A T H E S I S

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DEGREE OF DOCTOR OF PHILOSOPHY

by

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C O N T E N T S

SUMMARY

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Part I - The Structures of α-Amyrenol and Ursolic Acid.

Investigations have been carried out on the naturally occurring triterpene, α-amyrenol, $C_{30}H_{50}O$, and the related substance ursolic acid, $C_{30}H_{48}O_3$, with a view to the elucidation of the structure of the α-amyrin group of triterpenes.

Oxidation with chromic anhydride of the unsaturated hydrocarbon *l*-α-amyradiene $C_{30}H_{48}$, yielded *l*-α-amyradiene oxide, $C_{30}H_{48}O$, which on further oxidation with the same reagent was converted to *l*-α-amyrenone oxide $C_{30}H_{46}O_2$. In addition a number of well-defined mixed crystals of the hydrocarbon and each of its oxidation products were obtained, and one of these was identified with a product previously obtained by treatment of *l*-α-amyradiene with perbenzoic acid.

An examination of molecular rotational differences between certain derivatives of α-amyrenol and the corresponding derivatives of α-amyrenol has been made, from which, in conjunction with the oxidation experiments on *l*-α-amyradiene, a partial formulation for α-amyrenol has been derived.

Treatment of acetylursolyl chloride, $C_{32}H_{49}O_3Cl$, with phenylmagnesium bromide yielded the diphenylcarbinol,

$C_{44}H_{60}O_3$, which could not be dehydrated to the corresponding diphenylethylene derivative, and hence confirmed the tertiary nature of the carboxyl group of ursolic acid.

Ketoacetylursolic acid when refluxed with quinoline in presence of oxygen has been shown to yield nor-α-amyradienonyl acetate, $C_{31}H_{46}O_3$, while in the absence of air nor-α-amyrenonyl acetate was produced. Under similar conditions, ursonic acid $C_{30}H_{46}O_3$, acetylursolic acid $C_{32}H_{50}O_4$, and α-amyrenonyl acetate $C_{32}H_{50}O_3$ have been shown to be stable, from which the relative locations of the ketone group and the carboxyl group of ketoacetylursolic acid have been tentatively fixed.

Oxidation of acetylursolic acid with hydrogen peroxide produced an oxido-lactone $C_{32}H_{48}O_5$, identical to that obtained by Jeger, Borth, and Ruzicka (Helv.Chim. Acta,1946,29,1999). In addition, there was produced an acid which on methylation yielded methyl oxido-acetylursolate $C_{33}H_{52}O_5$, previously designated methyl ketodihydro-acetylursolate (Jeger, Borth, and Ruzicka,loc.cit.).

Methyl acetylursolate on treatment with hydrogen peroxide yielded methyl oxido-acetylursolate, $C_{33}H_{52}O_5$, which by the action of dilute mineral acid was converted to the isomeric methyl ketodihydro-acetylursolate. The compound described in the literature as methyl ketodihydro-acetylursolate was identified as methyl oxido-acetylursolate

described above. Treatment of methyl oxido-acetyl-ursolate and methyl ketodihydro-acetylursolate with bromine yielded the same bromo compound $C_{33}H_{51}O_5Br$, which on dehydrohalogenation gave methyl iso-ketoacetyl-ursolate, $C_{33}H_{50}O_5$. Oxidation of this with selenium dioxide gave methyl iso-ketodehydro-acetylursolate $C_{33}H_{48}O_5$, and from a comparison of the behaviour of this compound with that of similar derivatives of α- and β-amyrenol, a structure incorporating the partial structures already obtained, has been derived.

Part II - The resin from Canarium Schweinfurthii.

The resin from the tree Canarium Schweinfurthii has been examined analytically. The steam volatile portion was found to consist of the terpene d-limonene containing a trace of a phellandrene. The non-volatile portion contained the triterpenes α-amyrenol, β-amyrenol, α-elemolic acid, β-elemonic acid and a diol, $C_{30}H_{50}O_2$, which like α-elemolic and β-elemonic acids contained two unconjugated double bonds but which could not be identified with any of the known triterpene diols.

