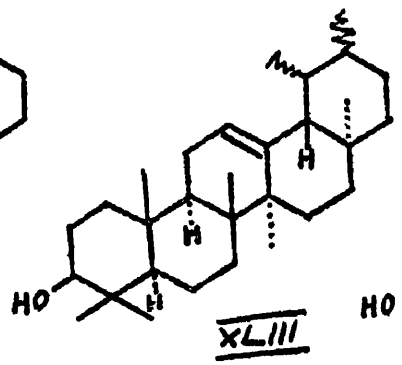
The "squalene hypothesis" for the biogenesis of triterpenes provides not only an explanation of the experimental results but also provides a means by which the structure of naturally-occurring triterpenes may be forecast and may thus facilitate the investigation of new compounds. We do not intend to dicuss the biogenesis of triterpenes beyond the limited field of squalene cyclisation. Regarding the biosynthesis of squalene (1), we refer the reader to the investigations carried out on acetic acid[79,80], β-hydroxy-β-methyl glutaric acid[81,82], β:β'-dimethyl acrylic acid[83,84], mevalonic acid[85-94], and farnesic acid[95]. The oxidative demethylation of lanosterol (XXI) has been described by Stokes, Fish and Hickey[96] and Gautschi and Bloch[97].

The Structure of the Amyrins

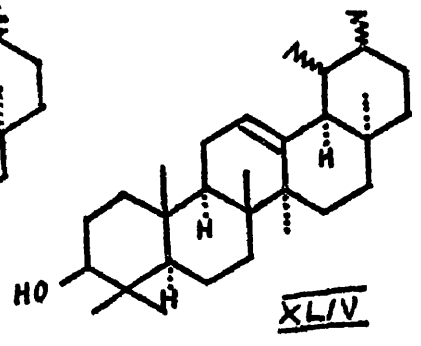
In 1955, at the beginning of the period covered by this research, the structure[98] of β-amyrin (VIII) had been established and the stereochemistry elucidated[99]. The structure of α-amyrin was at the same time in doubt, and four structures, (V)[100], (XLII)[101,102], (XLIII)[103] and (XLIV)[104], had been proposed. Evidence has since been presented[105-108] in support of structure (V), which has recently been established unequivocally by a partial synthesis of α-amyrin (V) from glycyrrhetic acid[109] (XLVI).



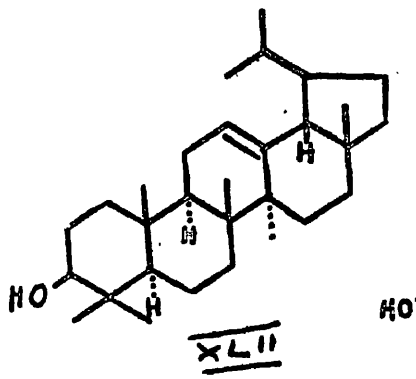
V



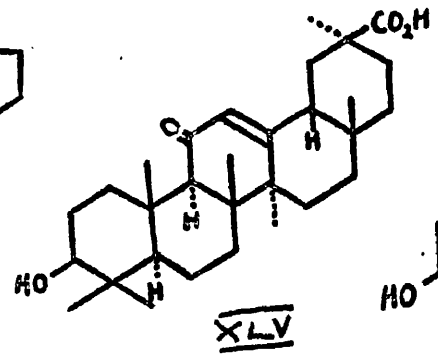
XLIII



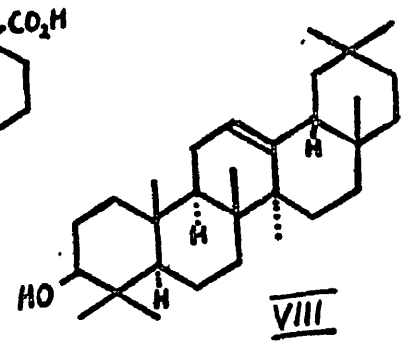
XLIV



XLII



XLV



VIII

SECTION I : Some Hexacyclic Derivatives of α-Amyrin.

Section I.

Some Hexacyclic Derivatives of ∝-Amyrin

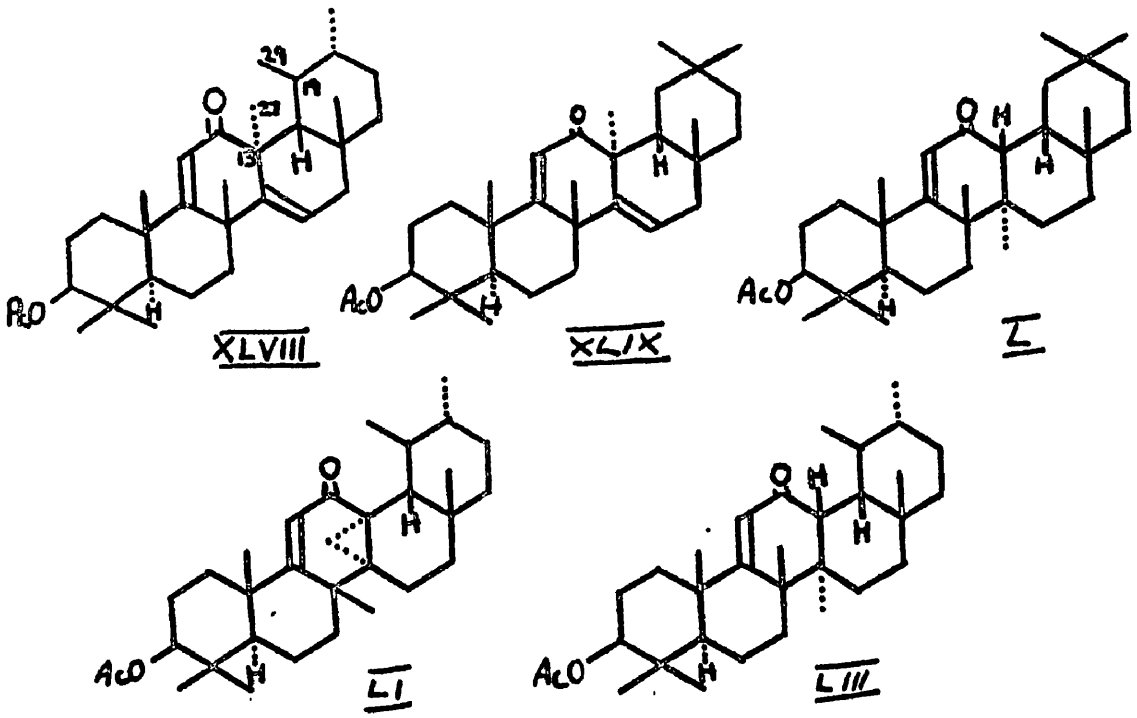
Preface

As would be expected of compounds based on such closely similar carbon-skeletons, the corresponding derivatives of ∝-amyrin (V) and β-amyrin (VIII) are frequently similar in physical and chemical properties. We felt justified by this similarity in using the existing knowledge of the chemistry of β-amyrin in this field in planning the course of our investigations. This has latterly involved us in carrying out a few experiments on β-amyrin derivatives and these are discussed wherever relevant and have not been segregated into a separate section.

12-Oxoisoursa-9(11):14-dien-3β-yl Acetate and 12-Oxoisooleana-9(11):14-dien-3β-yl Acetate.

Many of the compounds described below are oxidation products of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) and show general resemblence to the

corresponding derivatives[111] of 12-oxo*iso*oleana-9(11):14-dien-3β-yl acetate (XLIX). The chemical properties of these two oxo-dienyl acetates, (XLVIII) and (XLIX), do differ, however, in one important respect. 12-Oxo-*iso*oleana-9(11):14-dien-3β-yl acetate (XLIX), which is obtained by selenium dioxide oxidation of 12-oxoolean-9(11)-en-3β-yl acetate (L)[112], is stable to mineral acid[113]. On the other hand, 12-oxo*iso*oursa-9(11):14-dien-3β-yl acetate (XLVIII)[114] is easily isomerised to 12-oxo-13:27-*cyclo*ours-9(11)-en-3β-yl acetate (LI) on treatment with mineral acid[114,31].



The difference in stability of these two oxo-dienyl acetates is difficult to explain. It

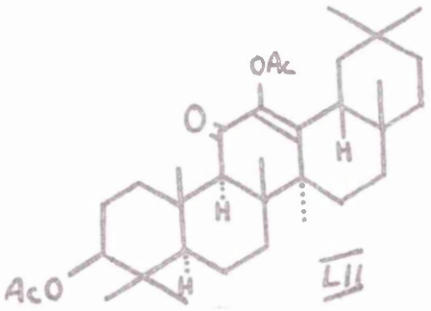
may be that in 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) interaction between the methyl groups, $C_{(27)}$ and $C_{(29)}$, attached to $C_{(13)}$ and $C_{(19)}$, constrains the methyl group, $C_{(27)}$, into a position favorable to cyclisation. In 12-oxoisooleana-9(11):14-dien-3β-yl acetate (XLIX), in which $C_{(19)}$ is unsubstituted, no such interaction takes place and the $C_{(13)}$-methyl group may be accommodated without constraint.

That some such interaction is present in 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) is implied by the abnormally low wavelength ($\lambda_{max.}$ 2350 Å) of the absorption due to the α:β-unsaturated ketone chromophore in this compound, compared with the absorption ($\lambda_{max.}$ 2450 Å) of the same chromophore in 12-oxoiso-oleana-9(11):14-dien-3β-yl acetate (XLIX). This may be due to ring-strain in ring C of the α-amyrin derivative, which could be due to distortion of the bond angles at $C_{(13)}$.

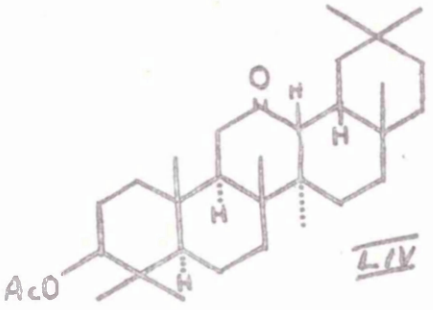
12-Oxoisoursa-9(11)14-dien-3β-yl acetate (XLVIII) is produced by oxidation of 12-oxours-9(11)-en-3β-yl acetate (LIII) with selenium dioxide in acetic acid and it is generally accompanied by varying amounts of 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (LI), from which it is freed only with difficulty. In our experience reduction of the reaction time to one hour reduces the yield of impurity (LI) and this can be

then removed by crystallisation.

We have confirmed the stability of 12-oxoisooleana-9(11):14-dien-3β-yl acetate (XLIX) to mineral acid. Examination of the water-insoluble product from the selenium dioxide oxidation of 12-oxoolean-9(11)-en-3β-yl acetate (L) confirmed that it consists entirely of 12-oxoisooleana-9(11):14-dien-3β-yl acetate (XLIX), the only impurity isolated being a small amount (less than 0.2%) of 12-acetoxy-11-oxoolean-12-en-3β-yl acetate (LII), which was probably derived from traces of 12-oxooleanan-3β-yl acetate (LIV) in the starting material.

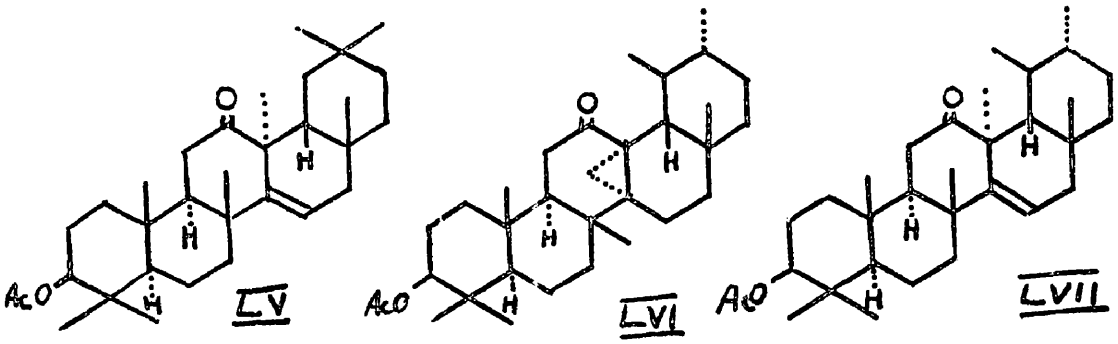


LII



LIV

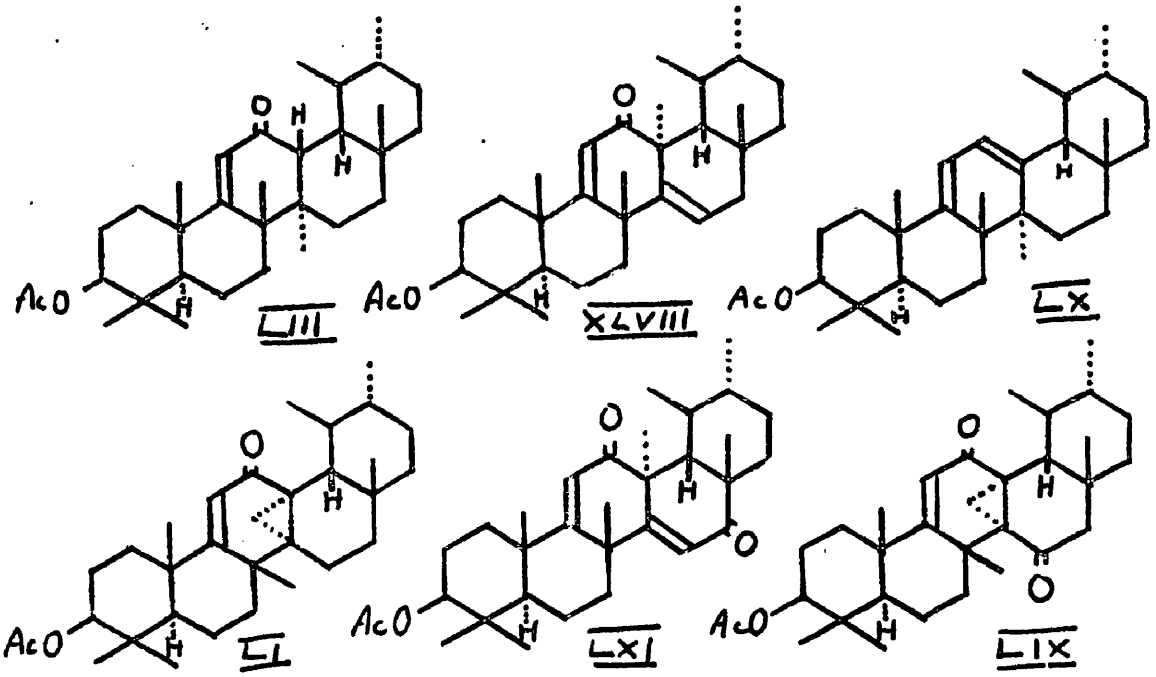
12-oxoisoolean-14-en-3β-yl acetate (LV)[115] was found to be stable to mineral acid under conditions which isomerise 12-oxoisours-14-en-3β-yl acetate (LVII) to 12-oxo-13:27-cycloursan-3β-yl acetate (LVI)



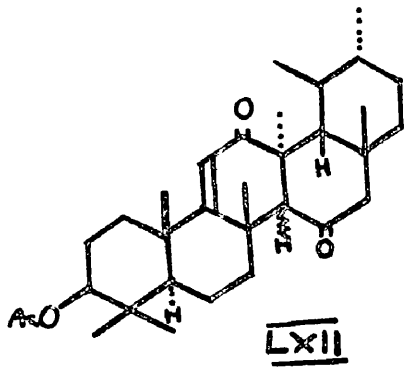
The Ketoacetate, $C_{32}H_{46}O_4$; derived from α-amyrin.

12-Oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) is oxidised by chromic acid to a ketoacetate, $C_{32}H_{46}O_4$, which is also produced by chromic acid oxidation of either 12-oxours-9(11)-en-3β-yl acetate (LIII), ursa-9(11):12-dien-3β-yl acetate (LX),[116] or 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (LI); it is also formed, with the isomeric 12:16-dioxoisoursa-9(11):14-dien-3β-yl acetate (LXI), by oxidation of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) with selenium dioxide[117,116].

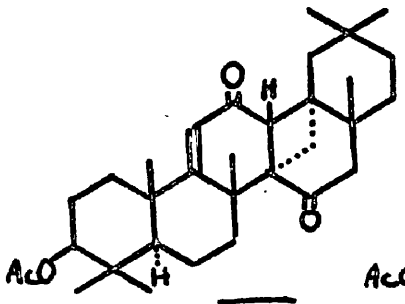
The formation of the ketoacetate $C_{32}H_{46}O_4$ by oxidation of 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (LI) might be taken to indicate that the ketoacetate, $C_{32}H_{46}O_4$, is hexacyclic, i.e. containing a



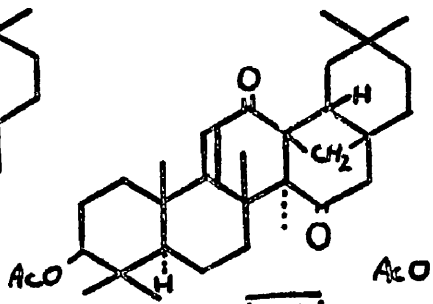
cyclopropane ring (LIX). However, while 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (LI) is itself stable to mineral acid, in the combined acidic and oxidative conditions of the chromic acid oxidation, fission of the cyclopropane ring could take place, leading to the formation of a pentacyclic compound (LXII), in which case the molecular formula of the product would be $C_{32}H_{48}O_4$.



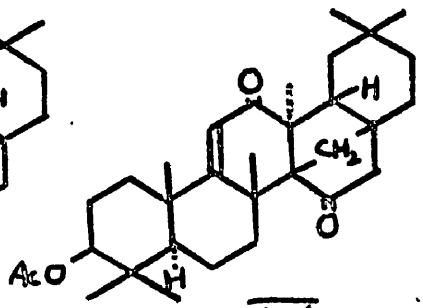
Oxidation of 12-oxo*iso*oleana-9(11):14-dien-3β-yl acetate (XLIX) with chromic acid gives a ketoacetate, $C_{32}H_{46}O_4$, which is strikingly similar in physical properties to the α-amyrin analogue described above. As experimental evidence appeared to exclude the presence of a *cyclo*propane ring in this β-amyrin derivative[111], this compound had been formulated[111] as (LXIII), (LXIV) or (LXV), which differ from the structure (LIX) ascribed to the ketoacetate, $C_{32}H_{46}O_4$, derived from α-amyrin, in the size and location of the ring superimposed upon the pentacyclic carbon skeletons. By analogy, three further structures (LXVI), (LXVII) and (LXVIII) might be considered for the ketoacetate, $C_{32}H_{46}O_4$, derived from α-amyrin, although it is difficult to discern a mechanism which could lead to these compounds in the oxidation of 12-oxo-13:27-*cyclo*urs-9(11)-en-3β-yl acetate (LI).



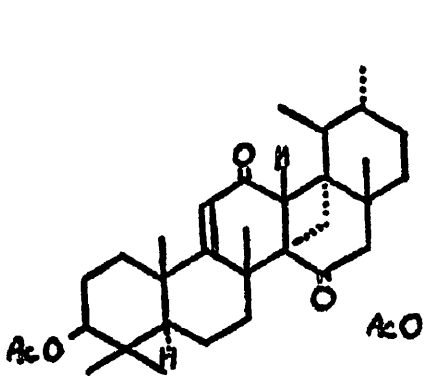
LXIII



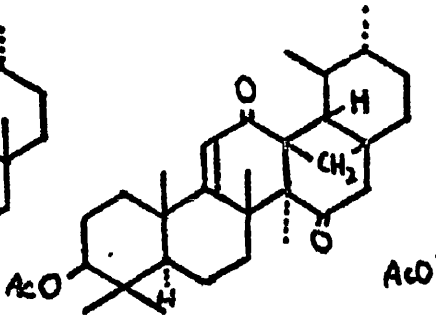
LXIV



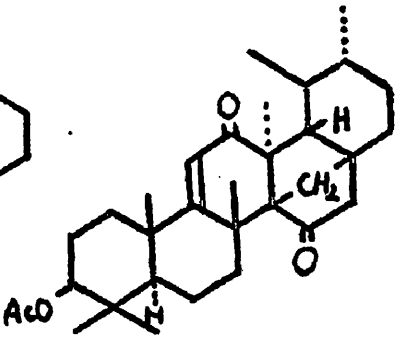
LXV



LXVI

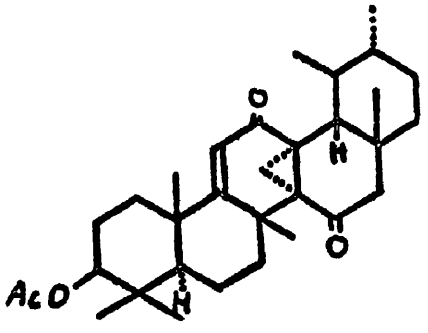


LXVII

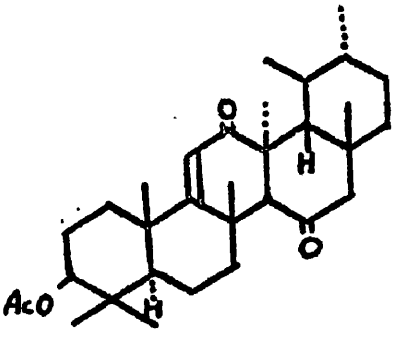


LXVIII

Thus five structures, (LIX), (LXII), (LXVI), (LXVII) and (LXVIII), could be proposed for the ketoacetate, $C_{32}H_{46}O_4$, derived from 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII), and the experiments described below were undertaken to differentiate between these five alternatives.



LIX



LXII

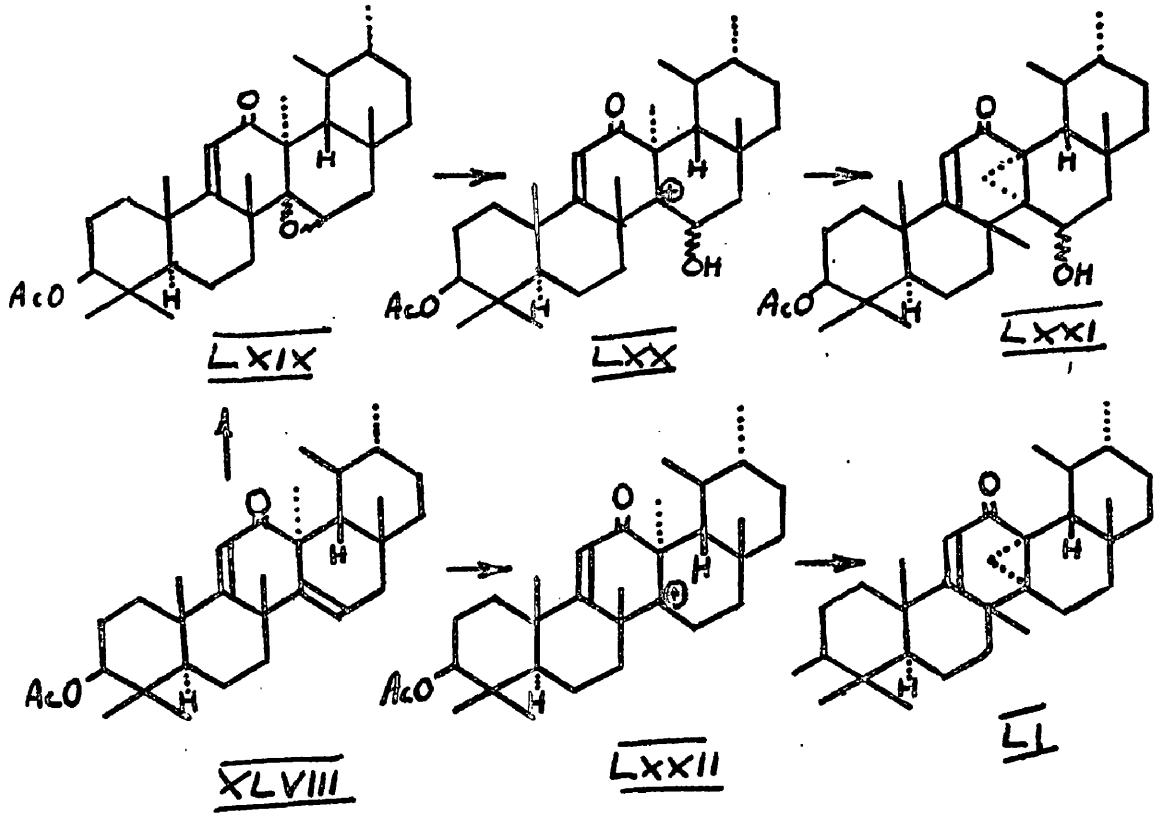
Synthesis of the Ketoacetate, $C_{32}H_{46}O_4$, from 12-Oxoisoursa-9(11):14-dien-3 -yl Acetate, via a Keto-diol Monoacetate

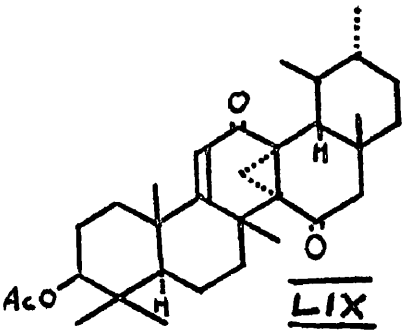
Oxidation of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) with hydrogen peroxide in acetic acid gave a mixture of 14§:15§-epoxy-12-oxoisours-9(11)-en-3β-yl acetate[118] (LXIX) and a keto-diol monoacetate, $C_{32}H_{48}O_4$. The keto-diol monoacetate, which was also obtained by treatment of the epoxide (LXIX) with mineral acid, gave a diacetate on acetylation and on treatment with chromium trioxide in pyridine it gave the ketoacetate, $C_{32}H_{46}O_4$. This behavior is analogous to the corresponding reactions in the β-amyrin series[111].