THE OCCURRENCE AND CHEMISTRY OF CERTAIN TRITERPENES WITH PARTICULAR REFERENCE TO THE α-AMYRIN GROUP.

Being a Thesis presented in Two Parts:-

PART I.

The Structures of α-Amyrenol and Ursolic Acid.

PART II.

The Resin from Canarium Schweinfurthii.

PART I

THE STRUCTURES OF α-AMYRENOL AND URSOLIC ACID.

HISTORICAL

The pentacyclic triterpenes although all alike in possessing a molecular formula containing thirty carbon atoms, can be divided into three distinct structural groups, the α-amyrin group, the β-amyrin group, and the lupeol group. Of these, the first two are thought to be related, in that they are supposed to have a structure containing five six-membered rings, whereas the third, the lupeol group, is believed to contain a five-membered ring in conjunction with four six-membered rings (9).

The two amyrin groups can be considered as being structurally derived from the isomeric secondary alcohols, α- and β-amyrin, more exactly designated α- and β-amyrenol (10), since each member of the groups can be converted, or simply related, to one or other of the amyrins. Thus, the four naturally occurring members of the α-amyrin group, ursolic acid, β-boswellic acid, uvaol, and α-amyrenol, are all inter-related. Uvaol (11) has been shown to have the same structure as ursolic acid, with the exception that it contains a $-CH_2OH$ grouping in place of the carboxyl group of the acid, while both ursolic acid (12) and β-boswellic acid (13) have been directly converted into α-amyrenol. The method employed has found general use throughout the

triterpene series for the conversion of mono-carboxylic acids into the corresponding compounds bearing a methyl group in place of the original carboxyl group. The acid with its hydroxyl group protected, is converted into the acid chloride, Rosenmund reduction of which gives the corresponding aldehyde which is then reduced by the Kischner-Wolff method to the deoxycompound:-

$$R\begin{cases}\text{-OAc}\\ \text{-COOH}\end{cases} \longrightarrow R\begin{cases}\text{-OAc}\\ \text{-COCl}\end{cases} \longrightarrow R\begin{cases}\text{-OAc}\\ \text{-CHO}\end{cases} \longrightarrow R\begin{cases}\text{-OH}\\ \text{-CH}_3\end{cases}$$

In the β-amyrin series, oleanolic acid (14), and α-boswellic acid (15) have each been converted to β-amyrenol by this method.

Although the precise relationships existing between the compounds within the two groups have thus been established, it has not been found possible to convert any compound of the one group into one of the other. In addition, no compound containing all or almost all of the original hydroaromatic structure is known which is common to both groups, and so although the structure of β-amyrenol has been almost completely elucidated, that of α-amyrenol is still in doubt.

Certain features of the α-amyrenol structure, however, have been definitely established, and these will now be discussed in the succeeding sections of the Historical part.

DEHYDROGENATION

The failure of early degradation experiments to throw any light on the nature of the carbon skeleton of the triterpenes can be attributed largely to the fact that there existed no reagent nor technique capable of producing stepwise degradation of the molecule. This in turn was at least partly due to the scarcity of functional groups within the molecule. Early degradation experiments involving oxidation (16) and bromination (17) were concentrated solely on these groups with the result that the carbon skeleton as a whole remained intact.