This synthesis of the ketoacetate, $C_{32}H_{46}O_4$, makes the pentacyclic structure (LXII) untenable. The oxidation of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) leads to the epoxide (LXIX) which under the acid conditions is converted to the cation (LXX) by protonation of the epoxide linkage. This cation (LXX) can be stabilised by the formation of either a double-bond or a new cycloalkane ring. As there is no evidence for the formation of a double-bond in the reaction, the keto-diol monoacetate is probably

hexacyclic.

The acid induced isomerisation of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) to 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (LI) must be initiated by protonation of the $\Delta^{14:15}$ double-bond to give the ion (LXXII). This ion (LXXII) is similar to the ion (LXX) which leads to the keto-diol monoacetate, and it would be expected that both would stabilise by the same mechanism. In such a case the cation (LXX) would lead to 12-oxo-13:27-cyclours-9(11)-ene-3β:15ξ-diol 3-monacetate (LXXI), which on oxidation would give a cyclopropanoid ketoacetate, $C_{32}H_{46}O_4$, (LIX).





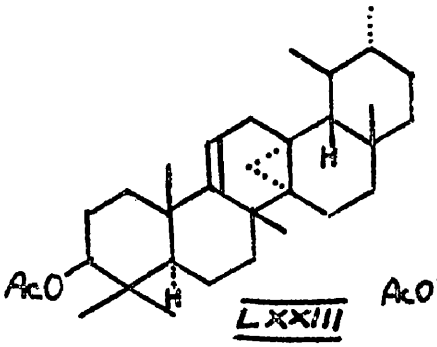
LIX

Reduction of the Ketoacetate, $C_{32}H_{46}O_4$, derived from ∝-Amyrin.

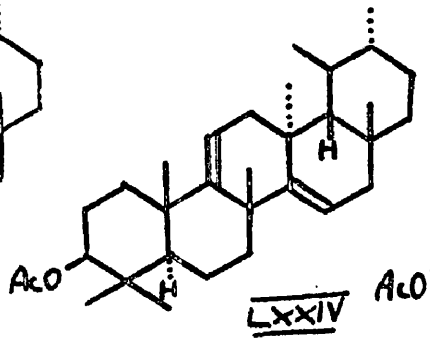
The synthesis of the ketoacetate, $C_{32}H_{46}O_4$, derived from ∝-amyrin, from 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) via the keto-diol monoacetate, is entirely analogous to a synthesis of the ketoacetate, $C_{32}H_{46}O_4$, derived from β-amyrin, described by Johnston and Spring[111]. As the experimental evidence regarding the reduction products of the latter ketoacetate apparently excluded a cyclopropanoid formulation for this compound, it was decided to investigate the reduction of the ketoacetate, $C_{32}H_{46}O_4$, derived from ∝-amyrin.

Catalytic hydrogenolysis of the ketoacetate, $C_{32}H_{46}O_4$, derived from ∝-amyrin, gave an acetate, $C_{32}H_{50-52}O_2$ which is transparent to light in the ultra-violet region above 2150 Å. This acetate, $C_{32}H_{50-52}O_2$, is not identical with any of the known mono-ethylenic or unconjugated di-ethylenic derivatives of ∝-amyrin.

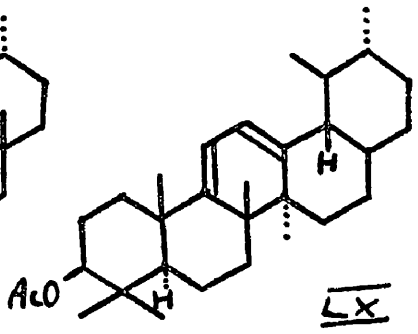
Nor is it identical with 13:27-cyclours-9(11)-en-3β-yl acetate, $C_{32}H_{50}O_2$, (LXXIII) which had been previously prepared by hydrogenolysis of 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (LI),[119] and which we reprepared by Wolff-Kishner reduction of the same compound (LI). Under acidic conditions in which both 13:27-cyclours-9(11)-en-3β-yl acetate (LXXIII)[119] and isoursa-9(11):14-dien-3β-yl acetate (LXXIV)[118] are isomerised to ursa-9(11):12-dien-3β-yl acetate (LX), the acetate, $C_{32}H_{50-52}O_2$, is stable. Alkaline hydrolysis of the acetate, $C_{32}H_{50-52}O_2$, gave a gummy alcohol from which the acetate was regenerated on acetylation.



LXXIII



LXXIV

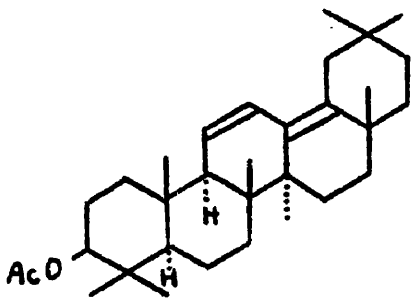


LX

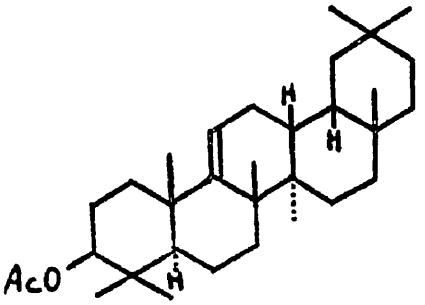
Wolff-Kishner reduction of the ketoacetate, $C_{32}H_{46}O_4$, derived from α-amyrin, gave two products, an acetate $C_{32}H_{48-50}O_3$, and the acetate, $C_{32}H_{50-52}O_2$, identical with the product of catalytic hydrogenolysis. The acetate, $C_{32}H_{48-50}O_3$, which gives an orange colour with

tetranitromethane in chloroform, shows only end-adsorption at 2070 Å (ε 5,000), and it is stable to mineral acid. Alkaline hydrolysis of the acetate, $C_{32}H_{48-50}O_3$, gave an alcohol, $C_{30}H_{46-48}O_2$, from which the acetate, $C_{32}H_{48-50}O_3$, is regenerated on acetylation. The infra-red spectrum of the acetate, $C_{32}H_{48-50}O_3$, includes a band at 1692 cm^{-1}, allocated to the unreduced carbonyl function.

In considering structures for these reduction products it is pertinent to examine the corresponding derivatives of β-amyrin described by Johnston and Spring[111]. The ketoacetate, $C_{32}H_{46}O_4$, derived from β-amyrin, gives two products on catalytic hydrogenolysis. The first product is a hexacyclic, mono-ethylenic acetate, $C_{32}H_{50}O_2$, which gives oleana-11:13(18)-dien-3β-yl acetate (LXXV) on treatment with mineral acid. The second product, an acetate, $C_{32}H_{52}O_2$, is stable to mineral acid[120]. Wolff-Kishner reduction of the ketoacetate, $C_{32}H_{46}O_4$, derived from β-amyrin, gives an acetate, $C_{32}H_{48}O_3$, showing no absorption in the ultra-violet region above 2100 Å, but giving an orange colour with tetranitromethane in chloroform. The acetate, $C_{32}H_{48}O_3$, is stable to mineral acid[111].

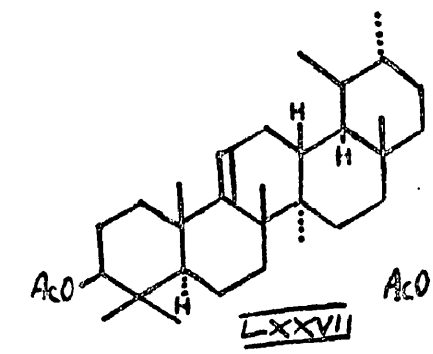


LXXV

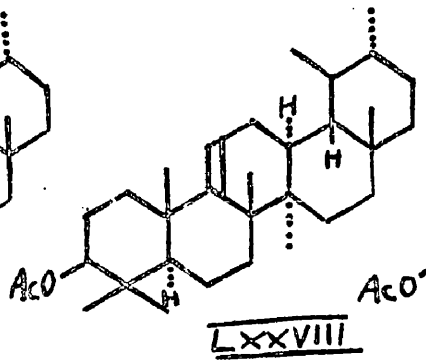


LXXVI

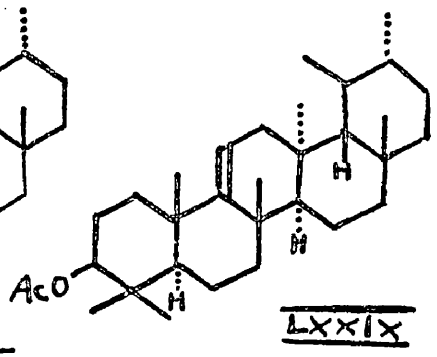
The acetate, $C_{32}H_{50-52}O_2$, obtained by catalytic hydrogenolysis and by Wolff-Kishner reduction of the ketoacetate, $C_{32}H_{46}O_4$, derived from α-amyrin, in our opinion, corresponds to the acid-stable acetate, $C_{32}H_{52}O_2$, obtained by catalytic hydrogenolysis of the ketoacetate, $C_{32}H_{46}O_4$, derived from β-amyrin[111]. Both these compounds must be produced by reductive fission of the cycloalkane ring in the parent ketoacetates, $C_{32}H_{46}O_4$. The acetate, $C_{32}H_{52}O_2$, derived from β-amyrin, is not identical with olean-9(11)-en-3β-yl acetate (LXXVI)[121]. By analogy, the acetate, $C_{32}H_{50-52}O_2$, derived from α-amyrin, which should now be formulated as $C_{32}H_{52}O_2$, is probably not urs-9(11)-en-3β-yl acetate (LXVII), and is to be formulated as either 13α-urs-9(11)-en-3β-yl acetate (LXXVIII), or isours-9(11)-en-3β-yl acetate (LXXIX).



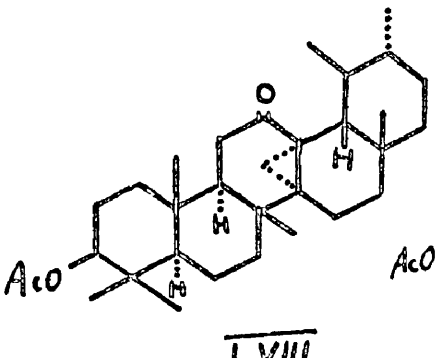
LXXVII



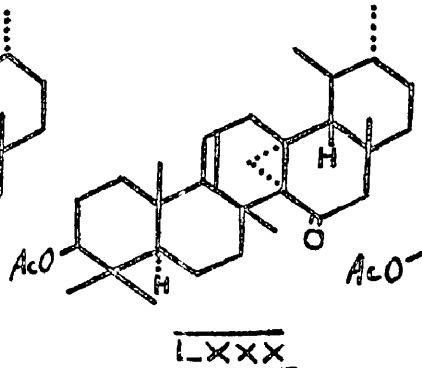
LXXVIII



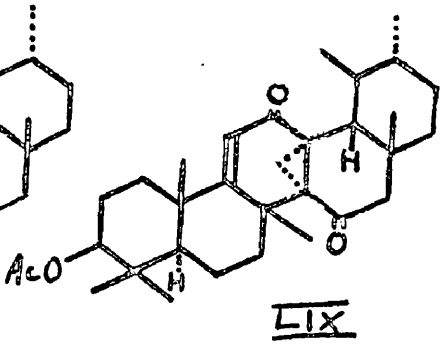
LXXIX



LVIII



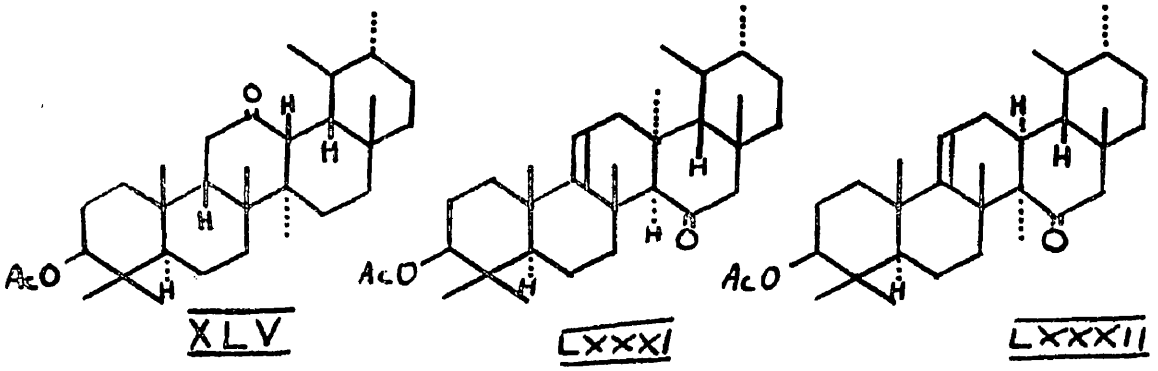
LXXX



LIX

The acetate, $C_{32}H_{48-50}O_3$, obtained by Wolff-Kishner reduction of the ketoacetate, $C_{32}H_{46}O_4$, derived from α-amyrin, must be formed by the reduction of the 12-ketone group since the ultra-violet absorption spectrum of the product no longer includes absorption due to the $\alpha:\beta$-unsaturated ketone chromophore. If we assume that no rearrangement of the hexacyclic carbon-skeleton of the ketoacetate, $C_{32}H_{46}O_4$, takes place during its reduction to the acetate, $C_{32}H_{48-50}O_3$, then the latter compound must be formulated as (LXXX), if the cyclopropanoid structure (LIX) for the ketoacetate, $C_{32}H_{46}O_4$, is correct.

The infra-red spectrum of the acetate, $C_{32}H_{48-50}O_3$, includes a band at 1692 $cm.^{-1}$, which is ascribed to the 15-ketone group. This band is similar in location to the ketonic band, at 1694 $cm.^{-1}$, in the spectrum of 12-oxoursan-3β-yl acetate(XLV)[122], and differs appreciably from the band, at 1681 $cm.^{-1}$, in the spectrum of 12-oxo-13:27-cycloursan-3β-yl acetate (LVIII), in which the ketone group is conjugated with a cyclopropane ring. On this basis, a cyclopropanoid structure (LXXX) seems unlikely for the acetate, $C_{32}H_{48-50}O_3$, and a pentacyclic structure, (LXXXI) or (LXXXII), seems to warrant consideration.

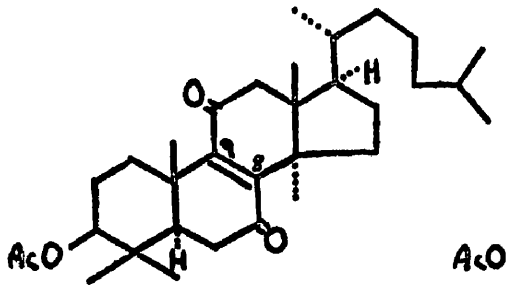


XLV LXXXI LXXXII

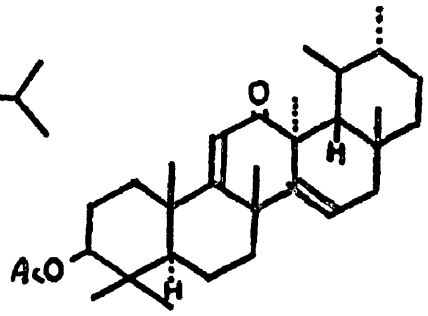
The ultra-violet spectrum of the acetate, $C_{32}H_{48-50}O_3$, supports the view that this compound does not contain a cyclopropane ring. Were the cyclopropanoid structure (LXXX) for this compound correct, the ultra-violet spectrum should show high-

intensity absorption near 2140 Å, by analogy with the absorption shown by 12-oxo-13:27-cycloursan-3β-yl acetate (LVIII), $\lambda_{max.}$ 2140 Å (ε 5250). The apparent maximum at 2070 Å (ε 5250) and the low intensity of absorption at 2140 Å (ε 2900) in the spectrum of the acetate, $C_{32}H_{48-50}O_3$, are more in keeping with a pentacyclic structure (LXXXI) or (LXXXII).

The pentacyclic formulation for the acetate (LXXXI) or (LXXXII), which should now be formulated as $C_{32}H_{50}O_3$, is in agreement with the pentacyclic structure, (LXXVIII) or (LXXIX), which was postulated above for the conascent acetate $C_{32}H_{52}O_2$. The ability of the cyclopropane ring in the parent ketoacetate, $C_{32}H_{46}O_4$, derived from α-amyrin, to undergo reductive fission in these reactions, is probably due to the activation of the ring by the adjacent 12:15-carbonyl groups. A similar effect is shown by the activation of the $\Delta^{8:9}$ double-bond in 7:11-dioxolanost-8(9)-en-3β-yl acetate (LXXXIII), where it enables reduction of the otherwise unreactive bond to take place[123-125].



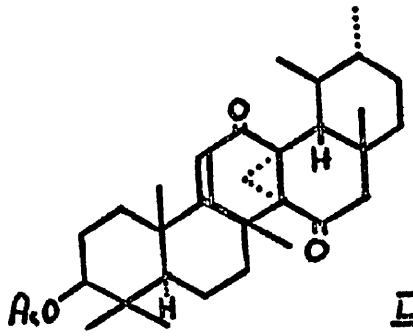
LXXXIII



XLVIII

The Structure of the Ketoacetate, $C_{32}H_{46}O_4$, derived from α-Amyrin : Conclusion.

In our opinion the ketoacetate, $C_{32}H_{46}O_4$, obtained by oxidation of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) with chromic acid, is correctly formulated as 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (LIX).

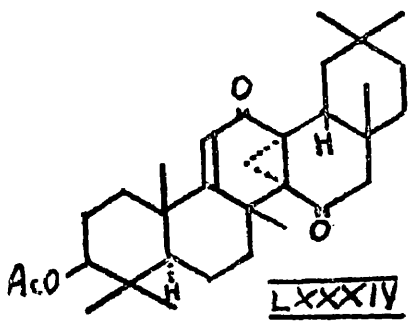


LIX

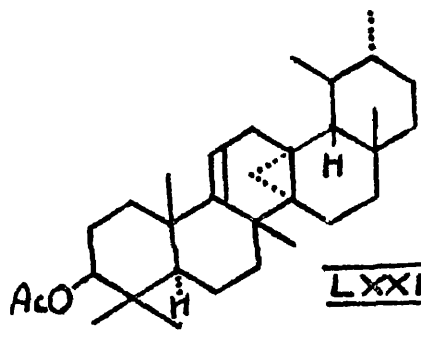
Remarks Regarding the Ketoacetate, $C_{32}H_{46}O_4$, derived from β-Amyrin.

As our conclusions regarding the structure of the ketoacetate, $C_{32}H_{46}O_4$, derived from α-amyrin,

are different from those of Johnston and Spring[111] regarding the structure of the corresponding ketoacetate, $C_{32}H_{46}O_4$, derived from β-amyrin, it is pertinent to examine the evidence on which the cyclopropanoid structure (LXXXIV) was excluded for the latter compound.



LXXXIV

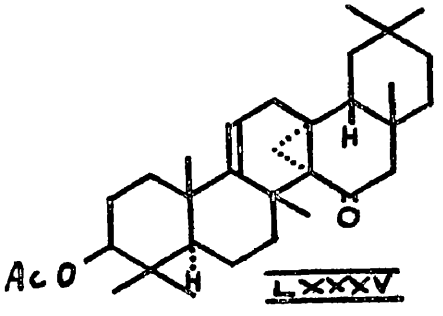


LXXIII

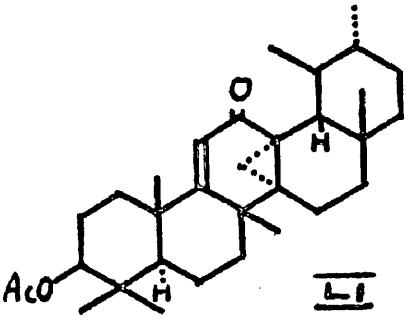
1. The infra-red spectrum of the hexacyclic acetate, $C_{32}H_{50}O_2$, obtained by catalytic hydrogenolysis of the ketoacetate, $C_{32}H_{46}O_4$, derived from β-amyrin[111] (see p. 29), does not show the absorption band at 1000 $cm.^{-1}$ expected in the spectra of compounds which contain a cyclopropane ring[126-129]. This band is intense, but it is difficult to detect in oxygenated compounds[130]. For comparison, we examined the spectrum of 13:27-cyclours-9(11)-en-3β-yl acetate (LXXIII) for this band. The band appeared in the spectrum of this compound, determined in nujol mull, and as a solution in carbon disulphide and carbon tetrachloride, as a slight shoulder on an adjacent band at 1024 $cm.^{-1}$ and

could have been overlooked in the spectrum of the acetate, $C_{32}H_{50}O_2$, described by Johnston and Spring.[111]

2. The optical properties of the hexacyclic derivatives of β-amyrin show no evidence for the presence of a cyclopropane ring. It is now known that the ultra-violet absorption of the $\alpha:\beta$-unsaturated ketone in ring C experiences only a slight hypsochromic shift on introduction of a cyclopropane ring in conjugation with the 12-ketone and that no separate band occurs which could be ascribed to the ketone in conjugation with the cyclopropane ring. Thus 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (LI) shows maximal absorption at 2360 Å (ε 11,000) only, which is similar to the absorption due to this chromophore in the spectra of all other hexacyclic derivatives of α-amyrin and β-amyrin[111].



LXXXV

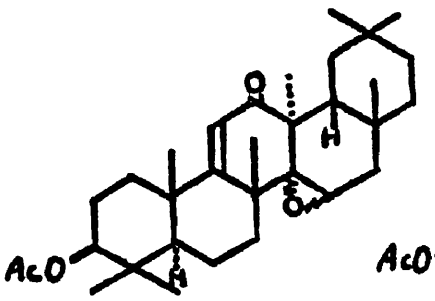


LI

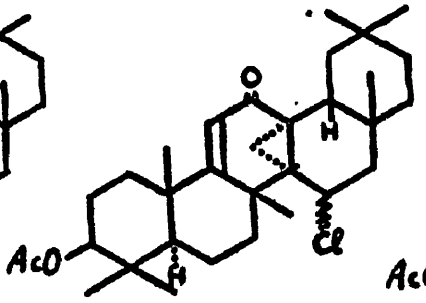
3. The acetate, $C_{32}H_{48}O_3$, obtained by Wolff-Kishner reduction of the ketoacetate, $C_{32}H_{46}O_4$, derived from β-amyrin (see p.29), was assumed to be hexacyclic, i.e. (LXXXV), if the parent ketoacetate were cyclopropanoid (LXXXIV). The acetate, $C_{32}H_{48}O_3$, did not, however, show the characteristic absorption in the ultra-violet region of a compound containing a ketone in conjugation with a cyclopropane ring. We are of the opinion that, by analogy to the corresponding derivative of α-amyrin, (LXXXI) or (LXXXII) (c.f. p.32), this compound should be formulated as $C_{32}H_{50}O_3$ and is, in fact, pentacyclic.

4. Treatment of 14:15-epoxy-12-oxoisoolean-9(11)-en-3β-yl acetate (LXXXVI) with hydrochloric acid was shown to give a chloro-acetate, $C_{32}H_{47}O_3Cl$, which contained the same hexacyclic carbon-skeleton as the ketoacetate, $C_{32}H_{46}O_4$, derived from β-amyrin. This chloro-acetate, $C_{32}H_{47}O_3Cl$, on treatment with collidine, gives an acetate, $C_{32}H_{46}O_3$, in which the presence of an isolated double-bond is indicated by ultra-violet absorption, and by a yellow colour with tetranitromethane in chloroform. If the cyclopropanoid formulation (LXXXIV) for the ketoacetate,

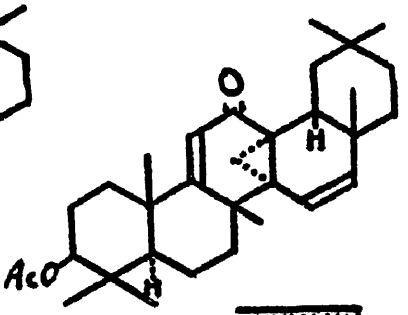
$C_{32}H_{46}O_4$, were correct, the chloro-acetate, $C_{32}H_{47}O_3Cl$, should be formulated as (LXXXVII), and the derived acetate, $C_{32}H_{46}O_3$, as (LXXXVIII). It might be expected that the $\Delta^{15:16}$ double-bond in the last compound (LXXXVIII) would be affected by the proximity of the cyclopropane ring and would not have the optical properties of an isolated double-bond, as is detected, experimentally, in the acetate, $C_{32}H_{46}O_3$.



LXXXVI



LXXXVII

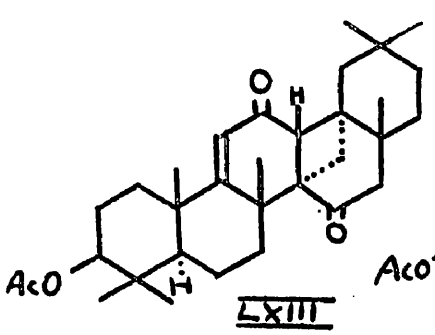


LXXXVIII

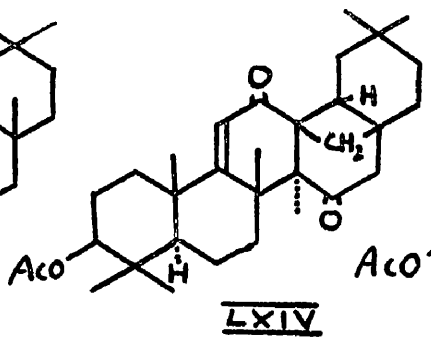
In our opinion, this is not necessarily the case. The ability of a cyclopropane ring to enter into electronic interaction with adjacent, unsaturated groupings is dependent on the stereochemistry of the chromophore as a whole. In relatively simple molecules in which the adjacent groups are capable of some movement relative to the plane of the three-ring, a cyclopropane ring is capable of both extending and transmitting electronic effects from contiguous unsaturated groupings[129-133].

In more complex molecules in which two carbon atoms of the cyclopropane ring form part of a larger cyclic system and adjacent groups are restrained from movement relative to the three-ring, the ability of a cyclopropane to transmit electronic effects is much impaired[131,134]. That a cyclopropane ring is capable of entering into conjugation with an adjacent ethylenic bond when both form part of a cyclohexene ring system, is undisputed[28,31,135,136], but we are of the opinion that in the environment of the highly unsaturated, and consequently rigid, decalin ring system, composing rings C and D of the acetate, $C_{32}H_{46}O_3$ (LXXXVIII), the ability of the cyclopropane ring to enter into conjugation with adjacent unsaturated groupings would be at a minimum and possibly insufficient to affect the properties of the $\Delta^{15:16}$ double-bond perceptibly.

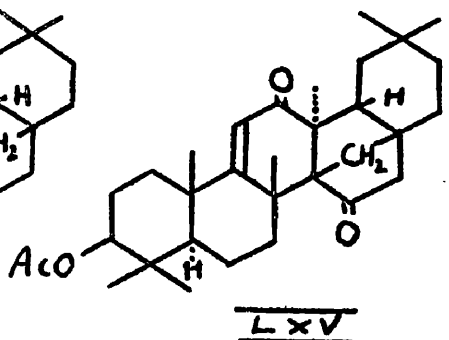
We are, therefore, of the opinion that it is unnecessary to postulate structures (LXIII), (LXIV) and (LXV), for the ketoacetate, $C_{32}H_{46}O_4$, derived from β-amyrin, as this compound is adequately represented by the cyclopropanoid structure (LXXXIV).



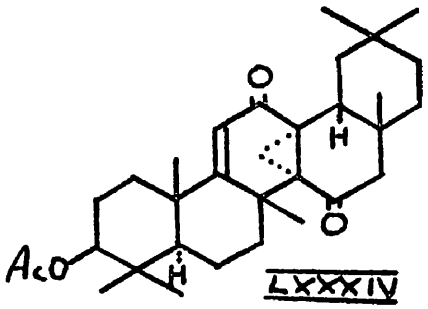
LXIII



LXIV



LXV



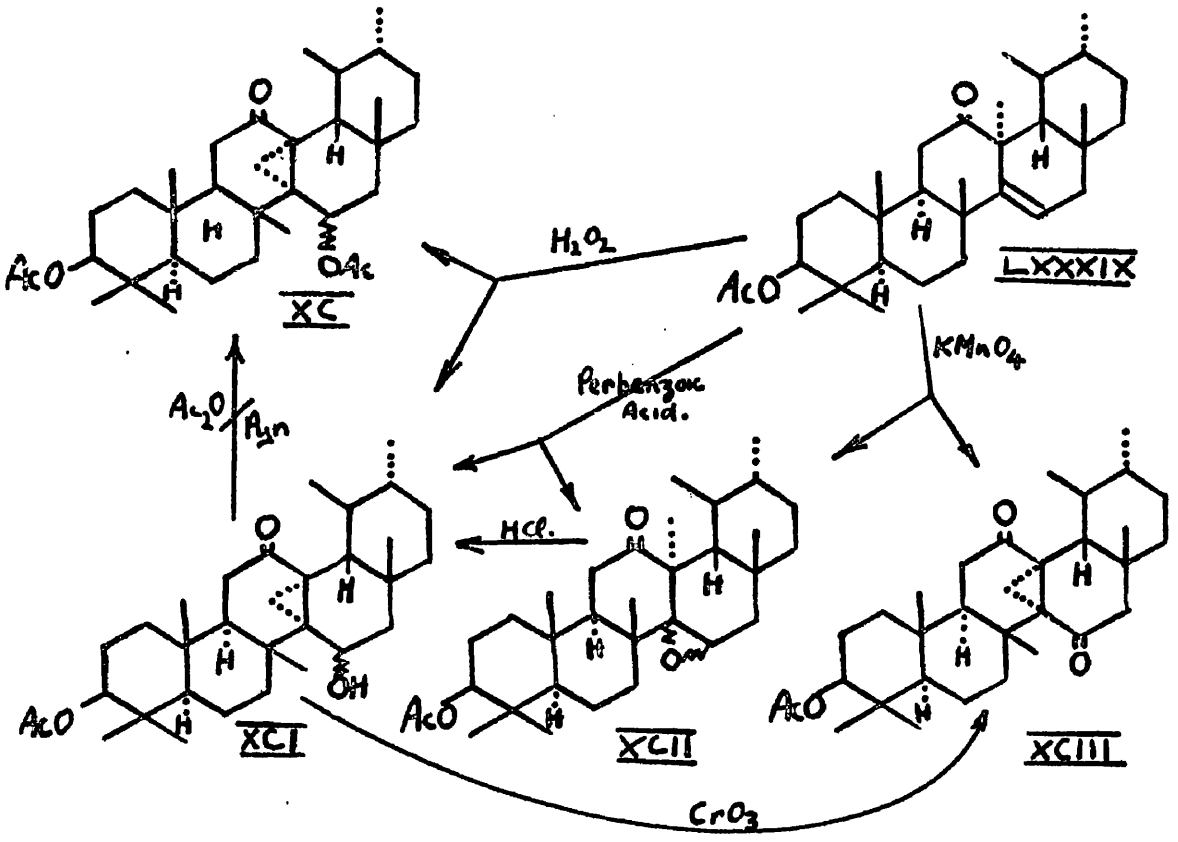
LXXXIV

The Oxidation of 12-Oxoisours-14-en-3β-yl Acetate.

Concurrently with the work described above, it was decided to supplement our knowledge of the oxidation of the $\Delta^{14:15}$ double-bond in derivatives of α-amyrin, by examining the oxidation of 12-oxoisours-14-en-3β-yl acetate (LXXXIX) with various reagents.

Oxidation of 12-oxoisours-14-en-3β-yl acetate (LXXXIX) with hydrogen peroxide gave two products, 12-oxo-13:27-cycloursan-3β:15ξ-diol diacetate (XC), and 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI). Acetylation of 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI) gave 12-oxo-13:27-cycloursan-3β:15ξ-diol diacetate (XC) in good yield.

Oxidation of 12-oxoisours-14-en-3β-yl acetate (LXXXIX)/.



(LXXXIX) with perbenzoic acid gave 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI), identical with that produced in the hydrogen peroxide oxidation, and 14:15-epoxy-12-oxoisoursan-3β-yl acetate (XCII), which on treatment with mineral acid gave 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI) in good yield.

Oxidation of 12-oxoisours-14-en-3β-yl acetate (LXXXIX) with potassium permanganate in acetic acid gave 14:15-epoxy-12-oxoisoursan-3β-yl acetate (XCII) and 12:15-dioxo-13:27-cycloursan-3β-yl acetate (XCIII).

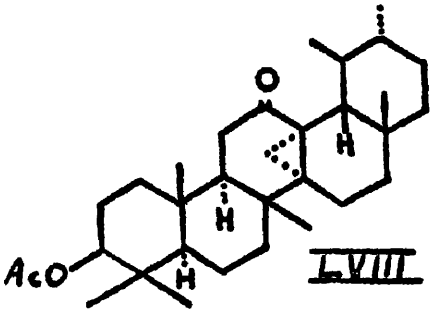
12:15-Dioxo-13:27-cycloursan-3β-yl acetate (XCIII) could also be prepared by oxidation of 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI) with chromium trioxide in pyridine.

No.	Compound	$\lambda_{max.}$ Å	ε
XCII	14:15-epoxy-12-oxoisoursan-3β-yl acetate	2020	1090
–	14:15-epoxy-12-oxoisoursan-3β-ol	2020	960
XC	12-oxo-13:27-cycloursan-3β:15ξ-diol diacetate	2100	5500
XCI	12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate	2100	4500
XCIII	12:15-dioxo-13:27-cycloursan-3β-yl acetate	2150	5750

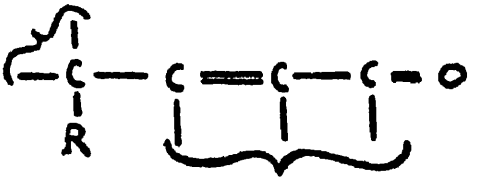
Table III. The ultra-violet spectra of oxidation products of 12-oxoisours-14-en-3β-yl acetate.

The ultra-violet spectra of these oxidation products of 12-oxoisours-14-en-3β-yl acetate (LXXXIX) are of particular interest (Table III). 14:15-epoxy-12-oxoisoursan-3β-yl acetate (XCII) shows only ketonic end-adsorption. 12-oxo-13:27-cycloursan-3β:15ξ-diol diacetate (XC) and 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI) show absorption, at 2100 Å, which can be ascribed to the ketone in conjugation with a

cyclopropane ring. The absorption is not due to an isolated ethylenic bond as these compounds do not give a colour with tetranitromethane in chloroform. This absorption, in the spectra of 12-oxo-13:27-cyclo-ursan-3β:15ξ-diol diacetate (XC) and 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI) is at a shorter wavelength than the absorption due to the same chromophore in 12-oxo-13:27-cycloursan-3β-yl acetate (LVIII), $\lambda_{max.}$ 2140 Å (ε 5500)[31]. It is known that a hydroxyl or acetoxyl substituent γ to an α:β-unsaturated ketone, i.e. R in (XCIV), can exert a considerable hypsochromic influence on the ultra-violet absorption of the α:β-unsaturated ketone chromophore[137]. The anomolous absorption of 12-oxo-13:27-cycloursan-3β:15ξ-diol diacetate (XC) and 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI) must be due to a similar effect exerted by the 15-acetoxyl or 15-hydroxyl substituents on the absorption due to the α:β-cyclo-propane ketone chromophore.



LVIII



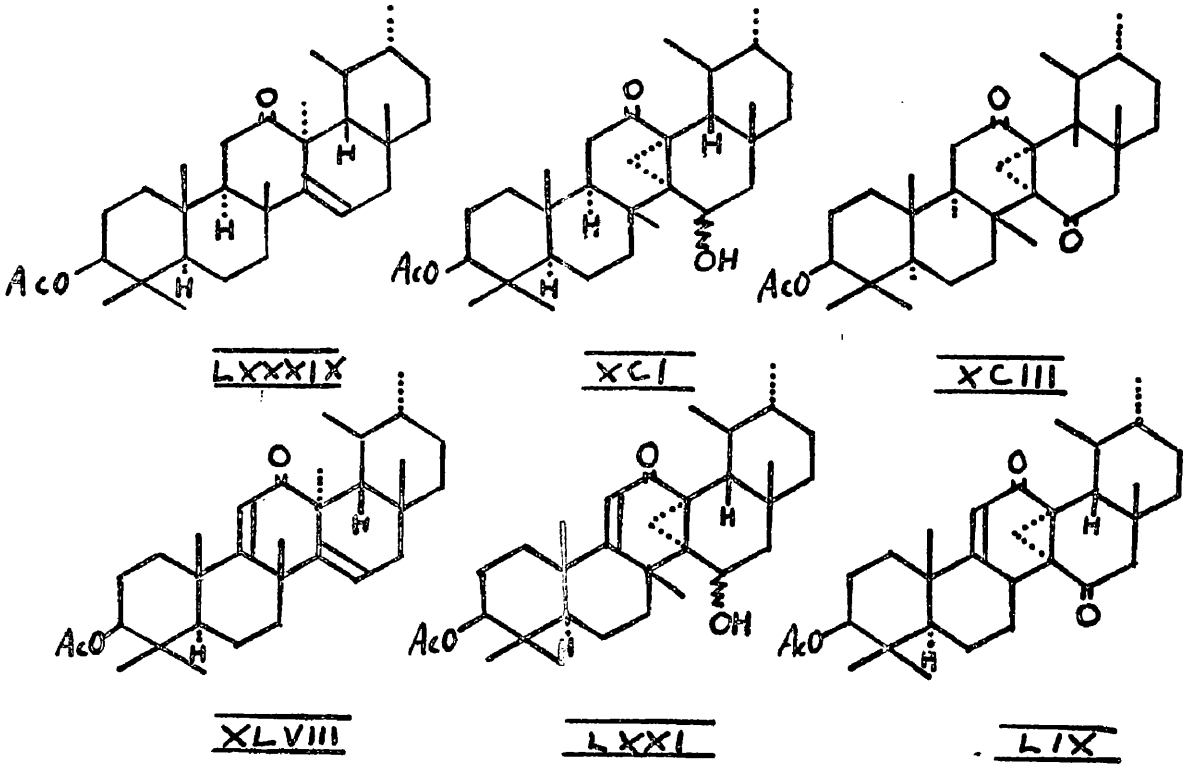
XCIV

12:15-Dioxo-13:27-cycloursan-3β-yl acetate (XCIII) contains two carbonyl groups adjacent to a cyclopropane ring. This chromophore, however, shows maximal absorption at 2150 Å, which differs only slightly from the absorption of a single ketone in conjugation with the cyclopropane ring in 12-oxo-13:27-cycloursan-3β-yl acetate (LVIII), $\lambda_{max.}$ 2140 Å. In the absorption of compounds containing an α:β -unsaturated ketone the introduction of an adjacent γ-ketone group (c.f. (XCIV) ; R = γ-substituent) effects a considerable bathochromic shift, and that a similar effect is not shown in the corresponding cyclopropanoid chromophore must be due to inability of the cyclopropane ring in 12:15-dioxo-13:27-cycloursan-3β-yl acetate (XCIII) to transmit conjugative effects between the contiguous ketone groups. (c.f. p. 38)

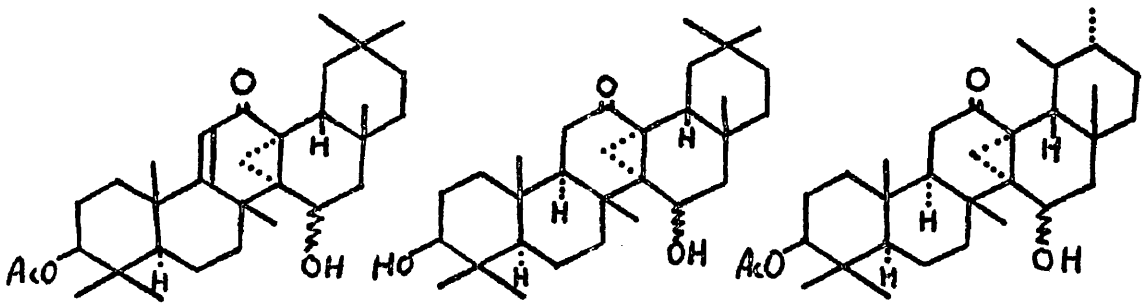
Conversely, in view of the theoretical[131] and experimental[134] indications that the cyclopropane ring in 12:15-dioxo-13:27-cycloursan-3β-yl acetate (XCIII) may, due to its environment, be incapable of transmitting such conjugative effects, the maximum absorption at 2150 Å shown by this compound can be taken as confirmation of the cyclopropanoid formulation.

The synthesis of 12:15-dioxo-13:27-cycloursan-3β-yl

acetate (XCIII) from 12-oxoisours-14-en-3β-yl acetate (LXXXIX) via 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI), is analogous to the synthesis of 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (LIX) from 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) via the keto-diol monoacetate, 12-oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol 3-acetate (LXXI) (see p.25); proof, therefore, that the oxidation products in the former reaction sequence are cyclopropanoid, can be taken to support the cyclopropanoid structures proposed for the corresponding compounds in the latter series and, in particular, it supports the formulation of the ketoacetate, $C_{32}H_{46}O_4$, derived from α-amyrin, as 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (LIX).



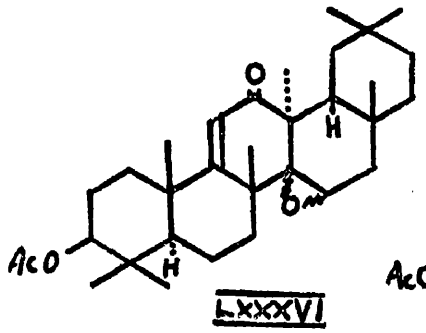
Some support for the extension of cyclopropanoid structures to the hexacyclic derivatives of β-amyrin has been forthcoming. 12-Oxo-13:27-cyclooleanan-9(11)-en-3β:15ξ-diol 3-acetate (XCV)[111] on reduction with lithium in liquid ammonia gave a keto-diol, 12-oxo-13:27-cyclooleanan-3β:15ξ-diol (XCVI) which shows maximal absorption at 2100 Å (ε 5100). The similarity of this absorption to that shown by 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (XCI) indicates that both compounds contain the same cyclopropanoid chromophore.



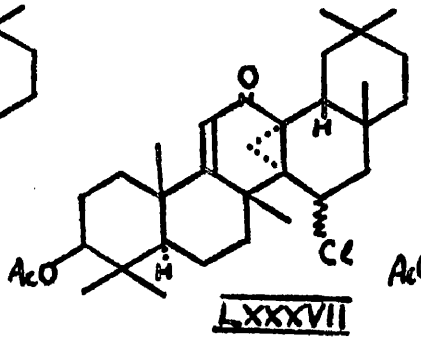
XCV XCVI XC

The Problem of the Chloro-acetates, $C_{32}H_{47}O_3Cl$, derived from α-Amyrin and β-Amyrin.

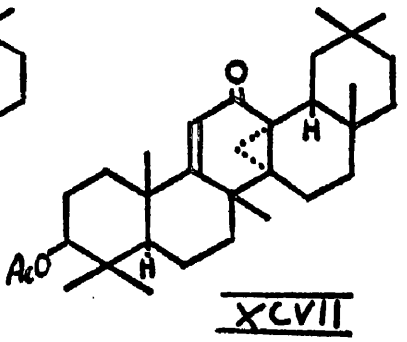
As mentioned above (p. 37), Johnston and Spring[111] showed that treatment of 14:15-epoxy-12-oxoisoolean-9(11)-en-3β-yl acetate (LXXXVI) with concentrated hydrochloric acid gives a chloro-acetate, $C_{32}H_{47}O_3Cl$, which we now formulate as 15-chloro-12-oxo-13:27-cycloolean-9(11)-en-3β-yl acetate (LXXXVII). Reduction of this chloro-acetate (LXXXVII) with activated zinc in ether-methanol gives an acetate, $C_{32}H_{48}O_3$, which Johnston and Spring[111] suggested would prove to be the β-amyrin analogue (XCVII) of 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (LI)[31], the structure of which was at that time unknown. It was decided to undertake an investigation of the corresponding series of reactions on α-amyrin derivatives.



LXXXVI



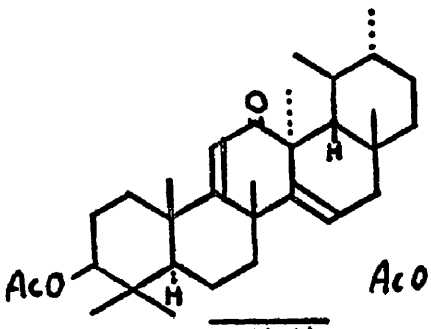
LXXXVII



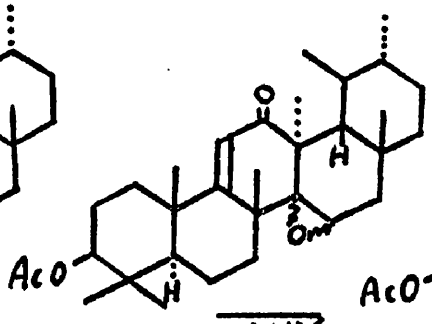
XCVII

Treatment of 14:15-epoxy-12-oxoisours-9(11)-en-3β-yl acetate (LXIX) with concentrated hydrochloric acid gave a chloro-acetate, $C_{32}H_{47}O_3Cl$, m.p. 210°, and, in very low yield, a substance, m.p. 294°.

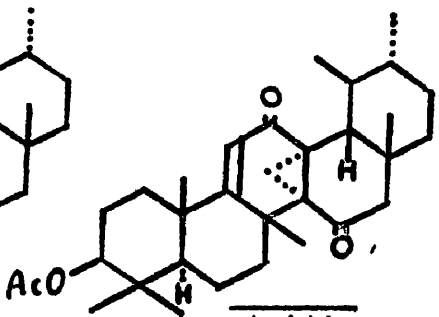
The substance, m.p. 294°, gives 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (LIX) on treatment with activated zinc in ether-methanol, and it is considered to be a mixed crystal (2:1) of this compound (LIX) and the chloro-acetate, m.p. 210°, formed from 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (LIX) present as an impurity in the starting material (LXIX). 14:15-Epoxy-12-oxoisours-9(11)-en-3β-yl acetate (LXIX) is obtained[118] by oxidation of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII) with potassium permanganate in acetic acid. As described above (p. 41), similar oxidation of 12-oxoisours-14-en-3β-yl acetate (LXXXIX) gives both 14:15-epoxy-12-oxoisoursan-3β-yl acetate (XCII) and 12:15-dioxo-13:27-cycloursan-3 -yl acetate (XCIII).



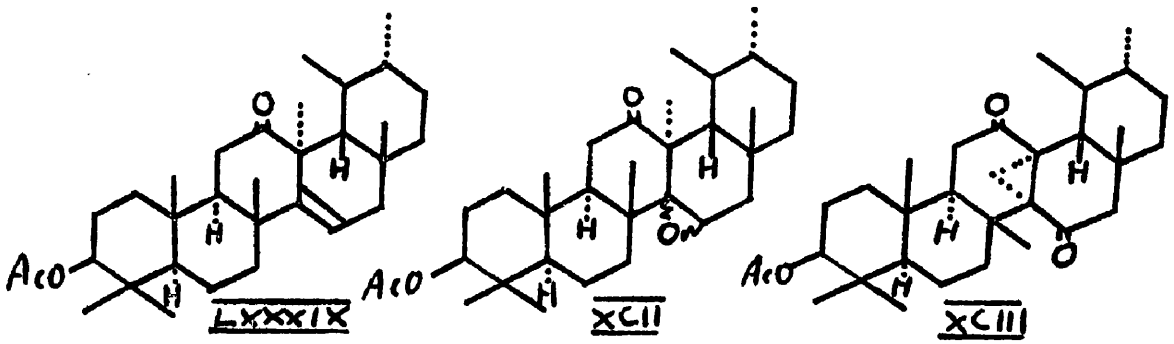
XLVIII



LXIX



LIX

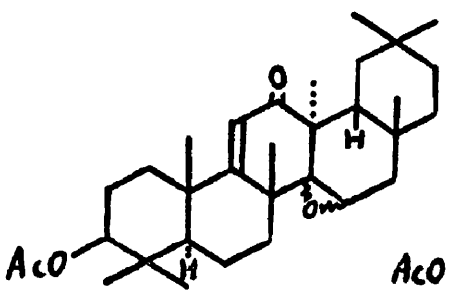


LXXXIX XCII XCIII

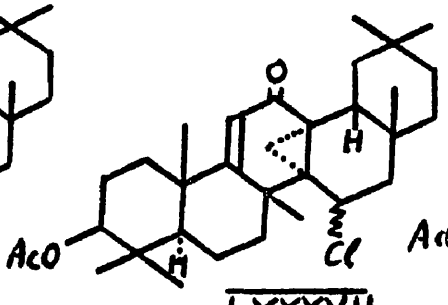
Reduction of the chloro-acetate, $C_{32}H_{47}O_3Cl$, m.p. 210°, which shows only one absorption band, $\lambda_{max.}$ 2390 Å (ε 11,000), in the ultra-violet region, and which gives no colour with tetranitromethane in chloroform, with activated zinc in ether-methanol, produced an acetate, $C_{32}H_{48}O_3$, m.p. 241°, $\lambda_{max.}$ 2070 Å and 2400 Å (ε 7300 and 10,150), which gives a yellow colour with tetranitromethane in chloroform. The acetate, $C_{32}H_{48}O_3$, m.p. 241°, on treatment with hydrochloric acid gives 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate, $C_{32}H_{48}O_3$ (LI), in good yield.

This sequence of reactions is not similar to the reactions of the corresponding derivatives of β-amyrin, described by Johnston and Spring[111], and it was decided to reinvestigate the reactions described by these authors[111].

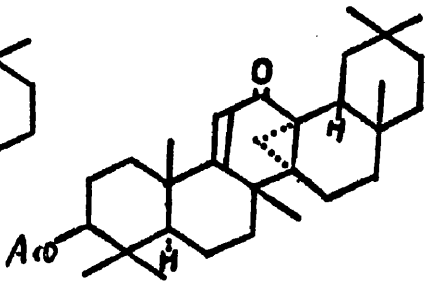
Treatment of 14:15-epoxy-12-oxoisoolean-9(11)-en-3β-yl acetate (LXXXVI) with hydrochloric acid gave, in our hands, a chloro-acetate, $C_{32}H_{47}O_3Cl$, m.p. 248°, $[\alpha]_D$ +146°, $\lambda_{max.}$ 2400 Å, which differs considerably from the chloro-acetate (LXXXVII), $C_{32}H_{47}O_3Cl$, m.p. 227-228°, $[\alpha]_D$ +118°, $\lambda_{max.}$ 2360 Å, isolated by Johnston and Spring[111] from the same reaction. Reduction of the latter chloro-acetate (LXXXVII), m.p. 227-228°, with zinc in ether methanol, is reported[111] to give an acetate, $C_{32}H_{48}O_3$, m.p. 274-275°, $[\alpha]_D$ +139° or +130°, λ_{max} 2350 Å, which is unaffected by treatment with mineral acid, and which, from its mode of synthesis, must be formulated as (XCVII). A similar reduction of the chloro-acetate, m.p. 248°, obtained by the author, gave an acetate, $C_{32}H_{48}O_3$, m.p. 207-209°, $[\alpha]_D$ -19°, $\lambda\lambda_{max.}$ 2070 Å and 2400 Å, which gives a yellow colour with tetranitromethane in chloroform, and which, on treatment with hydrochloric acid, gives an isomer, $C_{32}H_{48}O_3$, m.p. 275-276°, $[\alpha]_D$ +170°, $\lambda_{max.}$ 2360 Å, which gives no colour with tetranitromethane.



LXXXVI

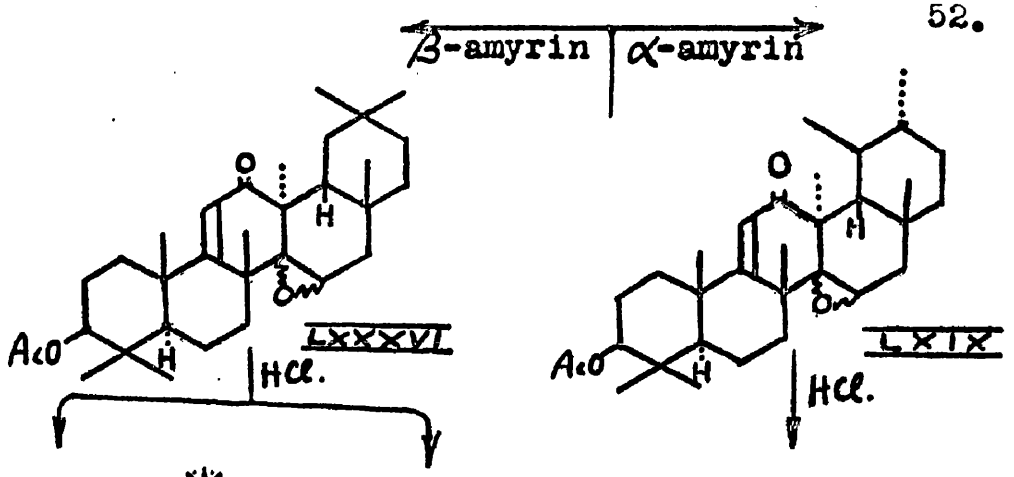


LXXXVII



XCVII

The formation and reactions of the chloro-acetates, $C_{32}H_{47}O_3Cl$, derived from α-amyrin and β-amyrin, are illustrated in table IV (p. 52), from which it will be seen that the results which we obtained in the α-amyrin series of derivatives, correspond closely to the results which we obtained on carrying out similar reactions in the β-amyrin series, but that they differ from the results described by Johnston and Spring[111] in the latter series. In view of this, it seems probable that the chloro-acetate, m.p. 210°, derived from α-amyrin, corresponds in structure to the chloro-acetate, m.p. 248°, derived from β-amyrin, and that the reduction products of these two chloro-acetates will also correspond in structure. This implies that the acetate, $C_{32}H_{48}O_3$, m.p. 275-276°, $[\alpha]_D$ +170°, derived from β-amyrin, must correspond in structure to 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (LI), and is, therefore, 12-oxo-13:27-cycloolean-9(11)-en-3β-yl acetate (XCVII). This structure (XCVII) has previously (see p. 47 and p. 50) been suggested for the acetate, $C_{32}H_{48}O_3$, m.p. 274-275°, $[\alpha]_D$ +130° or +139°, which was isolated by Johnston and Spring[111]. It is pertinent to note, therefore, that Johnston and Spring[111] quote optical rotation values for two



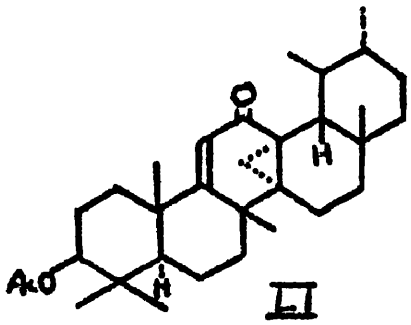
$C_{32}H_{47}O_3Cl$.*	$C_{32}H_{47}O_3Cl$.	$C_{32}H_{47}O_3Cl$.
m.p. 227-228°,	m.p. 248°,	m.p. 210°,
$[\alpha]_D$ +118°,	$[\alpha]_D$ +146°,	$[\alpha]_D$ +122°
$\lambda_{max.}$ 2360 Å.	$\lambda_{max.}$ 2400 Å.	$\lambda_{max.}$ 2390 Å.
↓ Zn/MeOH.	↓ Zn/MeOH	↓ Zn/MeOH.
$C_{32}H_{48}O_3$,*	$C_{32}H_{48}O_3$,	$C_{32}H_{48}O_3$,
m.p. 274-275°,	m.p. 207-209°,	m.p. 241°,
$[\alpha]_D$ +139° or +130°,	$[\alpha]_D$ -19°,	$[\alpha]_D$ ±0°,
$\lambda_{max.}$ 2350 A.	$\lambda_{max.}$ 2070 Å and 2400 Å.	$\lambda_{max.}$ 2070 Å and 2400 Å.
↓ HCl.	↓ HCl	↓ HCl
Unchanged	$C_{32}H_{48}O_3$ m.p. 275-276°, $[\alpha]_D$ +170°, $\lambda_{max.}$ 2360 Å.	$C_{32}H_{48}O_3$ (LI) m.p. 268-270°, $[\alpha]_D$ +156°, $\lambda_{max.}$ 2360 Å.

Table IV- Formation and reactions of the Chloro-acetates, $C_{32}H_{47}O_3Cl$, derived from α- and β-amyrin.

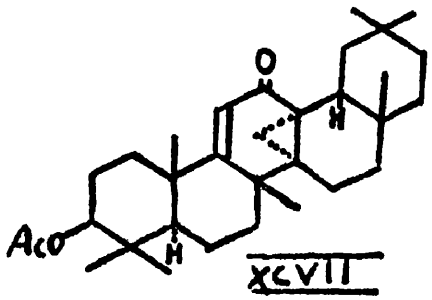
* = Reported by Johnston and Spring[111].

(LI)=12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate.

separate preparations of their acetate, m.p. 274-275°, which differ beyond the limit of experimental error, and this acetate, m.p. 274-275°, may not have been a pure substance, but may have been a mixture of the acetate, m.p. 275-276°, $[\alpha]_D$ +170°, with small amounts of the acetate m.p. 207-209°, $[\alpha]_D$ -19°.



LI



XCVII

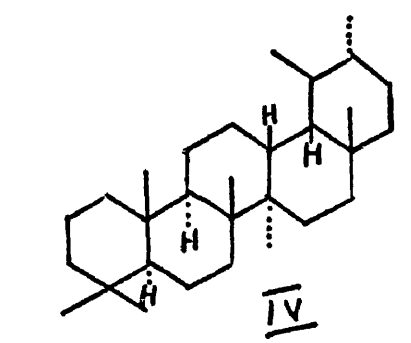
It is difficult to postulate plausible structures for the chloro-acetate, m.p. 210°, derived from α-amyrin, the chloro-acetate, m.p. 248°, derived from β-amyrin, and the acetates, m.p. 241° and 207-209°, obtained by reduction of these compounds.

The acetate, m.p. 241°, derived from α-amyrin, is pentacyclic, since on acid isomerisation it gives a hexacyclic derivative (LI) with the loss of a double-bond. By analogy, the acetate, m.p. 207-209°, derived from β-amyrin must also be pentacyclic. If we assume that the pentacyclic skeleton in, say, the acetate, m.p. 241°, derived from α-amyrin, is either the

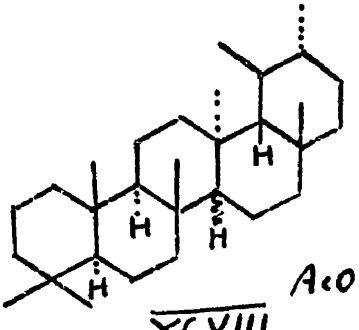
normal ursane skeleton (IV) or the "isoursane" skeleton (XCVIII), as exist in other pentacyclic derivatives of α-amyrin, then the most probable formulation for the acetate, m.p. 241°, is 12-oxoisoursa-9(11):15-dien-3β-yl acetate (XCIX). This formulation is, however, unsatisfactory, as it is difficult to discern why the $\Delta^{15:16}$ double-bond in such environment should be capable of isomerisation. It may be, however, that the locking of rings C and D in this compound (XCIX) is in an unstable configuration and, thus, provides a driving force for the isomerisation.

Were the formulation (XCIX) correct for the acetate, m.p. 241°, derived from α-amyrin, then an analogous structure, 12-oxoisooleana-9(11):15-dien-3 -yl acetate (C) would need to be ascribed to the acetate, m.p. 207-209°, derived from β-amyrin. The latter compound isomerises under acid conditions to give a hexacyclic derivative, 12-oxo-13:27-cycloolean-9(11)-en-3β-yl acetate (XCVII), and, if the formulation (C) is correct, this isomerisation would necessarily proceed through 12-oxoisooleana-9(11):14-dien-3β-yl acetate (XLIX), a compound which is known to be stable under acid conditions (see p.20). For these reasons we consider that a normal pentacyclic carbon-skeleton is

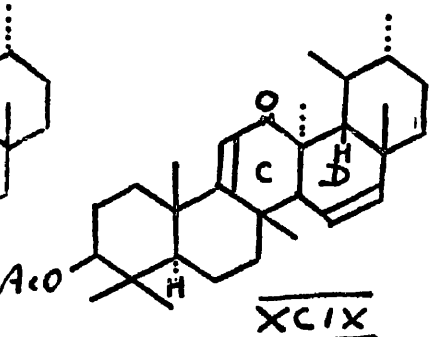
improbable for these compounds, the acetate, m.p. 241°, derived from α-amyrin, and the acetate, m.p. 207-209°, derived from β-amyrin.



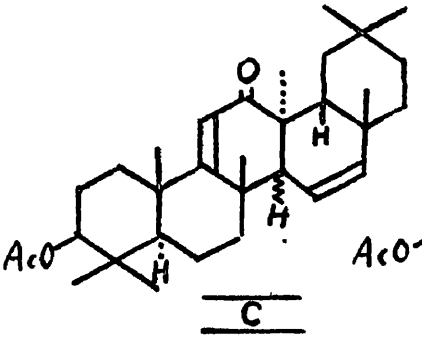
IV



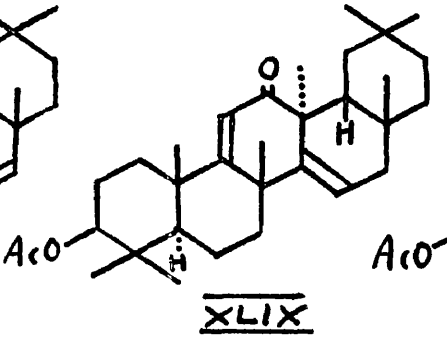
XCVIII



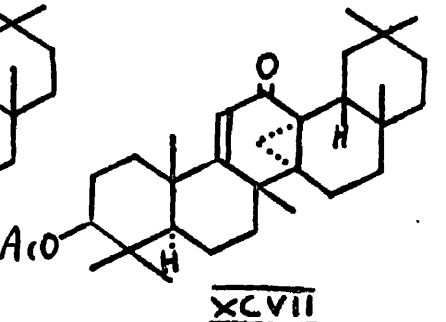
XCIX



C



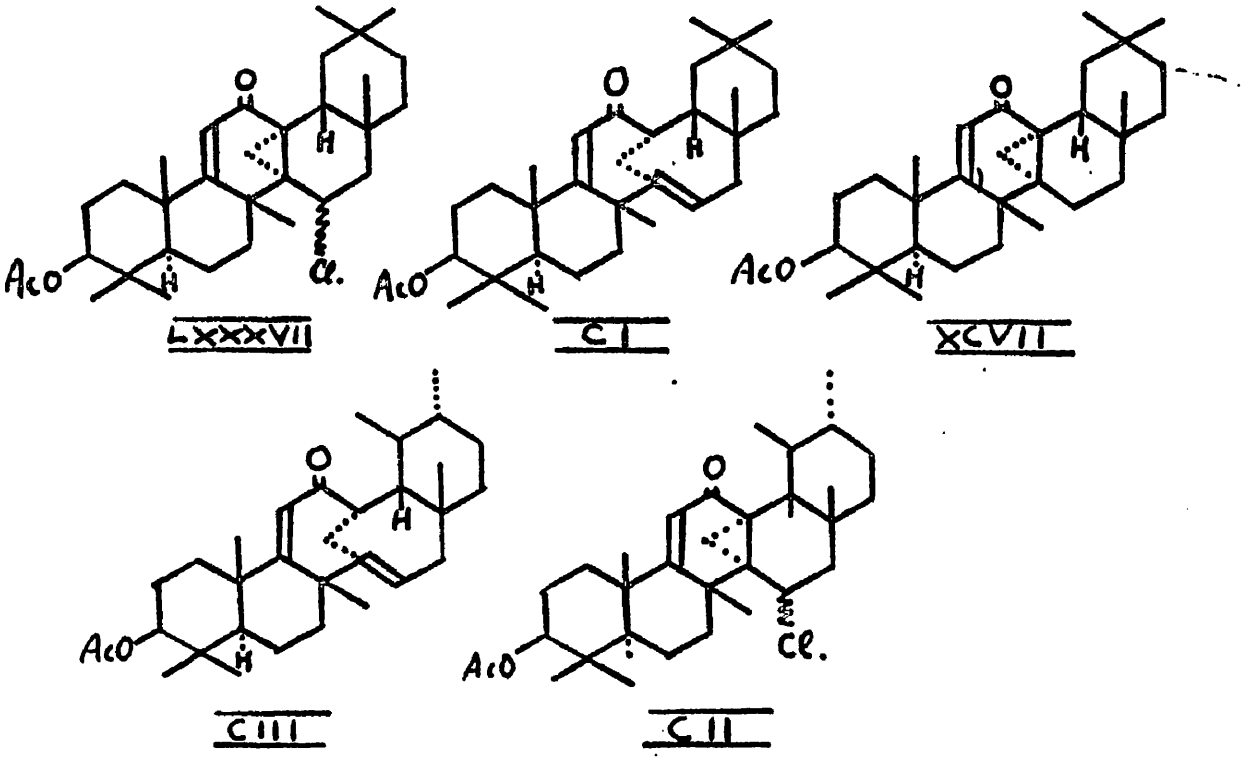
XLIX



XCVII

It is similarly difficult to formulate the parent chloro-acetates, as it is not certain whether these compounds are pentacyclic or hexacyclic. It is possible that they are hexacyclic, and that reduction of the chloro-acetates to pentacyclic products, e.g. the reduction of the chloro-acetate, m.p. 210°, derived from α-amyrin, to the acetate, m.p. 241°, may be accompanied by fission of some part of the carbon-skeleton. Johnston and Spring[111] proved the chloro-acetate,

m.p. 227-228°, $[\alpha]_D$ +118°, which they obtained, to be hexacyclic and, in view of our conclusions regarding the structure of related hexacyclic derivatives of β-amyrin (see p. 39), this chloro-acetate, m.p. 227-228°, must be formulated as cyclopropanoid (LXXXVII). We have indicated previously (p. 53) that the acetate, m.p. 274-275°, obtained by Johnston and Spring[111] by reduction of the chloro-acetate, m.p. 227-228°, (LXXXVII) with zinc in ether-methanol, could have been a mixture. Conceivably, therefore, the chloro-acetate, m.p. 227-228°, may have also been not homogeneous, and it may, in fact, have been partly composed of the chloro-acetate, m.p. 248°, which we obtained in trying to repeat this reaction. If this should be correct, the chloro-acetate, m.p. 248°, could be formulated as (LXXXVII), and reduction of this compound could lead to a pentacyclic acetate, m.p. 207-209°, (CI), formed by fission of the $C_{(13)}$-$C_{(14)}$ bond in the cyclopropanoid chloro-acetate (LXXXVII), and this reduction product (CI) could isomerise under acid conditions to 12-oxo-13:27-cycloolean-9(11)-en-3β-yl acetate (XCVII) by reformation of this bond. A similar structural sequence could be formulated for the corresponding derivatives of α-amyrin, giving the chloro-acetate, m.p. 210°, as (CII) and the derived acetate, m.p. 241°, as (CIII).



LXXXVII C I XCVII

C III C II

We do not regard these formulations as wholly satisfying explanations of our experimental results, which constitute, in any case, only a preliminary investigation of these compounds. The structural elucidation of these chloro-acetates, derived from both α-amyrin and β-amyrin, still presents many experimental and theoretical problems.

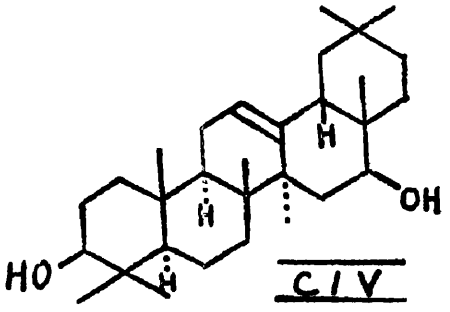
SECTION II : The Structure of Brein.

Section II

The Structure of Brein

Historical

Investigations of the chemistry of the triterpene diol, brein, have been continually hampered by the difficulty of the isolation of the free diol in reasonable quantity from Manila elemi resin, the only known source, in which it occurs in close association with maniladiol (CIV)[138-142].



CIV

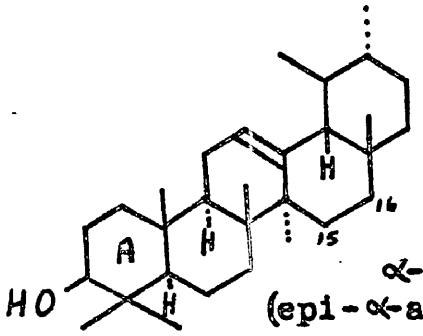
Vesterberg[4,5] showed brein to be a dihydric, triterpene alcohol, $C_{30}H_{50}O_2$, a result which was later confirmed by Rollett[143], who also showed the two hydroxyl groups to be secondary by preparation of a diketone, breindione.

Mladenovic and Hoffmann[144] demonstrated the presence of a hindered double-bond in brein by ozonisation of brein diacetate, and they suggested that the reactivity of this function is similar to that of the double-bond in α-amyrin. The presence of the ethylenic function was simultaneously demonstrated by Morice and Simpson[138], who oxidised brein diacetate to an α:β-unsaturated ketone. These last authors[138] also showed that Meerwein-Ponndorf reduction of breindione gives two isomeric keto-alcohols, $C_{30}H_{48}O_2$, which they called breinonol-A and breinonol-B.

Breinonol-B acetate was subsequently prepared by chromic acid oxidation of a monoacetyl derivative of brein[145], and it was reported that Wolff-Kishner reduction of both breinonol-A acetate or breinonol-B acetate gives epi-α-amyrin.[145] This indicated that brein must be 3α:X-dihydroxyurs-12-ene, but it was pointed out by Klyne and Stokes[147] that the molecular rotation differences between various derivatives of brein, and breinonol-B, in which, to account for its synthesis by oxidation of brein monoacetate, the 3-hydroxyl group must be in the same configuration as in brein, are more in keeping with a 3β-(equatorial)-hydroxyl group. They[147] suggested that the reduction of breinonol-B acetate to

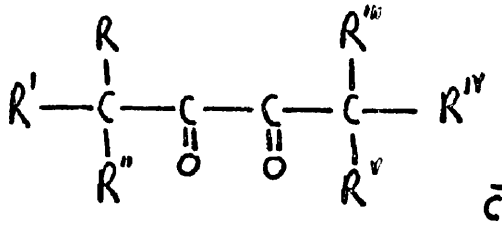
epi-α-amyrin must have been due to epimerisation of the 3-hydroxyl group during the reduction, or to contamination of the starting material with breinonol-A acetate. Laird[32] has now confirmed this suggestion since reduction of breinonol-B acetate by the Wolff-Kishner technique gave, in his hands, α-amyrin (V) only.

Laird[32] also showed that reduction of breindione with sodium borohydride gives breinonol-B, and, by the general rules[148] governing reduction of unhindered ketones with metal hydrides, the 3-hydroxyl group produced would be expected to be equatorial. The same reduction also gave a monoacetyl derivative of brein, brein monoacetate-II, which must be the x-epimer of the brein monoacetate which had been previously prepared by Buchi, Jeger and Ruzicka[145]. Comparision of the reactivities of the x-hydroxyl group in brein monoacetate and brein monoacetate-II led Laird[32] to conclude that the x-hydroxyl group in the former monoacetate, and therefore in brein, is equatorially oriented.



α- amyrin (V)
(epi-α-amyrin : 3α-hydroxyl).

Morice and Simpson[138] pointed out that as breindione does not show the chromophoric properties of an α- or a β-diketone, the x-hydroxyl group in brein cannot be located in ring A of the α-amyrin ($\underline{V}$) skeleton. Buchi, Jeger and Ruzicka[145] found that breinonol-B acetate gives an α-diketone on oxidation with selenium dioxide, which indicates the presence of a methylene group adjacent to the ketone group in breinonol-B. This α-diketone gave no colour with ferric chloride[145], implying that the carbon atoms adjacent to the α-diketone function must be fully substituted and, therefore, unable to donate the hydrogen atom necessary for enolisation.

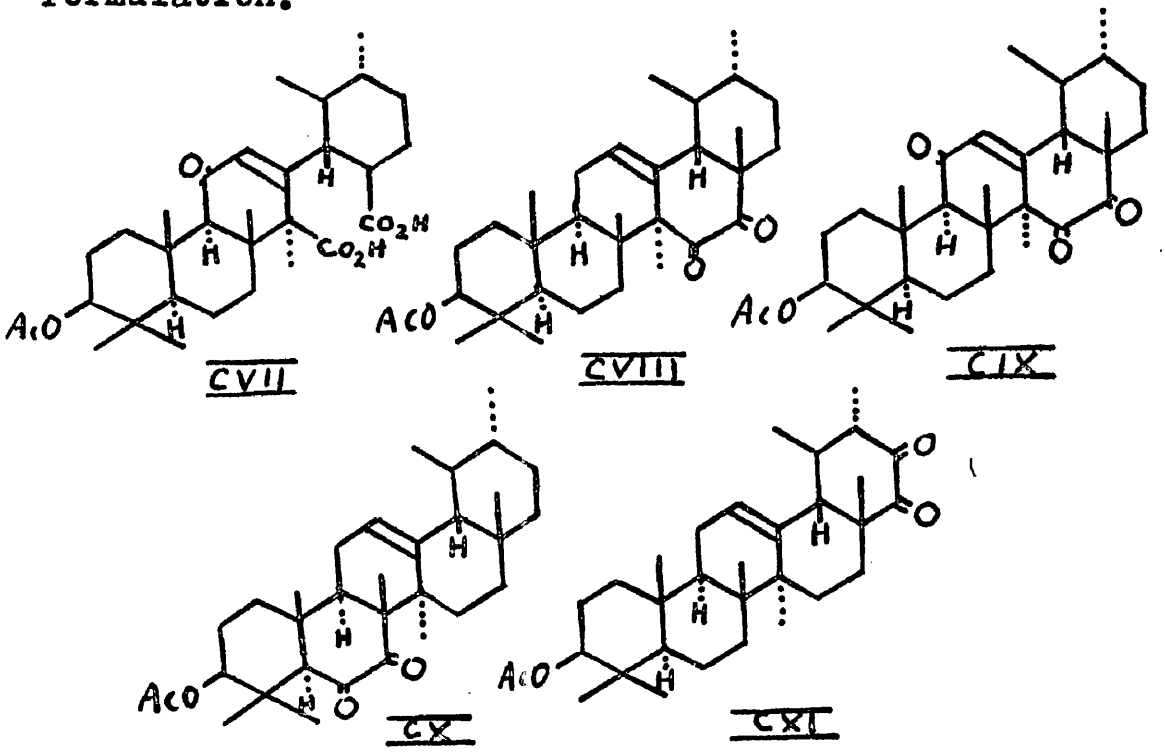


$R, R', R'', R''', R'', R^{V} \neq H.$

$\underline{CVI}$

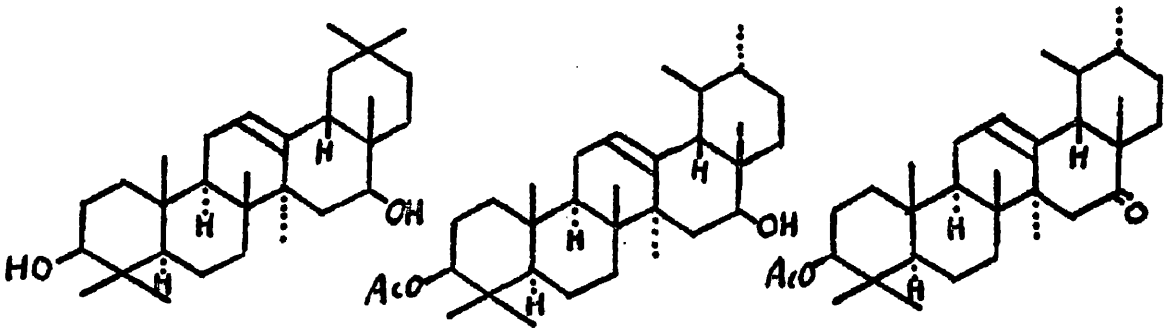
The α-diketone group in such an environment ($\underline{CVI}$) could only be located in ring D of the α-amyrin molecule ($\underline{V}$), i.e. at $C_{(15)}$ and $C_{(16)}$. It was later reported,[146] however, that a triketone, which may be prepared by chromic acid oxidation of this α-diketone, gave a violet colour after standing with ferric chloride for

four months. This triketone, presumably formed by oxidation of the allylic double-bond to an $\alpha:\beta$-unsaturated (11-)ketone, gives a dicarboxylic acid on oxidation with hydrogen peroxide.[146] It was suggested that the stability of this acid, which forms a stable anhydride,[146] is greater than would be expected of a vinylogous carboxylic acid, i.e. than of (CVII), if the α-diketone is (CVIII) and the triketone (CIX). Consequently, it was suggested[146] that the α-diketone must be (CX) or (CXI), and, as the 6:7-diketone system — as in (CX) — is known to enolise readily in comparable compounds, the location of the α-diketone function in ring E (CXI) was considered the more probable formulation.



CVII CVIII CIX

CX CXI



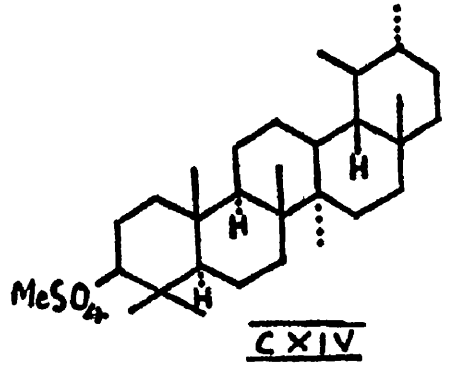
CIV CXII CXIII

Theoretical

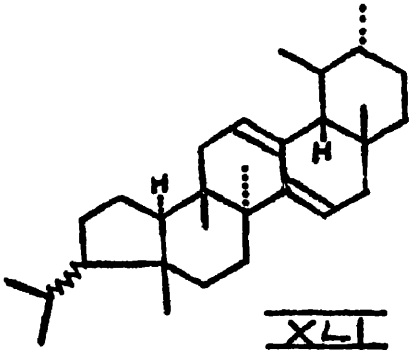
In planning this investigation of the chemistry of brein we assumed, due to the close natural association of brein with maniladiol (CIV), that the x-hydroxyl group in brein would prove to be located at $C_{(16)}$. This assumption, which proved to be justified, was made purely on the basis of biogenetic simplicity, and implies the formulation of brein as (CXII) and of breinonol-B as (CXIII).

Our first approach to the structural elucidation of brein (CXII) was to attempt the dehydration of breinonol-B with methanesulphonyl chloride. In connection with another problem, we had found that thermal decomposition of α-amyrin

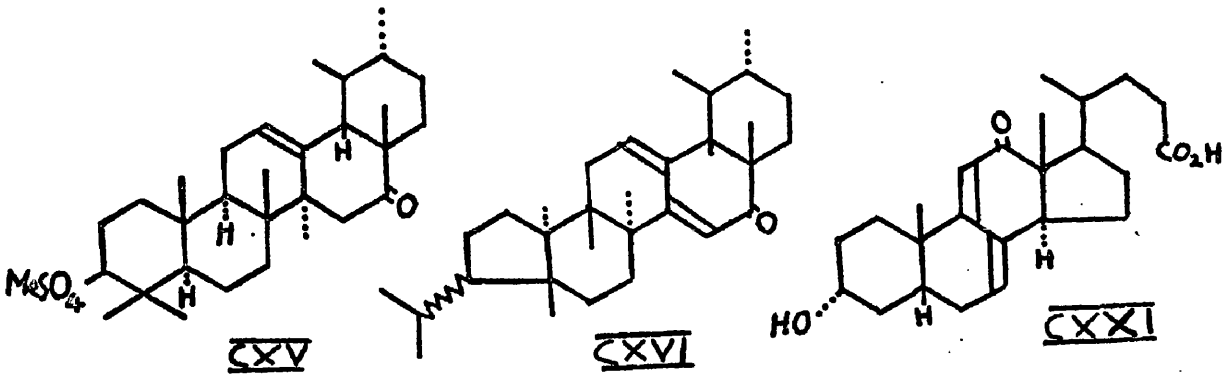
methanesulphonate (CXIV)[150] gives "1-α-amyradiene", 5ξ:8α:9β-trimethyl-10α-novursa-12:14-diene (XLI), in very high yield. If breinonol-B (CXIII) could be similarly dehydrated by decomposition of breinonol-b methanesulphonate (CXV), it would be expected to give 5ξ:8α:9β-trimethyl-10α-novursa-12:14-dien-16-one (CXVI), which contains a conjugated dienone chromophore, which is similar to the chromophore in 3α-hydroxy-12-oxo-7:9(11)-choladienic acid (CXXI) $\lambda\lambda_{max.}$ 2400 and 2930 Å (ε 3,700 and 12,900)[149], and which could therefore be detected by chromophoric examination. It had, in fact, been shown shortly before the beginning of this work that dehydration of breinonol-B (CXIII) with phosphoric acid gives an uncrystallisible gum, showing maximal absorption at 2400 Å and 2930 Å (ε 4,950 and 9,100)[32], and it was hoped to obtain the pure conjugated dienone (CXVI) by this alteration of the dehydrating agent.



CXIV



XLI



Breinonol-B methanesulphonate (CXV) could not, however, be obtained crystalline, and the product from repeated attempts to esterify breinonol-B (CXIII) with methanesulphonyl chloride in pyridine, was a gum, which developed a deep brown colour on standing at room temperature. Chromatography of the gummy "methanesulphonate" on alumina gave widely spread fractions and it seems probable that the product was decomposing at room temperature.

A freshly prepared sample of the gummy "methanesulphonate" obtained from breinonol-B, was heated at 80°, whereupon it decomposed with the evolution of acidic vapours. The decomposition product could not be crystallised, even after chromatographic separation, and showed continuous absorption in the ultra-violet region from 2000 Å to 3280 Å, with maxima at 2070, 2400 and 2890 Å (ε 7,000, 3,700 and 6,000) This ultra-violet spectrum of the product is unaffected by

treatment of the gum with mineral acid. Thus, if the dienone (CXVI) is formed in this reaction, it is formed in fairly low yield.

Our next approach to the problem was based on a product which may be obtained by ozonisation of brein diacetate (CXVII)[144]. This product gives no colour with tetranitromethane in chloroform, and shows ketonic end-absorption ($\varepsilon_{2040\ \text{Å}} \sim 700$) and a band at 2500 Å ($\varepsilon \sim 190$) in its ultra-violet spectrum. This optical property is comparable with that of 12-oxo-13α-ursan-3β-yl acetate (CXVIII), which is obtained by ozonisation of α-amyrin acetate (CXIX), and which shows end-absorption ($\varepsilon_{2030\ \text{Å}} \sim 1000$) and a similar unassigned band at 2500 A ($\varepsilon \sim 350$).[*]

The infra-red spectrum of the ozonisation product

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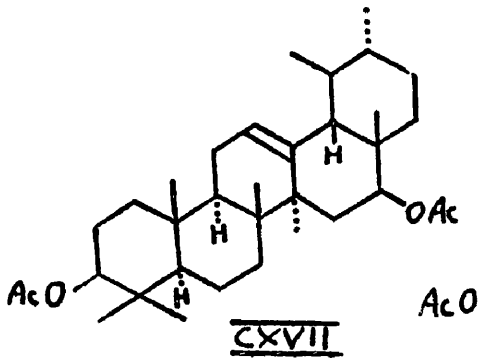
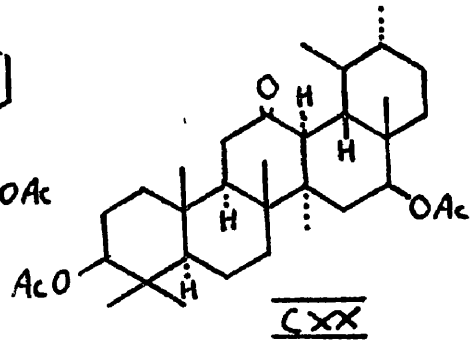
[*] The sample of 12-oxo-13α-ursan-3β-yl acetate, prepared by the author and on which these observations were made, had m.p. 208°, $[\alpha]_D$ +114°. Allan, Spring and Stevenson[152] report m.p. 210-211°, $[\alpha]_D$ +115°; Ruzicka, Jeger, Redel and Volli[153] report m.p. 204-205°, $[\alpha]_D$ +139°; Kaye, Fieser and Fieser[151] report m.p. 201-203°, $[\alpha]_D$ +124°, for the same compound. The last authors[151] also comment on the presence of an anomolous band at 2500 Å in the ultra-violet spectrum of their product.

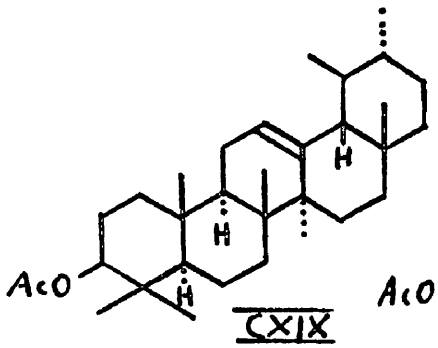
from brein diacetate (CXVII) includes a band at 1709 $cm.^{-1}$ This band is similar in location to the band at 1707 $cm.^{-1}$ in the infra-red spectrum of 12-oxo-13 - ursan-3β-yl acetate (CXVIII), in which it can be ascribed to the 12-ketone function. We are of the opinion, therefore, that the ozonisation of brein diacetate (CXVII) leads to the production of a saturated 12-ketone group, and we formulate the product as 12-oxo-13α-ursan-3β:16β-diol diacetate (CXX).



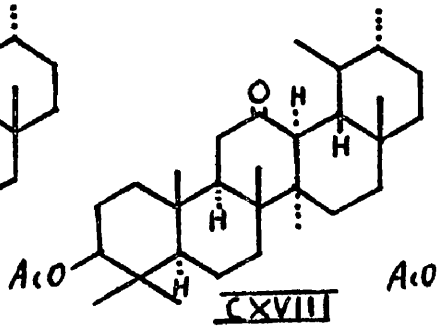
CXVII



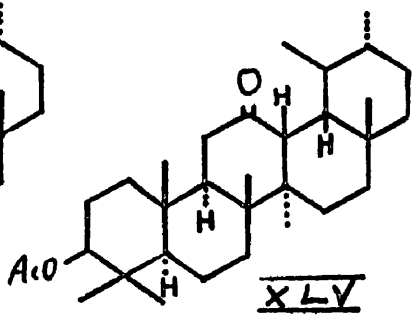
CXX



CXIX



CXVIII



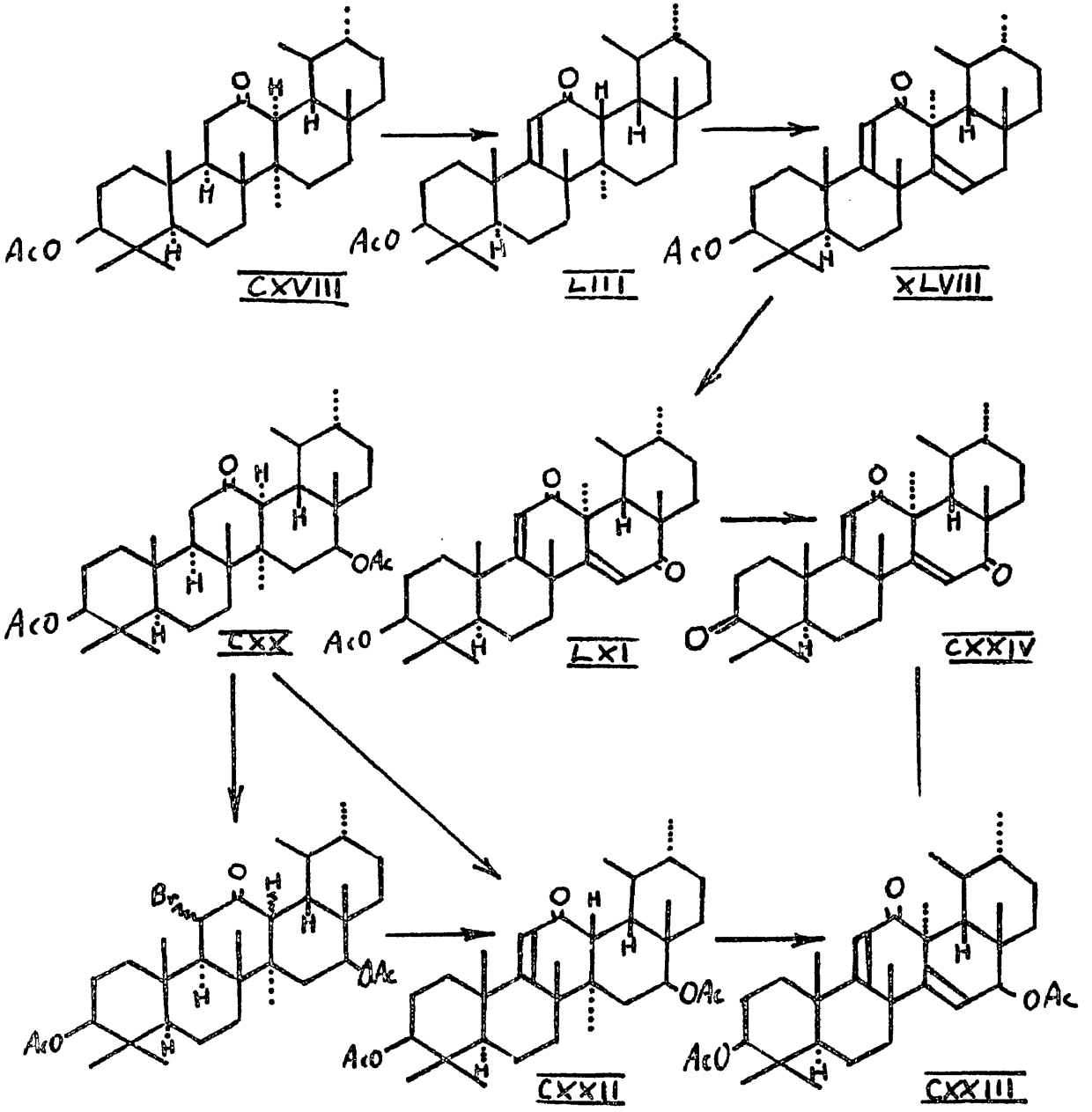
XLV

In assigning this structure to the ozonisation product from brein diacetate (CXVII) we have ascribed an α-configuration to the 13-hydrogen atom. The infra-red spectrum of the ozonisation product (CXX)

supports this formulation, since the ketonic band at 1709 $cm.^{-1}$ closely resembles the band , at 1707 $cm.^{-1}$, allocated to the 12-ketone group in the spectrum of 12-oxo-13α-ursan-3β-yl acetate (CXVIII), but differs from the ketonic band, at 1694 $cm.^{-1}$, in the spectrum of the 13β-isomer, 12-oxoursan-3β-yl acetate (XLV). Moreover, the change in optical rotation brought about by the ozonisation of brein diacetate (CXVII), $[\alpha]_D$ +76°, to the product (CXX), $[\alpha]_D$ +132°, is comparable with the change in optical rotation on oxidation of α-amyrin acetate (CXIX), $[\alpha]_D$ +78°, to 12-oxo-13α-ursan-3β-yl acetate (CXVIII), $[\alpha]_D$ +114°, while the change from α-amyrin acetate (CXIX) to 12-oxoursan-3β-yl acetate (XLV), $[\alpha]_D$ +18°,[152] is very different.

It was decided to use this ozonisation product, 12-oxo-13α-ursan-3β:16β-diol diacetate (CXX), as starting material for an attempt to synthesise 3:12:16-trioxoisoursa-9(11):14-diene (CXXIV). Treatment of 12-oxo-13α-ursan-3β-yl acetate (CXVIII) with bromine, and subsequent dehydrobromination of the crude α-bromo-ketone formed, by heating with mineral acid, is known to give 12-oxours-9(11)-en-3β-yl acetate (LIII), which, on oxidation with selenium dioxide, gives 12-oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII). A similar sequence

of reactions, starting from 12-oxo-13α-ursan-3β:16β-diol diacetate (CXX), should give 12-oxoisoursa-9(11):14-dien-3β:16β-diol diacetate,(CXXIII), which on hydrolysis and oxidation would give the triketone (CXXIV)



The same triketone (CXXIV) can be synthesised from α-amyrin (V) by an unambiguous route. 12-Oxoisoursa-9(11):14-dien-3β-yl acetate (XLVIII), on oxidation with selenium dioxide at high temperatures, gives 12:16-dioxoisoursa-9(11):14-dien-3β-yl acetate (LXI)[117], which on hydrolysis and oxidation should give the triketone (CXXIV). If the two triketones (CXXIV), derived by these syntheses, proved to be identical, the location of the x-hydroxyl group of brein (CXII) at $C_{(16)}$ would be established.

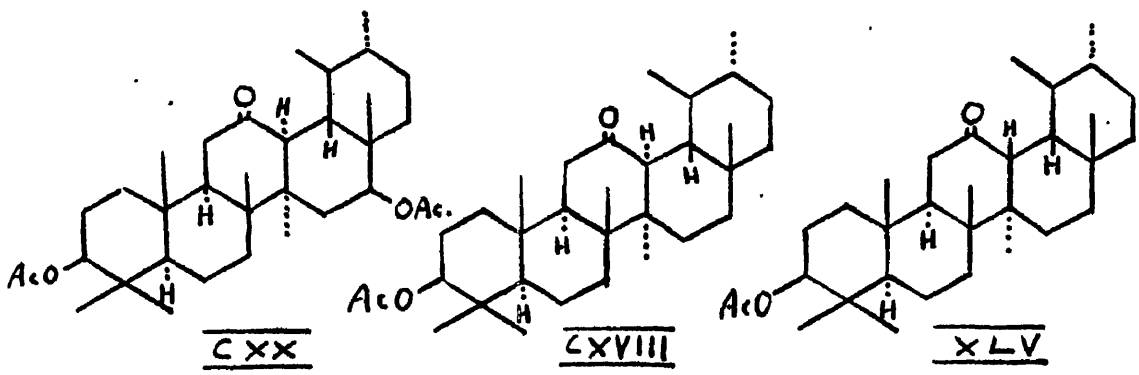
The projected synthesis of the triketone (CXXIV) from 12-oxo-13α-ursan-3β:16β-diol diacetate (CXX) failed in the first stage. Treatment of 12-oxo-13α-ursan-3β:16β-diol diacetate (CXX) with bromine in acetic acid gave the same uptake of bromine, as far as could be judged by the bleaching of the bromine solution, as was given by a control experiment on 12-oxo-13α-ursan-3β-yl acetate (CXVIII), but on heating to 100°, to effect dehydrobromination, a dark colour developed which did not appear in the control solution. On normal working up, a non-crystalline gum was obtained. The control experiment gave 12-oxours-9(11)-en-3β-yl acetate (LIII) in good yield. Chromatographic fractionation of the gum produced in this experiment, gave a high yield of

material, which showed maximal absorption at 2480 Å (ε 9,700), but which could not be obtained crystalline.

It seemed probable from these observations that a considerable amount of the desired $\alpha:\beta$-unsaturated ketone (CXXII) was being produced in the reaction, but that the product was so contaminated that it could not be obtained crystalline. As it was conceivable that this contamination occurred mainly in the attempted dehydrobromination of the intermediate α-bromo-ketone, it was decided to isolated the crude α-bromo-ketone and effect the dehydrobromination by refluxing with pyridine. The product from this reaction had similar physical and chromophoric properties to the gum obtained under acidic conditions, and, similarly, could not be obtained crystalline.

Attempts to form the $\alpha:\beta$-unsaturated ketone (CXXII) from 12-oxo-13α-ursan-3β:16β-diol diacetate (CXX) were discontinued at this stage, but it is of interest to note that treatment of the latter compound (CXX) with mineral acid gives rather anomolous results. 12-oxo-13α-ursan-3β:16β-diol diacetate (CXX) was treated with hydrochloric acid, under conditions which isomerise 12-oxo-13α-ursan-3β-yl acetate (CXVIII) to 12-oxoursan-3β-yl acetate (XLV)[151-153], and gave a

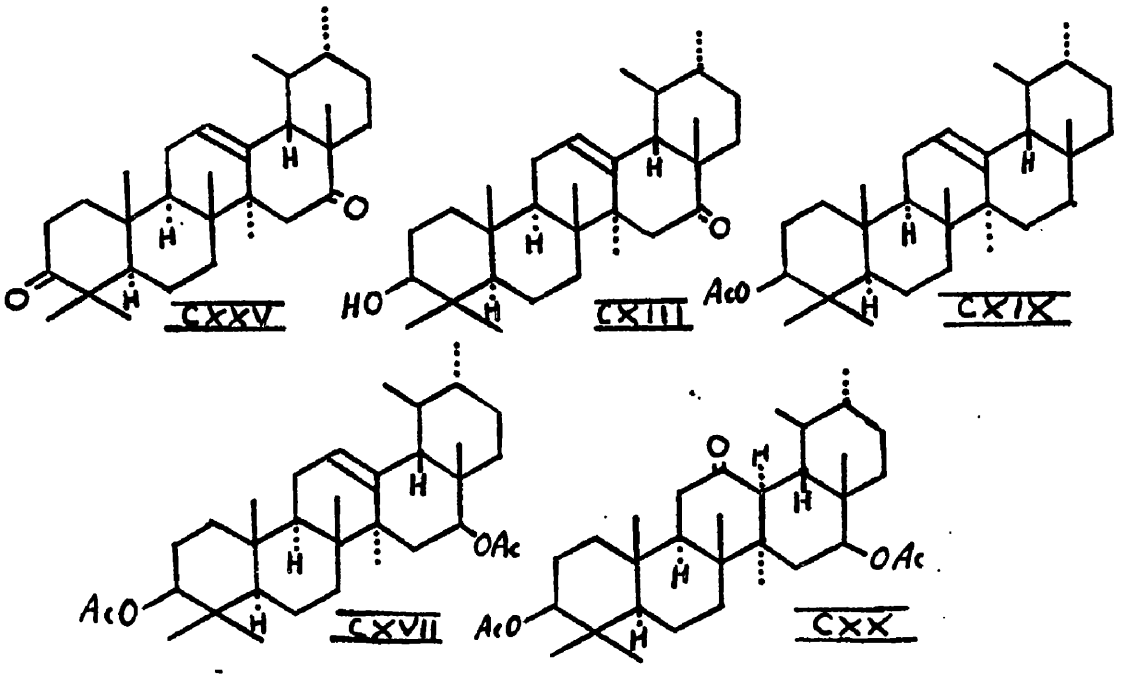
product, m.p. 192-199°, $[\alpha]_D$ +85°, which, due to the spread melting point, we took to be a mixture, although we could not alter the physical properties by attempted purification. On further acid treatment this product, m.p. 192-199°, gave a neutral substance, m.p. 259-260°, $[\alpha]_D$ +111°, which was transparent in the ultra-violet region between 2000 and 4000 Å. This product, m.p. 259-260°, appears to be richer in oxygen than the starting material. The infra-red spectrum of the product, m.p. 259-260°, includes bands at 1786, 905, 886, 876 and 847 $cm.^{-1}$, which do not appear in the spectrum of the starting material. The ketonic band, at 1709 $cm.^{-1}$, in the spectrum of the starting material does not appear in the spectrum of the product.



CXX CXVIII XLV

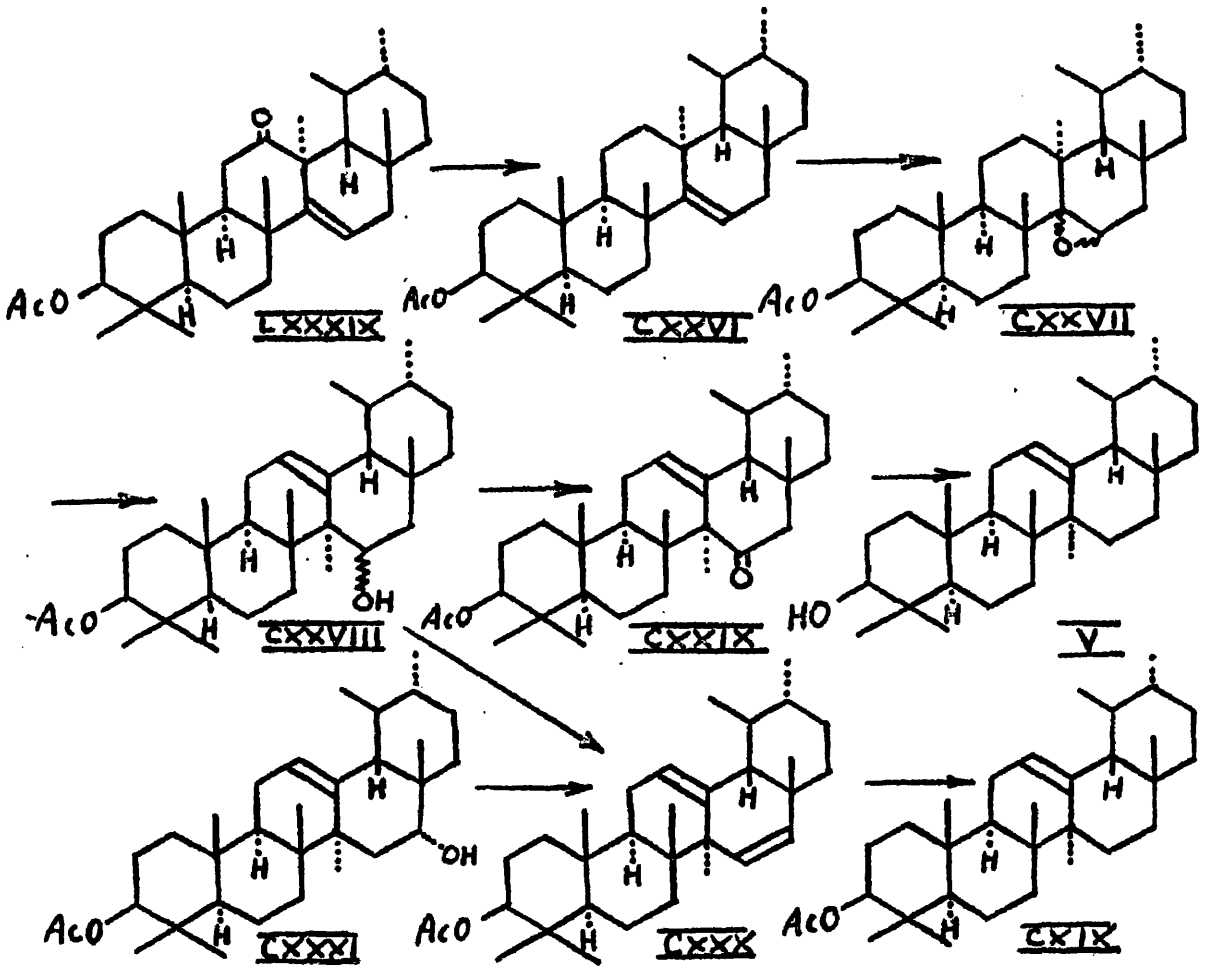
Under prolonged acid-treatment 12-oxo-13α-ursan-3β-yl acetate (CXVIII) is isomerised to 12-oxoursan-3β-yl acetate (XLV) and no further reaction takes

place. The instability of 12-oxo-13α-ursan-3β:16β-diol diacetate (CXX) and its consequent degradation to a compound, m.p. 259-260°, which, while we cannot formulate a structure which accounts both for its mode of formation and for its physical properties, is probably produced by a breakdown of the pentacyclic carbon-skeleton, must be due to some interaction between the 16-acetoxyl group and the 12-ketone group. It is pertinent to note, therefore, that Laird[32] observed that the absorption of the double-bond in breindione (CXXV) and breinonol-B (CXIII) in the ultra-violet region, compared with the absorption of the same chromophore in α-amyrin acetate (CXIX), indicates that there is some interaction between the $\Delta^{12:13}$ double-bond and the 16-ketone group in the brein derivatives. The reactivity of the $\Delta^{12:13}$ double-bonds in brein diacetate (CXVII) and α-amyrin acetate (CXIX) also differ, the former being less reactive than the latter[32], and it is, therefore, possible that the chemical properties of the reactive centre, $C_{(13)}$, in the acid-treatment of 12-oxo-13α-ursan-3β:16β-diol diacetate (CXX), are in some way affected by the presence of the 16-acetoxyl substituent.



The structure of brein (CXII) was finally established by the following method.

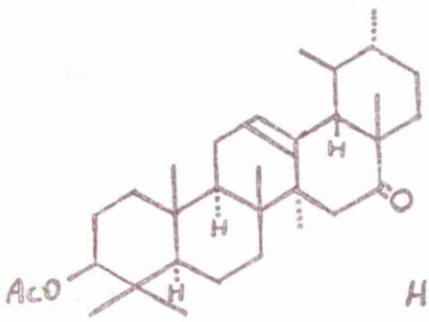
Wolff-Kishner reduction of 12-oxoisours-14-en-3β-yl acetate (LXXXIX), which can be synthesised from ∝-amyrin by an unambiguous route[31], gives isours-14-en-3β-yl acetate (CXXVI)[32]. The yield in this reaction was found to be much improved on modifying the reaction technique. Oxidation of isours-14-en-3β-yl acetate (CXXVI) with perbenzoic acid gives 14ξ:15ξ-epoxyisoursan-3β-yl acetate (CXXVII)[32], which on treatment with mineral acid gives urs-12-en-3β:15ξ-diol 3-acetate (CXXVIII)[32]. Oxidation of the last compound (CXXVIII) gives 15-oxours-12-en-3β-yl acetate (CXXIX), which yields ∝-amyrin (V) on Wolff-Kishner reduction[32].



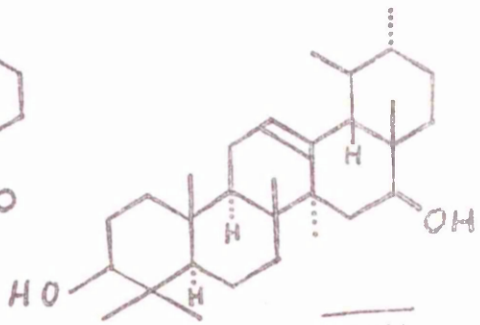
Dehydration of urs-12-en-3β:15ξ-diol 3-acetate (CXXVIII) with phosphorus oxychloride gave ursa-12:15-dien-3β-yl acetate (CXXX) which proved to be identical in m.p., mixed m.p., optical rotation, infra-red and ultra-violet absorption spectra, and analysis, with the dienyl acetate, m.p. 228-9°, $[\alpha]_D$ +40°, prepared by Laird[32] by dehydration of brein monoacetate-11 (CXXXI) Hydrogenation of ursa-12:15-dien-3β-yl acetate (CXXX) gave α-amyrin acetate (CXIX), proving that no rearrangement of the carbon-skeleton took place during

the dehydration of urs-12-en-3β:15ξ-diol 3-acetate (CXXVIII)

As 15-oxours-12-en-3β-yl acetate (CXXIX) is not identical with breinonol-b acetate (CXXXII), the x-oxygen function in brein derivatives cannot be located at $C_{(15)}$ and must, to account for the dehydration of brein monoacetate-II (CXXXI) to ursa-12:15-dien-3β-yl acetate (CXXX), be located at $C_{(16)}$. As has been mentioned previously (p.60) Laird[32] showed the x-hydroxyl group in brein to be equatorial, and so the structure of brein may be fully expressed as 3β:16β-dihydroxyurs-12-ene (CXII).



CXXXII



CXII

EXPERIMENTAL

Melting points were determined in capillary tubes (1 mm. bore) using a standard N. P. L. thermometer.

Specific rotations were measured in chloroform solution in a 1 dm. tube at approximately 15°.

Ultra-violet spectra were determine in ethanol solution, unless otherwise stated.

Infra-red spectra were determined in nujol mull.

The phrase 'normal working up' implies, in general, dilution with water, extraction with ether, washing consecutively with aqueous hydrochloric acid (5%), water, saturated aqueous sodium bicarbonate, and water, followed by drying of the ethereal extract with anhydrous sodium sulphate, filtration, and evaporation to dryness under reduced pressure.

The term 'petrol' signifies the light petroleum fraction, boiling point 60-80°.

For chromatography, Brockman Grade II alumina and solvents dried over sodium wire were used, except where otherwise stated.

EXPERIMENTAL

Section I

Selenium Dioxide Oxidation of 12-Oxours-9(11)-en-3β-yl Acetate : 12-Oxoisoursa-9(11):14-dien-3β-yl Acetate . - 12-Oxours-9(11)-en-3β-yl acetate (42 g.) in stabilised acetic acid (700 c.c.) was refluxed with selenium dioxide (65 g.) for 1 hr. Normal working up gave a solid, which was dissolved in petrol-benzene (9:1, 700 c.c.) and adsorbed on a column of alumina (1.5 kg.). Elution with petrol-benzene (1:1, 2500 c.c.) gave 12-oxoisoursa-9(11):14-dien-3β-yl acetate (27 g.) as needles (from chloroform-methanol), m.p. 220°, $[\alpha]_D$ +7.5° (c, 1.7) ; $\lambda\lambda_{max.}$ 2100 Å and 2360 Å (ε 7,000 and 12,000).

Literature[114, 118] gives m.p. 221-222°, $[\alpha]_D$ +7°, for this compound.

12-Oxoisooolean-14-en-3β-ol . - 12-Oxoisooleana-9(11):14-dien-3β-yl acetate (2 g.) in ether (75 c.c.) was added dropwise to a stirred solution of lithium (600 mg.) in liquid ammonia (400 c.c.) over 2 min. and stirring was continued for a further 2 min., when acetone (17 c.c.) was added. The ammonia was allowed to evaporate overnight and normal working up gave a

gum (2.1 g.) which was dissolved in boiling light petroleum (b.p. 80-100°, 150 c.c.). The solid which separated on cooling was crystallised from aqueous acetone to give 12-oxoisoolean-14-en-3β-ol (755 mg.) as needles, m.p. 285-286°, $[\alpha]_D$ -43° (c, 0.9) ; ε at 2040 Å = 5900 (Found : C, 81.6 ; H, 11.2 . Calc. for $C_{30}H_{48}O_2$: C, 81.8 ; H, 11.0%).

Literature[115] gives m.p. 276-278°, $[\alpha]_D$ -43°, for this compound.

12-Oxoisoolean-14-en-3 -yl Acetate . - 12-Oxoisoolean -14-en-3β-ol (700 mg.) was heated on a steam bath for 1 hr. with acetic anhydride (7 c.c.) and pyridine (7 c.c.). Normal working up gave a solid which crystallised from chloroform-methanol to give 12-oxoisoolean-14-en-3β-yl acetate (670 mg.) as needles, m.p. 297-300°, $[\alpha]_D$ -29° (c, 1.1) ; ε at 2040 Å = 4500.

Literature[115] gives m.p. 298-300°, $[\alpha]_D$ -30°, for this compound.

Treatment of 12-Oxoisoolean-14-en-3β-yl Acetate with Hydrochloric Acid . - 12-Oxoisoolean-14-en-3β-yl acetate (200 mg.) in glacial acetic acid (50 c.c.) and chloroform (10 c.c.) was treated with a slow stream of dry hydrogen chloride for 1 hr. and kept in a stoppered

flask at room temperature for 3 days. Evaporation to dryness under reduced pressure gave a solid (187 mg.) which crystallised from chloroform-methanol as needles, m.p. 298°, undepressed in m.p. on admixture with a sample of starting material. The ultra-violet spectrum of the product was identical with that of the starting material.

12:15-Dioxo-13:27-cyclours-9(11)-en-3β-yl Acetate (Ketoacetate, $C_{32}H_{46}O_4$) ; (a) A refluxing solution of 12-oxours-9(11)-en-3β-yl acetate (5 g.) in stabilised acetic acid (600 c.c.) was treated dropwise over 30 min. with chromium trioxide (5 g.) in stabilised acetic acid (90 c.c.) and refluxed for a further 1 hr. Normal working up gave a solid (4.6 g.) which crystallised from chloroform-methanol to give 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate as needles, m.p. 320-321°, $[\alpha]_D$ +93° (c, 0.9); λ_{max} 2360 Å (ε 12,000) (Found : C 77.8 ; H, 9.6 . Calc. for $C_{32}H_{46}O_4$: C, 77.7 ; H, 9.4%).

Literature[116] gives m.p. 321-324°, $[\alpha]_D$ +103°, for this compound.

(b) A refluxing solution of 12-oxoisoursa-9(11):14-dien-3β-yl acetate (5 g.) in stabilised acetic acid (600 c.c.) was treated dropwise over 30 min. with a solution of chromium trioxide (5 g.) in stabilised acetic acid (45 c.c) and water (5 c.c.), and refluxing continued for a further 30 min. Normal working up gave a solid (4.5 g.) which crystallised from chloroform-methanol to give 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate as needles, m.p. and mixed m.p. 320-322°, $[\alpha]_D$ +92° (c, 1.1); $\lambda_{max.}$ 2360 Å (ε 11,500).

(c) A refluxing solution of 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (7 g.) in stabilised acetic acid (750 c.c.) was treated dropwise over 45 min. with a solution of chromium trioxide (7 g.) in stabilised acetic acid (50 c.c.) and water (7 c.c.) and refluxing was continued for a further 1 hr. Normal working up gave a solid (6 g.) which crystallised from chloroform-methanol to give 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate as needles, m.p. and mixed m.p. 321-322°, $[\alpha]_D$ +89°, +92° (c, 0.8 1.0) ; $\lambda_{max.}$ 2360 Å (ε 11,700).

12:15-Dioxo-13:27-cyclours-9(11)-en-3β-ol . - 12:15-Dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (100 mg.) in methanol (10 c.c.) was refluxed with potassium hydroxide (100 mg.) for 2 hr. Normal working up gave a gum which crystallised from aqueous acetone to give 12:15-dioxo-13:27-cyclours-9(11)-en-3β-ol (86 mg.) as needles, m.p. 233-234°, $[\alpha]_D$ +76°, +75° (c, 1.0, 1.1) ; $\lambda_{max.}$ 2360 Å (ε 12,250) (Found : C, 79.9, 79.3 ; H, 9.6, 10.0 . $C_{30}H_{44}O_3$ requires C, 79.6 ; H, 9.8%)

12:15-Dioxo-13:27-cyclours-9(11)-en-3β-ol (74 mg.) was heated at 100° for 1 hr. with acetic anhydride (7 c.c.) and pyridine (7 c.c.). Normal working up gave a solid which crystallised from chloroform-methanol to give 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (67 mg.) as needles, m.p. and mixed m.p. 321-322°, $[\alpha]_D$ +92° (c, 1.3) ; $\lambda_{max.}$ 2340 Å (ε 12,250).

Oxidation of 12-Oxoisoursa-9(11):14-dien-3β-yl Acetate with Hydrogen Peroxide ; 12-Oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol 3-Acetate (Keto-diol monoacetate, $C_{32}H_{48}O_4$) . - 12-oxoisoursa-9(11):14-dien-3β-yl acetate (2 g.) in glacial acetic acid (300 c.c.) was treated at 95° over 2 hr. with hydrogen peroxide (200 c.c.)

in glacial acetic acid (200 c.c.) and the solution stirred for a further 3 hr. at the same temperature. Normal working up gave a gum which was dissolved in benzene (50 c.c.) and adsorbed on a column of alumina (170 g.). Elution with benzene (2700 c.c.) gave a solid (500 mg.) which was dissolved in boiling petroleum ether (b.p. 80-100 , 30 c.c.). The solid which separated from this solution on cooling was crystallised from aqueous methanol to give 12-oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol 3-acetate (130 mg.) as needles, m.p. 262-265°, $[\alpha]_D$ +156°, +155° (c, 1.3, 1.8) ; $\lambda_{max.}$ 2360 Å (ε 11,600) (Found : C, 77.7 ; H 9.8 . $C_{32}H_{48}O_4$ requires C, 77.4 ; H 9.7%). This compound gives no colour with tetranitromethane in chloroform.

The petroleum ether solution was adsorbed on a column of alumina (7 g.). Elution with petrol-benzene (3:2, 900 c.c.) gave a gum which crystallised from methanol to give 14:15-epoxy-12-oxoisours-9(11)-en-3β-yl acetate as needles, m.p. 281-283°, $[\alpha]_D$ +56° (c, 1.2)

Literature[118] gives m.p. 280-283°, $[\alpha]_D$ +56 , for this compound.

12-Oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol Diacetate . - 12-Oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol 3-acetate (80 mg.) was heated on a steam-bath for 1 hr. with acetic anhydride (10 c.c.) and pyridine (10 c.c.). Normal working up through ether gave a solid (82 mg.) which crystallised from chloroform-methanol to give 12-oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol diacetate as needles, m.p. 148°, $[\alpha]_D$ +123°, +123° (c, 1.2, 1.4) ; $\lambda_{max.}$ 2360 A (ϵ 11,400) (Found : C, 75.65 ; H, 9.5 . $C_{34}H_{50}O_5$ requires C, 75.8 ; H, 9.4%).

Oxidation of 12-Oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol 3-Acetate with Chromium Trioxide : 12:15-Dioxo-13:27-cyclours-9(11)-en-3β-yl Acetate . - 12-Oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol 3-acetate (148 mg.) in pyridine (3 c.c.) was added to chromium trioxide (150 mg.) - pyridine (1.5 c.c.) complex and the mixture left at room temperature for 8 hr. Normal working up gave a product (110 mg.) which crystallised from chloroform-methanol to give 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate as needles, m.p. and mixed m.p. 320-321°, $[\alpha]_D$ +90.5° (c, 1.1), identical in infra-red spectrum with a sample prepared by the oxidation of

12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate with chromic acid.

Catalytic Hydrogenolysis of 12:15-Dioxo-13:27-cyclours-9(11)-en-3β-yl Acetate ; Acetate $C_{32}H_{50-52}O_2$. - 12:15-Dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (400 mg.) in glacial acetic acid (100 c.c.) was added to a suspension of platinum (from 200 mg. platinum oxide) in acetic acid (20 c.c.) and the mixture shaken with hydrogen at atmospheric pressure for 36 hr. Evaporation of the filtered solution to dryness under reduced pressure gave a solid, which crystallised from chloroform-methanol to give an acetate (230 mg.) as blades, m.p. 208-210°, $[\alpha]_D$ +35°, +34°, +33° (c, 1.6, 1.5 ,1.0) ; ε at 2080 Å = 7,500 (Found : C, 82.1 ; H 11.0 . $C_{32}H_{50}O_2$ requires C, 82.3; H, 10.8 . $C_{32}H_{52}O_2$ requires C, 82.0 ; H, 11.2%). This compound gives an orange colour with tetranitromethane in chloroform.

Treatment of the Acetate, $C_{32}H_{50-52}O_2$, m.p. 208-210°, with Hydrochloric Acid . - The acetate, m.p. 208-210°, (85 mg.) obtained in the

previous experiment, in acetic acid (45 c.c.) was treated with concentrated hydrochloric acid (3.8 c.c.) and heated on a steam-bath for 5 hr. Normal working up gave a gum which was dissolved in petrol (15 c.c.) and adsorbed on a column of alumina (3.5 g.). Elution with petrol (200 c.c.) gave a solid which crystallised from chloroform-methanol as blades (45 mg.) m.p. 207-210°, $[\alpha]_D$ +34° (c, 1.2), which showed no depression in m.p. on admixture with a sample of starting material.

Attempted Hydrolysis of the Acetate, $C_{32}H_{50-52}O_2$, m.p. 208-210°. - Acetate, m.p. 208-210°, (70 mg.) in methanol (7 c.c.) and potassium hydroxide (70 mg.) was refluxed for 1 hr. Normal working up gave a gum (67 mg.) which could not be crystallised, even after chromatography, but which on acetylation with acetic anhydride (5 c.c.) and pyridine (5 c.c.) at 100° gave a solid which crystallised from chloroform-methanol as blades, m.p. 207-210°, $[\alpha]_D$ +33° (c, 0.7), which showed no depression in m.p. on admixture with a sample of the starting material.

Wolff-Kishner Reduction of 12-Oxo-13:27-cyclours-9(11)-en-3β-yl Acetate . - 12-Oxo-13:27-cyclours-9(11)-en-3β-yl acetate (1.5 g.) was added to a solution of sodium (2 g.) in diethylene glycol (110 c.c.) and the mixture heated to 180°. Anhydrous hydrazine was distilled into the mixture in a stream of nitrogen until it refluxed gently at 180°and refluxing was continued at this temperature for 18 hr. The mixture was distilled until the temperature rose to 215° and refluxing was continued at this temperature for a further 22 hr. Normal working up gave a gum which was acetylated with acetic anhydride (15 c.c.) and pyridine (15 c.c.) at room temperature overnight. Normal working up of the acetylation gave a product (1.2 g.) which was dissolved in petrol-benzene (9:1, 110 c.c.) and adsorbed on a column of alumina (35 g.). Elution with petrol-benzene (9:1, 600 c.c. ; 4:1, 500 c.c. ; 2:1, 1000 c.c.) gave a gum which crystallised from chloroform-methanol to give 13:27-cyclours-9(11)-en-3β-yl acetate as blades, m.p. 250-251°, $[\alpha]_D$ +90° (c, 1.0) ; ε at 2060 Å = 5,300 .

Literature[119] gives m.p. 253-256°, $[\alpha]_D$ +88°, for this compound.

Wolff-Kishner Reduction of 12:15-Dioxo-13:27-cyclours-9(11)-en-3β-yl Acetate : Acetate $C_{32}H_{46-50}O_3$. - A solution of sodium (1.0 g.) in diethylene glycol (50 c.c.) was heated to 180° and anhydrous hydrazine added by distillation in a stream of nitrogen until the mixture refluxed at this temperature. 12:15-Dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (650 mg.) was added to the cooled solution which was then refluxed for 18 hr. The solution was then distilled until the boiling point rose to 218° and refluxed at this temperature for a further 24 hr.

Normal working up through chloroform-ether (2:1) gave a product (640 mg.) which was acetylated with pyridine (10 c.c.) and acetic anhydride (10 c.c.) on a steam-bath for 2 hr. Normal working up of the acetylation gave a product which was dissolved in petrol (120 c.c.) and adsorbed on a column of alumina (16 g.). Elution with petrol (600 c.c.) gave a gum (203 mg.) which was dissolved in petrol and adsorbed on a column of alumina (9 g.) from which elution with the same solvent gave a solid (162 mg.) which crystallised from chloroform-methanol to give an acetate

as blades, m.p. 210-212°, $[\alpha]_D$ +33° (c, 1.1), undepressed in melting point on admixture with a sample of the acetate, $C_{32}H_{50-52}O_2$, m.p. 208-210°, obtained by hydrogenolysis of the starting material.

Elution of the original column with petrol (400 c.c.) gave a solid which crystallised from chloroform-methanol to give 12:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (40 mg.) as needles, m.p. and mixed m.p. (with starting material) 320-322°.

Elution with petrol-benzene (9:1, 1500 c.c. ; 3:1, 800 c.c.) gave a solid (210 mg.) which was dissolved in petrol (10 c.c.) and adsorbed on a column of alumina (7 g.). Elution of this column with petrol-benzene (4:1, 2000 c.c.) gave a solid which crystallised from chloroform-methanol to give an acetate as needles, m.p. 254-256°, $[\alpha]_D$ -7° (c, 1.1) ; ε at 2070 Å = 5,250 .(Found : C, 79.9 ; H, 10.4 . $C_{32}H_{48}O_3$ requires C, 79.95 ; H, 10.1 . $C_{32}H_{50}O_3$ requires C, 79.6 ; H, 10.4%). This compound gives an orange colour with tetranitromethane in chloroform.

Treatment of the Acetate, $C_{32}H_{48-50}O_3$, m.p. 254-256°, with Hydrochloric Acid . - The acetate, m.p.

254-256°, obtained in the previous reaction (70 mg.), in acetic acid (40 c.c.) was treated with concentrated hydrochloric acid (3.4 c.c.) and heated on a steam-bath for 5 hr. The product, obtained by normal working up, was dissolved in petrol and adsorbed on a column of alumina (2 g.). Elution with petrol-benzene (1:1, 750 c.c.) gave a solid which crystallised from methanol as needles (47 mg.), m.p. 253-256°, undepressed in m.p. on admixture with starting material.

Hydrolysis of the Acetate, $C_{32}H_{48-50}O_3$, m.p. 254-256°. - The acetate, m.p. 254-256°, (67 mg.) was refluxed with potassium hydroxide (70 mg.) in methanol (45 c.c.) for 1 hr. Normal working up gave a gum (60 mg.) which crystallised from petrol to give an alcohol as needles, m.p. 197-198°, $[\alpha]_D$ -13° (c, 0.7) (Found :C, 82.05 ;H, 10.8 . $C_{30}H_{46}O_2$ requires C, 82.1 ; H, 10.6 . $C_{30}H_{48}O_2$ requires C, 81.8 ; H, 11.0%).

14:15-Epoxy-12-oxoisoursa-9(11)-en-3β-yl Acetate . - 12-Oxoisoursa-9(11):14-dien-3β-yl acetate (1 g.) in stabilised acetic acid (300 c.c.) was treated at room temperature over 30 min. with stirring with

potassium permanganate (270 mg.) in water (40 c.c.) and stirring continued for a further 30 min. Normal working up gave a solid which crystallised from chloroform-methanol to give 14:15-epoxy-12-oxoisours-9(11)-en-3β-yl acetate (810 mg.) as blades, m.p. 280-283°, $[\alpha]_D$ +57° (c, 0.9).

Literature[118] gives m.p. 280-283°, $[\alpha]_D$ +56°, for this compound.

Treatment of 14:15-Epoxy-12-oxoisours-9(11)-en-3β-yl Acetate with Mineral Acid : (a) with Hydrochloric Acid in Methanol - 14:15-Epoxy-12-oxoisours-9(11)-en-3β-yl acetate (75 mg.) in methanol (45 c.c.) and chloroform (20 c.c.) was treated with concentrated hydrochloric acid (2 c.c.) and water (2 c.c.) and allowed to stand at room temperature for 18 hr. Evaporation to dryness under reduced pressure gave a gum which crystallised from aqueous acetone-methanol to give 12-oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol 3-acetate (45 mg.) as needles, m.p. 262-263°, $[\alpha]_D$ +157° (c, 1.1), undepressed in m.p. on admixture with a sample of the same compound obtained by oxidation of 12-oxoisoursa-9(11):14-dien-3β-yl acetate with

hydrogen peroxide.

(b) with concentrated Hydrochloric Acid in Acetic Acid :Chloro-acetate, $C_{32}H_{47}O_3Cl$, m.p. 210°. - 14:15-Epoxy-12-oxoisours-9(11)-en-3β-yl acetate (600 mg.) in stabilised acetic acid (50 c.c.) and chloroform (10 c.c.) was kept at 50-60° with concentrated hydrochloric acid (5 c.c.) for 2 hr. Normal working up through chloroform gave a gum which was dissolved in boiling petrol (20 c.c.) and the solution allowed to cool overnight. The solid which separated out was crystallised from chloroform-methanol to give a substance (65 mg.) as needles, m.p. 294-296°, $[\alpha]_D$ +101° (c, 1.3) ; $\lambda_{max.}$ 2360 Å (ε 12,000) (Found : C, 77.0 ; H, 9.5 ; Cl, 2.3 . $C_{32}H_{47}O_3Cl.2C_{32}H_{46}O_4$ * requires C, 76.65 ; H, 9.3 ; Cl, 2.4 %).

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*We are of the opinion that this substance is a mixed-crystal, c.f. p.48.
Reduction of this substance, m.p. 294-296°,(350 mg.) with activated zinc dust (4g.) in refluxing ether (50 c.c.) -methanol (50 c.c.) for 5 hr., gave 12;:15-dioxo-13:27-cyclours-9(11)-en-3β-yl acetate (200 mg.) after normal working up and crystallisation from chloroform-methanol.

The petrol solution was evaporated to dryness under reduced pressure and gave a solid (370 mg.) which crystallised from aqueous acetone-methanol to give a chloro-acetate as needles, m.p. 210°, $[\alpha]_D$ +122° (c, 1.0); $\lambda_{max.}$ 2390 Å (ε 11,000) (Found: C, 74.6 ; H, 9.25 ; Cl 6.6 . $C_{32}H_{47}O_3Cl$ requires C, 74.6 ; H, 9.2 ; Cl, 6.9%). This compound gives no colour with tetranitromethane in chloroform.

Treatment of the Chloro-acetate, $C_{32}H_{47}O_3Cl$, m.p. 210°, with Activated Zinc* in Ether-methanol . - The chloro-acetate, m.p. 210°, (500 mg.) in ether (75 c.c.) and methanol (75 c.c.) was refluxed with freshly activated zinc dust (5 g.) for 3½ hr., allowed to cool overnight, and filtered through kieselguhr. Normal working up gave a solid (650 mg.) which was dissolved in benzene (200 c.c.) and adsorbed on a column of alumina (15 g.). Elution with benzene (800 c.c.)

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* Activated zinc was prepared by heating zinc dust with aqueous ammonium chloride (10%) on a steam-bath for 30 min., followed by washing by decantation with warm distilled water.

gave a gum (450 mg.) which crystallised from chloroform-methanol to give an acetate as needles, m.p. 241-242°, $[\alpha]_D$ ±0°, ±0° (c, 1.1, 1.6) ; $\lambda\lambda_{max.}$ 2400 Å and at 2070 Å (ε 10,150 and 7,300)(Found C, 79.9 ; H, 10.4 . $C_{32}H_{48}O_3$ requires C, 79.95 ; H, 10.1%). This compound gives a yellow colour with tetranitromethane in chloroform.

Concentration of the mother-liquors of this crystallisation gave 12-oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol 3-acetate (60 mg.), m.p. and mixed m.p. 261-264°, $[\alpha]_D$ +157° (c, 1.7). This compound gives a large depression in m.p. when mixed with a sample of 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate, m.p. 269-271°, $[\alpha]_D$ +157° .111, 109.

Treatment of the Acetate, $C_{32}H_{48}O_3$, m.p. 241-242°, with Mineral Acid . - The acetate (51 mg.), m.p. 241-242°, obtained in the previous experiment, in glacial acetic acid (13 c.c.) and chloroform (3 c.c.) was treated with a slow stream of dry hydrogen chloride for 30 min. and kept in a stoppered flask for 60 hr. Evaporation to dryness under reduced pressure gave a solid which crystallised from chloroform-methanol to

give 12-oxo-13:27-cyclours-9(11)-en-3β-yl acetate (43 mg.) as needles, m.p. and mixed m.p.(with an authentic specimen) 268-269°, $[\alpha]_D$ +154° (c, 1.4) ; λ_{max} 2360 Å (ε 10,500). The product melted at 245-258 when mixed with a sample of 12-oxo-13:27-cyclours-9(11)-en-3β:15ξ-diol 3-acetate.

14:15-Epoxy-12-oxoisoolean-9(11)-en-3β-yl Acetate . - A stirred solution of 12-oxoisooleana-9(11):14-dien-3β-yl acetate (30 g.) in stabilised acetic acid (2.5 litre) was treated dropwise over 30 min. at room temperature with a solution of potassium permanganate (7.92 g.) in water (500 c.c.) and stirring was continued for a further 30 min. Normal working up gave a solid which crystallised from chloroform-methanol to give 14:15-epoxy-12-oxoisoolean-9(11)-en-3β-yl acetate (24 g.) as blades, m.p. 280-283°, $[\alpha]_D$ -15° (c, 1.7).

Literature[111] gives m.p. 281-282°, $[\alpha]_D$ -12.5°, for this compound.

Treatment of 14:15-Epoxy-12-oxoisoolean-9(11)-en-3β-yl Acetate with Hydrochloric Acid : Chloro-acetate, $C_{32}H_{47}O_3Cl$, m.p. 248°. - 14:15-Epoxy-12-oxoisoolean-9(11)-en-3β-yl acetate (15 g.) in chloroform (250 c.c.)

and acetic acid (625 c.c.) was kept at 55-60° with concentrated hydrochloric acid (62 c.c.) for 2 hr. Normal working up gave a gum which crystallised from chloroform-methanol to give a chloro-acetate as needles, m.p. 248°, $[\alpha]_D$ +146° (c, 1.5) ; $\lambda_{max.}$ 2400 Å (ε 9,900)(Found : C, 74.8 ; H, 9.5 ; Cl, 6.9 . $C_{32}H_{47}O_3Cl$ requires C, 74.6 ; H, 9.2 ; Cl, 6.9%). This compound gives no colour with tetranitromethane in chloroform.

Treatment of the Chloro-acetate, $C_{32}H_{47}O_3Cl$, m.p. 248°, with Activated Zinc* in Ether-Methanol . - The chloro-acetate (7 g.), m.p. 248°, obtained in the previous experiment, in ether (500 c.c.) and methanol (500 c.c.) was refluxed with activated zinc (70 g.) for 5 hr. The reaction mixture was allowed to cool overnight and filtered through kieselguhr. Normal working up gave a solid (9 g.) which was dissolved in benzene (200 c.c.) and adsorbed on a column of alumina (250 g.). Elution with benzene (1500 c.c.) gave a gum (5.2 g.) which crystallised from chloroform-methanol to give an acetate as plates, m.p. 207-209°,

*See page 93.

$[\alpha]_D$ -19°, -20° (c, 1.5, 1.4) ; $\lambda\lambda_{max.}$ 2400 Å and at 2070 Å (ε 9,700 and 6,200)(Found : C, 78.5 ; H, 9.8 . $C_{32}H_{48}O_3.\frac{1}{2}CH_3OH$ requires C, 78.6 ; H, 9.8%). This compound gives a yellow colour with tetranitromethane in chloroform.

Concentration of the mother-liquors of the above crystallisation gave 12-oxo-13:27-cycloolean-9(11)-en-3β:15ξ-diol 3-acetate, m.p. 315-319°, $[\alpha]_D$ +156° (c, 1.3) ; $\lambda_{max.}$ 2380 Å (ε 10,800).

Literature[111] gives m.p. 318-319°, $[\alpha]_D$ +155°, for this compound.

Treatment of the Acetate, $C_{32}H_{48}O_3$, m.p. 207-209°, with Mineral Acid. - The acetate (200 mg.), m.p. 207-209°, obtained in the previous reaction, in chloroform (10 c.c.) and glacial acetic acid (50 c.c.) was treated with a slow stream of dry hydrogen chloride for 45 min. and kept in a stoppered flask for 18 hr. Evaporation to dryness under reduced pressure gave a gum which crystallised from chloroform-methanol to give an acetate as needles, m.p. 275-276°, $[\alpha]_D$ +170°, +172° (c, 1.1, 1.3) ;

$\lambda_{max.}$ 2360 Å (ε 11,900)(Found : C, 79.8 ; H, 10.2 . $C_{32}H_{48}O_3$ requires C, 79.95 ; H, 10.1%). This compound gives no colour with tetranitromethane in chloroform.

Oxidation of 12-Oxoisours-14-en-3β-yl Acetate : (a) with Potassium Permanganate - 12-Oxoisours-14-en-3β-yl acetate (500 mg.) in stabilised acetic acid (200 c.c.) was treated at room temperature with stirring with a solution of potassium permanganate (135 mg.) in water (20 c.c.), added dropwise over 30 min., and stirring was continued for a further 30 min. Normal working up gave a solid which was dissolved in petrol-benzene (4:1, 110 c.c.) and adsorbed on a column of alumina (20 g.). Elution with the same solvent mixture gave a gum (300 mg.) which crystallised from chloroform-methanol to give 14:15-epoxy-12-oxoisoursan-3β-yl acetate as needles, m.p. 239°, $[\alpha]_D$ +38°, +38°, +37° (c, 0.9, 1.3, 0.4) ; $\lambda_{max.}$ 2800 Å (ε 110), ε at 2020 Å = 1090 ;(Found : C, 77.0 ; H, 10.4 . $C_{32}H_{50}O_4$ requires C, 77.1 ; H, 10.1%)

Elution with petrol-benzene (1:1, 500 c.c. ; 1:2, 500 c.c. ; 1:4, 250 c.c.) gave a solid (216 mg.) which was dissolved in petrol-benzene (7:3, 25 c.c.)

and adsorbed on a column of alumina (7 g.), from which the same solvent mixture eluted a solid which crystallised from aqueous acetone to give 12:15-dioxo-13:27-cycloursan-3β-yl acetate as needles, m.p. 347-349°, $[\alpha]_D$ +40°,+40.5° (c, 1.0, 0.5) ; $\lambda_{max.}$ 2150 Å (ε 5,700) (Found : C, 77.4 ; H, 10.0. $C_{32}H_{48}O_4$ requires C, 77.4 ; H, 9.8%). This compound gives no colour with tetranitromethane in chloroform.

(b) with Hydrogen Peroxide - A stirred solution of 12-oxoisours-14-en-3β-yl acetate (500 mg.) in glacial acetic acid (75 c.c.) at 95° was treated with a mixture of hydrogen peroxide (50 c.c.) and glacial acetic acid (50 c.c.), added dropwise over 2 hr., and the mixture was stirred for a further 3 hr. at the same temperature. Normal working up gave a gum (350 mg.) which was dissolved in petrol-benzene (1:1, 30 c.c.) and adsorbed on a column of alumina (12 g.). Elution with petrol-benzene (1:1, 900 c.c.) gave a gum (78 mg.) which crystallised from chloroform-methanol to give 12-oxo-13:27-cycloursan-3β:15ξ-diol diacetate as plates, m.p. 252-254°, $[\alpha]_D$ +40° (c, 1.5) ; λ_{max} 2800 Å and 2100 Å (ε 100

and 5,000)(Found : C, 75.8 ; H, 9.7 . $C_{34}H_{52}O_5$ requires C, 75.5 ; H, 9.7%). This compound gives no colour with tetranitromethane in chloroform.

Elution with petrol-benzene (1:4, 600 c.c.) gave a gum (90 mg.) which crystallised from aqueous acetone to give 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate as blades, m.p. 295°, $[\alpha]_D$ +53° (c, 2.4) ; $\lambda_{max.}$ 2100 Å (ε 5,500)(Found : C, 77.3 ; H, 10.1 . $C_{32}H_{50}O_4$ requires C, 77.1 ; H, 10.1%). This compound gives no colour with tetranitromethane in chloroform.

Elution with benzene, ether and methanol, gave fractions (190 mg.) which could not be crystallised.

(c) with Perbenzoic Acid - 12-Oxoisours-14-en-3β-yl acetate (650 mg.) was treated at room temperature with a solution of perbenzoic acid (275 mg.) in chloroform (25 c.c.) for 24 hr. The solution was washed with saturated aqueous sodium bicarbonate and water and dried over anhydrous sodium sulphate. Evaporation to dryness under reduced pressure gave a solid which was dissolved in petrol-benzene (7:3, 100 c.c.) and adsorbed on alumina (10 g.). Elution with petrol-benzene (7:3, 150 c.c.; 1:1, 150 c.c.; 3:7, 150 c.c.) gave a solid (200 mg.) which

crystallised from chloroform-methanol as needles, m.p. 219-220°, $[\alpha]_D$ -24° (c, 1.1), undepressed in m.p. on admixture with a specimen of starting material.

Elution with petrol-benzene (1:9, 150 c.c.) and benzene (150 c.c.) gave a gum which crystallised from chloroform-methanol to give 14:15-epoxy-12-oxoisoursan-3β-yl acetate as needles, m.p. and mixed m.p. (with a sample from the potassium permanganate oxidation) 238°, $[\alpha]_D$ +38° (c, 1.3).

Further elution with benzene (150 c.c.) gave a gum (23 mg.) which crystallised from methanol to give 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate as blades, m.p. and mixed m.p. (with a sample from the hydrogen peroxide oxidation) 294°.

14:15-Epoxy-12-oxoisoursan-3β-ol . - 14:15-Epoxy-12-oxoisoursan-3β-yl acetate (100 mg.) was refluxed with methanolic potassium hydroxide (5%, 5 c.c.) for 1 hr. Normal working up gave a gum which crystallised from aqueous acetone to give 14:15-epoxy-12-oxo-isoursan-3β-ol (70 mg.) as needles, m.p. 228-230°, $[\alpha]_D$ +30° (c, 0.9) ; $\lambda_{max.}$ 2800 Å (ϵ 100), ϵ at 2020 Å = 960 (Found : C, 79.1 ; H, 10.8 .

$C_{30}H_{48}O_3$ requires C, 78.9 ; H, 10.6%).

Acetylation of 14:15-epoxy-12-oxoisoursan-3β-ol (25 mg.) with pyridine (1 c.c.) and acetic anhydride (1 c.c.) at 95° for 1 hr. gave 14:15-epoxy-12-oxoisoursan-3β-yl acetate as needles (from chloroform-methanol), m.p. 238-239°, $[\alpha]_D$ +36° (c, 1.6), undepressed in m.p. on admixture with a sample of the original acetate, while a mixture with 14:15-epoxy-12-oxoisoursan-3β-ol melt at 223°.

Treatment of 14:15-Epoxy-12-oxoisoursan-3β-yl Acetate with Hydrochloric Acid . - A solution of 14:15-epoxy-12-oxoisoursan-3β-yl acetate (100 mg.) in methanol (50 c.c.) and chloroform (20 c.c.) containing hydrychloric acid (2 c.c.) and water (2 c.c.) was kept at room temperature for 18 hr. Normal working up gave a gum (100 mg.) which was dissolved in petrol-benzene (1:1, 20 c.c.) and adsorbed on a column of alumina (3 g.). Elution with the same solvent mixture (160 c.c.) gave a solid (17 mg.) which crystallised from aqueous acetone as needles, m.p. 241-260°, ε at 2060 Å =4,000 , which were not further investigated.

Elution with petrol-benzene (1:3, 100 c.c.) gave a solid (45 mg.) which crystallised from chloroform-methanol to give 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate as needles, m.p. and mixed m.p., 293-295°, $[\alpha]_D$ +52° (c, 1.0).

Acetylation of 12-Oxo-13:27-cycloursan-3β:15ξ-diol 3-Acetate : 12-Oxo-13:27-cycloursan-3β:15ξ-diol Diacetate . - 12-Oxo-13:27-cycloursan -3β:15ξ-diol 3-acetate (45 mg.) was kept at room temperature with acetic anhydride (4 c.c.) and pyridine (4 c.c.) for 18 hr. Normal working up through ether gave a gum which crystallised from chloroform-methanol to give 12-oxo-13:27-cycloursan-3β:15ξ-diol diacetate (27 mg.) as needles, m.p. 250-252 , $[\alpha]_D$ +39.5° (c, 1.5), undepressed in m.p. on admixture with a sample of the diacetate obtained by oxidation of 12-oxoisours-14-en-3β-yl acetate with hydrogen peroxide.

Oxidation of 12-Oxo-13:27-cycloursan-3β:15ξ-diol 3-Acetate with Chromium Trioxide : 12:15-dioxo-13:27-cycloursan-3β-yl Acetate . - 12-oxo-13:27-cycloursan-3β:15ξ-diol 3-acetate (45 mg.) in

pyridine (1 c.c.) was added to a complex of chromium trioxide (90 mg.) and pyridine (0.9 c.c.) and the mixture was allowed to stand at room temperature for 18 hr. The mixture was poured into water (70 c.c.) and extracted with chloroform-ether (1:1). The extract was washed consecutively with aqueous sodium hydroxide (20%), water, aqueous hydrochloric acid (5%), water, and saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The product (34 mg.) crystallised from chloroform-methanol to give 12:15-dioxo-13:27-cycloursan-3β-yl acetate as needles, m.p. 347° (decomp.), $[\alpha]_D$ +42° (c, 0.9), undepressed in m.p. on admixture with a sample of the same compound obtained by potassium permanganate oxidation of 12-oxoisours-14-en-3 -yl acetate.

Reduction of 12-Oxo-13:27-cycloolean-9(11)-en-3β:15ξ-diol 3-Acetate with Lithium in Liquid Ammonia . - 12-Oxo-13:27-cycloolean-9(11)-en-3β:15ξ-diol 3-acetate (850 mg.) in dry ether (120 c.c.) was added over 2 min. to a stirred solution of lithium

(300 mg.) in liquid ammonia (200 c.c.) and stirring was continued for a further 3 min. Acetone (23 c.c.) was added and the ammonia allowed to evaporate overnight. Normal working up through ether-chloroform gave a gum which crystallised from ether to give 12-oxo-13:27-cyclooleanan-3β:15ξ-diol (430 mg.) as needles, m.p. 288-289°, $[\alpha]_D$ +26° (c, 0.6) ; $\lambda_{max.}$ 2100 Å (ε 5,100) (Found : C, 79.1 ; H, 10.55 . $C_{30}H_{48}O_3$ requires C, 78.9 ; H, 10.6%). This compound gives no colour with tetranitromethane in chloroform.

EXPERIMENTAL

Section II.

α-Amyrin Methanesulphonate . - α-Amyrin (5 g.) in pyridine (30 c.c.) and methanesulphonyl chloride (3 c.c.) was kept at room temperature overnight. The solution was carefully diluted with water and extracted with ether. The ethereal extract was washed with aqueous hydrochloric acid (5%), water, saturated aqueous sodium bicarbonate, and water, and concentrated to small bulk. The warm ethereal solution was poured into warm methanol (300 c.c.) and the mixture distilled at atmospheric pressure until free of ether. On cooling α-amyrin methanesulphonate (3.4 g.) separated out as plates, m.p. 119°, $[\alpha]_D$ +74° (c, 1.4).

Literature[150] gives m.p. 116-118° for this compound.

Action of Heat on α-Amyrin Methanesulphonate . - α-Amyrin methanesulphonate (5 g.) was heated at 87° in an air-oven for 12 min. The charred residue was crystallised from acetone to give 1-α-amyradiene (5ξ:8α:9β-trimethyl-10α-novursa-12:15-diene)(4.25 g.) as flattened needles, m.p. and mixed m.p. (with an authentic specimen) 192°, $[\alpha]_D$ -107° (c, 1.0).

Literature[154] gives m.p. 193-194°, $[\alpha]_D$ -105°, for this compound.

Treatment of 16-Oxours-12-en-3β-ol (Breinonol-B) with Methanesulphonyl Chloride . - 16-Oxours-12-en-3β-ol (200 mg.) in pyridine (3 c.c.) and methanesulphonyl chloride (0.3 c.c.) was kept at room temperature for 17 hr. The mixture was diluted with water (150 c.c.) and extracted with ether. The ethereal extract was washed consecutively with aqueous hydrochloric acid (5%), water, saturated aqueous sodium bicarbonate and water.

(a) The extract was concentrated to small bulk and poured into warm methanol. The mixture was distilled until free from ether and allowed to cool overnight. A gum separated out and was unchanged by attempted crystallisations from methanol, aqueous methanol, aqueous acetone, ethanol and petrol.

(b) In a second preparation the ether extract was dried over anhydrous sodium sulphate and evaporated to dryness under 15°. The residue was dissolved in petrol (30 c.c.) and adsorbed on a column of alumina

(9 g.). Elution with petrol, petrol-benzene, benzene, ether and methanol, gave fractions which could not be crystallised from normal solvents.

Note . The product was a colourless gum on attempted crystallisation from ether-methanol but rapidly developed a brown colour on standing at room temperature.

(c) The effect of heat on the product of the above reaction - 16-Oxours-12-en-3β-ol (150 mg.) in pyridine (2 c.c.) and methanesulphonyl chloride (0.2 c.c.) was kept at room temperature overnight. Normal working up gave a gum (140 mg.) which was heated at 85° for 15 min. The product was dissolved in petrol (12 c.c.) and adsorbed on a column of alumina (6 g.). Elution with petrol (150 c.c.) gave a gum (106 mg.) which showed no absorption in the ultra-violet region above 2100 Å. Elution with petrol-benzene (4:1, 200 c.c.) gave a gum (34 mg.) which was dissolved in petrol-benzene (9:1, 10 c.c.) and adsorbed on a column of alumina (12 g.) from which the same solvent mixture eluted a gum (33 mg.) which could not be crystallised. The light absorption properties of this gum were, $\lambda\lambda_{max.}$ 2890 Å, 2400 Å and 2070 Å (ε 6,000, 3,700

and 7,000).

The gum (106 mg.), eluted (above) with petrol, was heated on a steam-bath with glacial acetic acid (75 c.c.) and concentrated hydrochloric acid (2.5 c.c.) for 2 hr., when a further addition of concentrated hydrochloric acid (2.5 c.c.) was made and heating continued for a further 2 hr. Evaporation to dryness under reduced pressure gave a gum which was identical in light absorption properties with the starting material. A similar treatment of the gum which had been eluted with petrol-benzene and rechromatographed , effected no alteration in the light absorption properties of the material.

Treatment of Urs-12-en-3β:16β-diol Diacetate (Brein Diacetate) with Ozone : 12-Oxo-13α-ursan-3β:16β-diol Diacetate . - Urs-12-ene-3β:16β-diol diacetate (1 g.) in carbon tetrachloride (50 c.c.) was treated at room temperature with a slow stream of ozonised oxygen (containing circa 2% ozone) until the solution no longer gave a yellow colour with tetranitromethane. The solution was evaporated to dryness under reduced pressure and the residue was refluxed with distilled

water (200 c.c.) for 2 hr. The cooled mixture was extracted with ether and the extract was washed with saturated aqueous sodium bicarbonate and water, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure to give a gum (1.15 g.).

The combined sodium bicarbonate and water washings were acidified with dilute hydrochloric acid and extracted with ether. Evaporation of the extract to dryness under reduced pressure gave a gum (35 mg.) which was not further investigated.

The neutral product was dissolved in petrol (7 c.c.) and adsorbed on a column of alumina (35 g.).

Elution with petrol (1400 c.c.) gave only traces of material which were not further investigated.

Elution with petrol-benzene (9:1, 1400 c.c.; 4:1, 1200 c.c.; 3:2, 1800 c.c.; 2:3, 1400 c.c.; 1:4 600 c.c.) gave a gum (860 mg.).

Elution with benzene (400 c.c.) and benzene-ether (9:1, 1400 c.c.) gave a gum (150 c.c.) which could not be crystallised and which was not further investigated.

The material (860 mg.) eluted with petrol-benzene mixtures was dissolved in petrol (50 c.c.) and adsorbed on a column of acid-washed* alumina (24 g.).

* See overleaf.

Elution with petrol (600 c.c.) gave a gum (470 mg.) which crystallised from methanol to give urs-12-en-3β:16β-diol diacetate as prisms, m.p. and mixed m.p. (with a sample of starting material) 198°, $[\alpha]_D$ +76° (c, 1.3).

Literature[31] gives m.p. 197-198°, $[\alpha]_D$ +74°, for this compound.

Further elution with the same solvent (1400 c.c.) gave a gum (375 mg.) which crystallised from aqueous methanol to give 12-oxo-13α-ursan-3β:16β-diol diacetate as needles, m.p. 206-208°, $[\alpha]_D$ +130°, +132°, +132° (c, 1.4, 1.0, 1.2); $\lambda_{max.}$ 2500 Å (ε 150), ε at 2040 Å = 600 (Found : C, 75.1 ; H, 10.0 . $C_{34}H_{54}O_5$ requires C, 75.2 ; H, 10.0%). The infra-red spectrum of this compound includes a peak at 1709 $cm.^{-1}$ not included in the spectrum of the starting material. This compound gives no colour with tetranitromethane in chloroform.

Mladenovic and Hoffmann[144] give m.p. 211° for an "ozonide" prepared by ozonolysis of brein diacetate in acetic acid.

* Acid-washed alumina was prepared by shaking alumina with 1 part aqueous acetic acid (10%) and 9 parts petrol for 30 min. The alumina was washed with dry petrol before use.

Elution with ether (200 c.c.) gave a solid (80 mg.) which crystallised from methanol with considerable loss to give a substance as needles, m.p. 296-300°(decomp.), $[\alpha]_D$ +30° (c, 0.9); $\lambda_{max.}$ 2480 Å (ε 10,000)(Found : C, 75.2 ; H, 9.5 . $C_{34}H_{52}O_5$ requires C, 75.5 ; H, 9.7%). This compound gave no colour with tetranitromethane in chloroform.

Note - Under the same conditions α-amyrin acetate (urs-12-en-3β-yl acetate) on ozonisation gave 12-oxo-13α-ursan-3β-yl acetate, m.p. 206-207°, $[\alpha]_D$ +114° (c, 2.1) ; $\lambda_{max.}$ 2500 Å (ε 350, in chloroform), ε at 2030 Å = 1,000, which showed a peak at 1707 $cm.^{-1}$ in its infra-red spectrum.

Attempted Bromination and Dehydrobromination of 12-Oxo-13α-ursan-3β:16β-diol Diacetate . -

(a) 12-Oxo-13α-ursan-3β:16β-diol diacetate (180 mg.) in stabilised acetic acid (6 c.c.) containing a trace of hydrobromic acid was treated dropwise at 60° over 10 min. with a solution of bromine in stabilised acetic acid (4% by volume : 0.6 c.c.), heated on a steam-bath for 2 hr. and allowed to cool overnight. Normal working

up gave a gum which could not be crystallised from normal solvents. The gum (190 mg.) was dissolved in petrol (10 c.c.) and adsorbed on a column of alumina (6 g.). Elution with petrol (250 c.c.) gave a solid (17 mg.) which crystallised from from methanol to give a substance as blades, m.p. 258-259°, $[\alpha]_D$ +119° (c, 0.6) ; transparent to light between 2000 Å and 4000 Å.

Elution with petrol-benzene (2:1, 350 c.c. ; 1:1, 500 c.c.) gave a gum (147 mg.) which could not be crystallised and was dissolved in petrol (10 c.c.) and adsorbed on a column of alumina (5 g.) from which elution with petrol-benzene (3:1, 600 c.c.) gave a gum (125 mg.) which could not be crystallised. The light absorption properties of this gum were, $\lambda_{max.}$ 2500 Å (ε 7,000), ε at 2050 Å = 6,000.

(b) To a solution of 12-oxo-13α-ursan-3β:16β-diol diacetate (150 mg.) in stabilised acetic acid (4 c.c.) was added a solution of hydrobromic acid in stabilised acetic acid (1% HBr by volume, 1 c.c.) and the mixture was treated at 60° with a solution of bromine in stabilised acetic acid (4% by volume, 0.48 c.c.),

added dropwise over 10 min. The solution was heated on a steam-bath for 14 hr. and worked up in the normal manner to give a gum (145 mg.) which could not be crystallised. The light absorption properties of this gum were, $\lambda_{max.}$ 2480 Å (ε 9,700), ε at 2040 Å = 4000.

(c) 12-Oxo-13α-ursan-3β:16β-diol diacetate (137 mg.) in stabilised acetic acid (7.5 c.c.), containing a trace of hydrobromic acid, was treated at 60° with a solution of bromine in stabilised acetic acid (4% by volume, 0.44 c.c.) added dropwise over 10 min. The reaction mixture was diluted with water (100 c.c.) and the precipitate filtered off and washed with water (3 X 100 c.c.). This solid product was dissolved in pyridine (20 c.c.) and refluxed for 4 hr. Normal working up gave a gum (125 mg.) which could not be crystallised. The light absorption properties of this gum were, $\lambda_{max.}$ 2480 Å (ε 9,300), ε at 2040 Å = 3,500.

Note - Treatment of 12-oxo-13α-ursan-3β-yl acetate as described above, (a) and (c), gave 12-oxours-9(11)-en-3β-yl acetate, m.p. 285°, $[\alpha]_D$ +84° (c, 1.2), in good yield. The compound shows maximal absorption at

2480 Å (ϵ 10,250).

Treatment of 12-Oxo-13α-ursan-3β:16β-diol Diacetate with Mineral Acid . - 12-Oxo-13α-ursan-3β:16β-diol diacetate (150 mg.) in chloroform (1 c.c.) and glacial acetic acid (2 c.c.) containing concentrated hydrochloric acid (0.1 c.c.) was kept at 40° for 30 min. Normal working up gave a solid which crystallised from aqueous methanol as needles, m.p. 192-199°, $[\alpha]_D$ +85° (c, 2.1). The physical properties of this substance were unchanged by crystallisation from methanol or ethanol, or by chromatography on a column of alumina (5 g.)

The total product obtained above was dissolved in chloroform (1 c.c.) , added to a solution of concentrated hydrochloric acid (0.1 c.c.) in glacial acetic acid (2 c.c.) and kept at 40° for a further 4 hr. Water (75 c.c.) was added and the product, which was recovered by filtration, was crystallised from aqueous acetone to give a substance as needles, m.p. 259-260°, $[\alpha]_D$ +111°, +111° (c, 1.0, 1.6)(Found : C, 73.1 ; H, 9.6 . $C_{34}H_{52}O_6$

requires C, 73.3 ; H, 9.4 . $C_{34}H_{54}O_6$ requires C, 73.1 ; H, 9.7%). This compound shows no absorption in the ultra-violet region between 2000 Å and 4000 Å, and it gives no colour with tetranitromethane in chloroform. The infra-red spectrum of this compound includes bands at 1786 $cm.^{-1}$ (strong), 905 $cm.^{-1}$ (medium), 886 $cm.^{-1}$ (medium), 876 $cm.^{-1}$ (medium) and 847 $cm.^{-1}$ (medium), which are not present in the spectrum of the starting material. The band at 1709 $cm.^{-1}$, in the spectrum of the starting material, is not included in the infra-red spectrum of this product. The m.p. of this product is not depressed on admixture with a sample of the material, m.p. 258-259°, $[\alpha]_D$ +119°, isolated from the attempted bromination and dehydrobromination of 12-oxo-13α-ursan-3β:16β-diol diacetate [Method (a), page 112] .

Wolff-Kishner Reduction of 12-Oxoisours-14-en-3β-yl Acetate . - Anhydrous hydrazine was distilled into a solution of sodium (2 g.) in diethylene glycol (200 c.c.) at 180° until the mixture refluxed gently at this temperature. 12-oxoisours-14-en-3β-yl acetate

(1.5 g.) was added to the cooled solution and the mixture was refluxed for 20 hr. The solution was distilled until the temperature rose to 215° and anhydrous hydrazine distilled into the reaction mixture until it again refluxed at 180°. Refluxing was continued at 180° for a further 18 hr., the mixture was distilled until the temperature rose to 220° and refluxing was continued at this temperature for a further 48 hr. All the above operations were carried out in an atmosphere of nitrogen.

Normal working up gave a gum which was heated on a steam-bath with pyridine (25 c.c.) and acetic anhydride (25 c.c.) for 1 hr. Normal working up of the acetylation gave a gum which was dissolved in petrol (35 c.c.) and adsorbed on a column of alumina (45 g.). Elution with petrol (1500 c.c.) gave a solid (890 mg.) which crystallised from chloroform-methanol to give isours-14-en-3β-yl acetate (760 mg.) as plates, m.p. 214-215°, $[\alpha]_D$ +35° (c. 2.1). This compound was found to vary in crystalline shape depending on the technique of crystallisation. Crystallisation from a boiling solution gives plates . Crystallisation from a cool solution gives fine needles.

Literature[32] gives m.p. 214-216°, $[\alpha]_D$ + 36°, for

this compound.

Elution with petrol-benzene (9:1, 1000 c.c.) gave a gum (419 mg.) which crystallised from methanol as needles, m.p. 226-227°, undepressed in m.p. on admixture with a sample of the starting material.

Elution with benzene-methanol (1:1, 1000 c.c.) gave a gum (110 mg.) which could not be crystallised.

Urs-12-en-3β:15ξ-diol 3-Acetate . - isoUrs-14-en-3β-yl acetate (650 mg.) in chloroform (25 c.c.) containing perbenzoic acid (275 mg. ≡ 2 mol.) was kept in darkness at 0° for 18 hr. The solution was washed with saturated aqueous sodium bicarbonate solution and water, and evaporated to dryness under reduced pressure. The residue was dissolved in chloroform (100 c.c.) and methanol (100 c.c.), and concentrated hydrochloric acid (12 c.c.) and water (12 c.c.) added. The mixture was allowed to stand at room temperature for 18 hr., when normal working up gave a gum (605 mg.) which was dissolved in petrol-benzene (9:1, 40 c.c.) and adsorbed on a column of

(21 g.). Elution with petrol-benzene (9:1, 2000 c.c.) gave a solid (165 mg.) which crystallised from chloroform-methanol to give 14:15-epoxy*iso*oursan-3β-yl acetate as plates, m.p. 247-249°, $[\alpha]_D$ +58° (*c*, 1.8).

Literature[32] gives m.p. 249-251°, $[\alpha]_D$ +57°, for this compound.

Elution with petrol-benzene (1:4, 1000 c.c.), benzene (750 c.c.) and benzene-ether (19:1, 1000 c.c.) gave a gum (405 mg.) which was dissolved in petrol-benzene (19:1, 20 c.c.) and adsorbed on a column of alumina (16 g.), from which elution with the same solvent mixture gave a little 14:15-epoxy*iso*oursan-3β-yl acetate, m.p. and mixed m.p. 248-249°. Elution with petrol-benzene (1:9, 1250 c.c.) and benzene (1000 c.c.) gave a gum (360 mg.) which crystallised from aqueous methanol to give urs-12-en-3β:15ξ-diol 3-acetate as needles, m.p. 223-224°, $[\alpha]_D$ +72° (*c*, 1.6); ε at 2040 Å ≈ 5,500 .

Literature[32] gives m.p. 223-225°, $[\alpha]_D$ +75°, for this compound.

Dehydration of Urs-12-en-3β:15ξ-diol 3-Acetate with Phosphorus Oxychloride : Ursa-12:15-dien-3β-yl Acetate . - Urs-12-en-3β:15β-diol 3-acetate (2.1 g.) in pyridine (30 c.c.) was refluxed with phosphorus oxychloride (30 c.c.) for 30 min. Normal working up gave a gum which was dissolved in petrol (100 c.c.) and adsorbed on a column of alumina (120 g.). Elution with petrol gave a gum which could not be crystallised and was not further investigated.

Elution with petrol-benzene (9:1, 1200 c.c.; 3:1, 1700 c.c.) gave a gum (725 mg.).

Elution with benzene (2000 c.c.) gave a gum (240 mg.) which could not be crystallised and which was not further investigated.

The fraction (725 mg.), eluted with petrol-benzene mixtures, was dissolved in petrol-benzene (19:1, 300 c.c.) and adsorbed on a column of alumina (40 g.). Elution with petrol-benzene (19:1, 1000 c.c.; 12:1, 450 c.c.; 9:1, 600 c.c.; 4:1, 450 c.c.) gave a gum (300 mg.) which on crystallisation from aqueous methanol gave a gum which on standing in the mother-liquor for 8 days deposited amorphous nodules of solid. This solid was separated from the residual gum

by hand, washed with methanol and crystallised from methanol and chlroform-methanol, with considerable loss, to give ursa-12:15-dien-3β-yl acetate (60 mg.) as needles, m.p. 229°, $[\alpha]_D$ +42.5° (c, 1.1) ; ε at 2040 A = 4,000 (Found ; C, 82.2 ; H, 10.8 . $C_{32}H_{50}O_2$ requires C, 82.3 ; H, 10.8%). This product was undepressed in m.p. on admixture with a sample of the dienyl acetate, m.p. 228-229°, $[\alpha]_D$ +40°, prepared by dehydration of brein monoacetate-II (urs-12-en-3β:16α-diol 3-acetate) with phosphorus oxychloride in pyridine[32]. The infra-red spectra of the two dienyl acetates are also identical.

Elution of the column with petrol-benzene (7:3, 450 c.c.) gave a gum which on crystallisation from acetone-methanol gave a substance (10 mg.) as plates, m.p. 183° ; ε at 2030 Å = 5500 (Found : C, 77.2 ; H, 10.3%). This substance was not further investigated.

Note - In a second reaction under the same conditions, urs-12-en-3β:15ξ-diol 3-acetate (290 mg.) gave ursa-12:15-dien-3β-yl acetate (9 mg.) as needles, m.p. 223-225°, $[\alpha]_D$ +45° (c, 0.5).

Treatment of Ursa-12:15-dien-3β-yl Acetate with Hydrochloric Acid . - Ursa-12:15-dien-3β-yl acetate (20 mg.) in chloroform (1 c.c.) and stabilised acetic acid (5 c.c.) was treated with a slow stream of dry hydrogen chloride for 30 min. and kept in a stoppered flask overnight. Evaporation to dryness under reduced pressure gave a solid which crystallised from chloroform-methanol as needles (16 mg.), m.p. 224-227°, undepressed in m.p. on admixture with a sample of starting material.

Hydrogenation of Ursa-12:15-dien-3β-yl Acetate . - Ursa-12:15-dien-3β-yl acetate (30 mg.) in glacial acetic acid (30 c.c.) containing platinum catalyst (from 20 mg. PtO_2) was shaken under an atmosphere of hydrogen for 30 hr. The filtered solution was evaporated to dryness under reduced pressure, giving a solid which crystallised from chloroform-methanol to give α-amyrin acetate (urs-12-en-3β-yl acetate,(13 mg.) as blades, m.p. and mixed m.p. (with an authentic specimen) 224°, $[\alpha]_D$ +80° (c, 0.7).

- BIBLIOGRAPHY.-

Bibliography

1. Baup, Jahresberichte fur Chemie, 1851, 528.
2. Tschirch and Cremer, Arch. fur Pharm., 1902, 313.
3. Rose, Ann., 1839, 22, 297.
4. Vesterberg, Ber., 1887, 20, 1242.
5. idem, Ber., 1906, 39, 2467.
6. Mladenovic and Lieb, Monatsh., 1940, 73, 228.
7. Heilbron, Kamm and Owens, J., 1926, 1630.
8. Heilbron, Hilditch and Kamm, J., 1926, 3131.
9. Dietrich and Lederer, Compt.Rendu., 1952, 234, 637.
10. idem, Helv. Chim. Acta., 1952, 35, 1148.
11. Jeger and Ruzicka, ibid., p.817.
12. Barton and Overton, Chem. and Ind., 1955, 654.
13. idem, J., 1955, 2639.
14. Ruzicka and Marxer, Helv. Chim. Acta., 1939, 22, 195.

15. Prelog, Norymberski and Jeger, ~~Hlv~~ ibid., 1946, 29, 360.

16. Diener, Jeger and Ruzicka, ibid., 1950, 33, 896.

17. Barton and Brooks, J., 1951, 257.

18. Schaffner, Caglioti, Arigoni and Jeger, Helv. Chim. Acta., 1958, 41, 152.

19. Dunstan, Fazakerley, Halsall and Jones, Croat. Chem. Acta., 1957, 29, 173.

20. Schaffner, Caglioti, Arigoni, Jeger, Fazakerley, Halsall and Jones, Proc. Chem. Soc., 1957, 353.

21. Mills and Werner, J., 1955, 3132.

22. Barton, de Mayo and Orr, J., 1958, 2239.

23. Ryabinin and Matyukhina, Zhur. obshchei. Khim., 1957, 27, 227.

24. Barton and Bruun, J., 1952, 1683.

25. Asahina and Akagi, Ber., 1938, 71, 980.

26. Asahina and Yosioka, ibid., 1940, 73, 742.

27. Orr, Parks, Dunker and Uhl, J. Amer. Pharm. Assoc. Sci. Ed., 1945, 34, 39.

28. Zurcher, Jeger and Ruzicka, Helv. Chim. Acta., 1954, 37, 2145.

29. Barton and de Mayo, J., 1953, 2178.

30. Barton, Page and Warnhoff, J., 1954, 2715.

31. Beaton, Easton, Macarthur, Spring and Stevenson, J., 1955, 3992.

32. Laird, Ph.D. Thesis, Glasgow, 1956.

33. Buchi, Jeger and Ruzicka, Helv. Chim. Acta., 1946, 29, 442.

34. Ruzicka and Wirz, ibid., 1939, 22, 948.

35. Goodson, J., 1938, 999.

36. Dawson, Halsall and Swayne, J., 1953, 590.

37. Cox, King and King, Proc. Chem. Soc., 1957, p.290.

38. Djerassi, Mills and Villotti, J. Amer. Chem. Soc., 1958, 80, 1006.

39. Mazur, Weizmann and Sondheimer, ibid., p. 1007.

40. Langdon and Bloch, J. Biol. Chem., 1953, 200, 135.

41. Tomkins, Dauben, Shepperd and Chaikoff, ibid., 1953, 202, 487.

42. Clayton and Bloch, ibid., 1956, 218, 305.

43. idem, ibid., p. 319.

44. Schneider, Clayton and Bloch, ibid., 1957, 224, 175.

45. Tchen and Bloch, ibid., 1957, 226, 921.

46. idem, ibid., p. 931.

47. Ruzicka, Experientia, 1953, 9, 357.

48. Ruzicka, Eschenmoser, and Heusser, ibid., p. 362.

49. Woodward and Bloch, J. Amer. Chem. Soc., 1953, 75, 2023.

50. Dauben, Abraham, Hotta, Chaikoff, Bradlow and Soloway, ibid., p. 3038.

51. Stork and Burgsthaler ibid., 1955, 77, 5068.

52. Eschenmoser, Ruzicka, Jeger and Arigoni, Helv. Chim. Acta., 1955, 38, 1890.

53. Ruzicka, "Perspectives in Organic Chemistry", Interscience Publishers Ltd, London, 1956, p. 265.

54. Tsujimoto, J. Ind. and Eng. Chem., 1916, 8, 889.

55. Taufel, Thaler and Schreyegg, Fettchem. Umschau., 1936, 43, 26.

56. Fitelson, J. Ass. Official. Agric. Chem., 1943, 26, 499.

57. Dimter, Z. physiol. Chem., 1941, 270, 247.

58. Mackenna, Wheatly and Wormall, J. Invest. Dermat., 1950, 15, 33.

59. Sobel, ibid., 1949, 13,333.

60. Mihelic and Momirovic, Fram. Glasnik., 1955, 11, 191.

61. Heller, Heller Springer and Clark, Nature, 1957, 179, 919.

62. Dickhart, Amer. J. Pharm., 1955, 127, 359.

63. Nicolaides and Laves, J. Amer. Chem. Soc., 1954, 76, 2596.

64. Dauben and Takemura, ibid., 1953, 75, 6302.

65. Tchen and Bloch, ibid., 1956, 78, 1516.

66. Bruun, Acta. Chem. Scand., 1954, 8, 1291.

67. Brooks, J., 1955, 1675.

68. Beaton, Spring, Stevenson and Stewart, J., 1955, 2131.

69. Ames and Jones, Nature, 1949, 164, 1090.

70. Vesterberg, Ber., 1891, 24, 3835.

71. Fayez, Grigor, Spring, and Stevenson, J., 1955, 3378.

72. Dauben, Ban and Richards, J. Amer. Chem. Soc., 1956, 78, 5329.

73. idem, ibid., 1957, 79, 968.

74. Dauben, Fonken and Boswell, ibid., p. 1000.

75. Danielson and Bloch, ibid., p. 500.

76. Alexander, Gold and Schwenk, ibid., p. 2967.

77. Alexander and Schwenk, ibid., p. 4554.

78. Arigoni, Experientia, 1958, 14, 153.

79. Cornforth, Gore and Popjak, Biochem. J., 1956, 64, 38p.

80. idem., ibid., 1957, 65, 94.

81. Rabinowitz and Gurin, J. Biol. Chem., 1954, 208, 307.

82. Bloch, Clark and Harary, ibid., 1954, 211, 687.

83. Rudney, J. Amer. Chem. Soc., 1954, 76, 2595.

84. idem, ibid., 1955, 77, 1698.

85. Tavormina, Gibbs and Huff, ibid., 1956, 78, 4498.

86. Wolf, Hoffmann, Aldrich, Skeggs, Wright, and Folkers, ibid., p. 4499

87. Wright, Cresson, Skeggs, Macrae, Hoffmann, Wolf, and Folkers, ibid., p. 5273.

88. Tavormina and Gibbs, ibid., p. 6210.

89. Amdur, Rilling and Bloch, ibid., 1957, 79, 2646.

90. Dituri, Gurin and Rabinowitz, ibid., p. 2650.

91. Wright, Federation Proc., 1957, 16, 274.

92. Cornforth, Cornforth, Popjak and Youhotsky-Gore, Biochem. J., 1957, 66, 10p.

93. Gould and Popjak, ibid., p. 51p.

94. Isler, Ruegg, Wursch, Gey and Pletscher, Chimia (Switz), 1957, 11, 167.

95. Dituri, Rabinowitz, Hullin and Gurin, J. Biol. Chem., 1957, 229, 825.

96. Stokes, Fish and Hickey, ibid., 1956, 220, 415.

97. Gautschi and Bloch, J. Amer. Chem. Soc., 1957, 79, 684.

98. Haworth, Ann. Reports, 1937, 34, 32.

99. Barton, Tilden Lecture, J., 1953, 1027.

100. Corey and Ursprung, Chem. and Ind., 1954, 1387.

101. Allan, Beaton, Shaw, Spring, Stevenson, Stewart and Strachan, ibid., 1955, 281.

102. Beaton, Spring, Stevenson and Strachan, J., 1955, 2610.

103. Beton and Halsall, Chem. and Ind., 1954, 1560.

104. Zurcher, Jeger and Ruzicka, Helv. Chim. Acta., 1954, 37, 2145.

105. Corey and Ursprung, J. Amer. Chem. Soc., 1956, 78, 183.

106. Melera, Arigoni, Eschenmoser, Jeger, and Ruzicka, Helv. Chim. Acta., 1956, 39, 441.

107. Meisels, Ruegg, Jeger and Ruzicka, ibid., 1955, 38, 1298.

108. Cole, Thornton and White, Chem. and Ind., 1956, 795.

109. Corey and Cantrall, J. Amer. Chem. Soc., 1958, 80, 499.

110. Phillips and Tuiten, ibid., 1956, 78, 5438.

111. Johnston and Spring, J., 1954, 1556.

112. Green, Mower, Picard and Spring, J., 1944, 527.

113. Budziarek, Johnston, Manson and Spring, J., 1951, 3019.

114. Ruzicka, Ruegg, Volli and Jeger, Helv. Chim. Acta., 1947, 30, 140.

115. Beaton, Spring, Stevenson and Stewart, J., 1955, 2131.

116. Shaw, Ph.D. Thesis, Glasgow, 1956.

117. Allan, Ph.D. Thesis, Glasgow, 1955.

118. Easton and Spring, J., 1955, 2120.

119. Easton, Ph.D. Thesis, Glasgow, 1953.

120. Johnston, Ph.D. Thesis, Glasgow, 1953.

121. Picard and Spring, J., 1941, 35.

122. Meyer, Jeger, Prelog, and Ruzicka, Helv. Chim. Acta., 1951, 34, 747.

123. Dorée, McGhie and Kurzer, J., 1945, 988.

124. idem, J., 1949, 570.

125. Cavalla, McGhie and Pradhan, J., 1951, 3142.

126. Derfer, Pickett and Boord, J. Amer. Chem. Soc., 1949, 71, 2482.

127. Barton J., 1951, 1444.

128. Slabey, J. Amer. Chem. Soc., 1954, 76, 3604.

129. Mohrbacher and Cromwell, ibid., 1957, 79, 401.

130. Cole, J., 1954, 3807.

131. Cromwell and Hudson, J. Amer. Chem. Soc., 1953, 75, 872.

132. Cromwell and Graff, J. Org. Chem., 1952, 17, 414.

133. Rogers, J. Amer. Chem. Soc., 1947, 69, 2544.

134. Eastmann, ibid., 1954, 76, 4115.

135. Klotz, ibid., 1944, 66, 88.

136. Smith and Rogier, ibid., 1951, 73, 3840.

137. Dorfmann, Chem. Rev., 1953, 53, 72.

138. Morice and Simpson J., 1940, 798.

139. idem, J., 1942, 198.

140. Jeger, Montavon, and Ruzicka, Helv. Chim. Acta., 1946, 29, 1124.

141. Jeger, Nisoli and Ruzicka, ibid., p. 1183.

142. Bischof, Jeger and Ruzicka, ibid., 1949, 32, 1911.

143. Rolett, Monatsh., 1929, 53-54, 231.

144. Mladenović and Hoffmann, Ber., 1940, 73, 200.
Sitzberichte Akad. Wiss. Wein, Math-Naturwissenschafte Klasse, Abt. IIb, 149, p.144 (1940).

145. Buchi, Jeger and Ruzicka, Helv. Chim. Acta., 1946, 29, 442.

146. idem, ibid., 1948, 31, 139.

147. Klyne and Stokes, J., 1954, 1979.

148. Barton J., 1953, 1027.

149. Heusser, Eichenberger, Kurath, Dallenbach and Jeger, Helv. Chim. Acta., 1951, 34, 2106.

150. Noller and Hearst, J. Amer. Chem. Soc., 1950, 72, 625.

151. Kaye, Fieser and Fieser, ibid., 1955, 77, 5936.

152. Allan, Spring and Stevenson J., 1955, 3072.

153. Ruzicka, Jeger, Redel and Volli, Helv. Chim. Acta., 1945, 28, 119.

154. Ewen, Gillam and Spring, J., 1944, 28.

155. Seymour, Sharples and Spring, J., 1939, 1075.