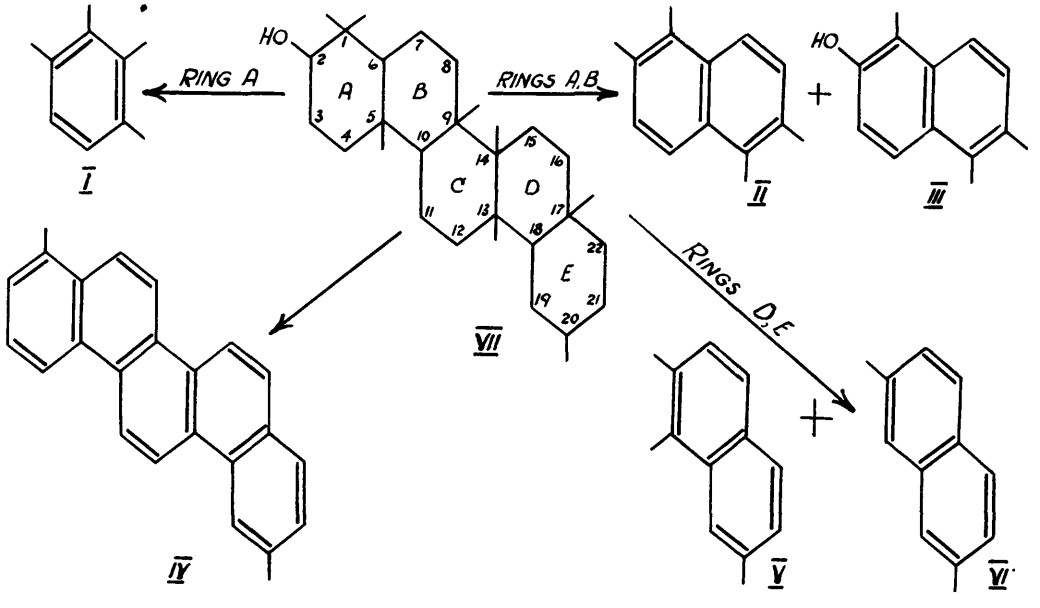
The first real indication that the triterpene molecule could be split into identifiable fragments was obtained by Ruzicka in 1929 (18); after vigorous heat treatment of a mixture of α- and β-amyrenol, he obtained various hydrocarbons which yielded naphthalene homologues on treatment with sulphur or selenium. Following this, he subjected the same mixture of triterpenes to dehydrogenation with selenium, using a technique which he had developed as a result of similar experiments in the diterpene field. The product isolated in this manner from a number of triterpenes was shown to be 1:2:7-trimethylnaphthalene (V, p.6).

Further experiments, again involving a mixture of

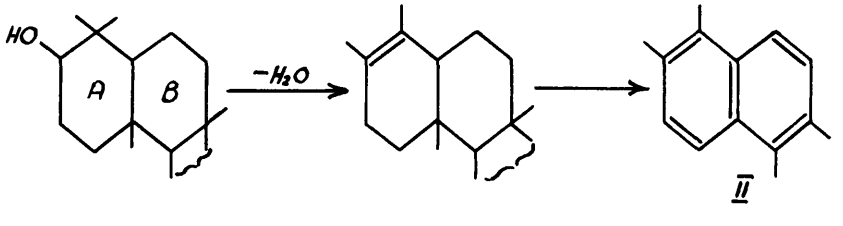
the isomeric amyrenols, resulted in the identification of the following products:- 1:2:3:4-tetramethylbenzene (I)(19), 2:7-dimethylnaphthalene (VI)(19), 1:2:5:6-tetramethylnaphthalene (II)(19), and 1:5:6-trimethyl-2-naphthol (III)(20). Later work has confirmed the production of these compounds in the dehydrogenation of pure α-amyrenol (21) with the exception of 1:2:3:4-tetramethylbenzene and 2:7-dimethylnaphthalene which have however been obtained by dehydrogenation of ursolic acid (22). No exhaustive search has been made for 1:2:3:4-tetramethylbenzene amongst the dehydrogenation products of the α-amyrin group, and it is generally assumed that its occurrence amongst the products from the mixed amyrenols is in part due to the α-isomer.

Consideration of these naphthalene dehydrogenation products led Ruzicka, in 1932, (23) to propose a basic hydropicene structure for the triterpenes of the α- and β-amyrin groups. When, however, 1:8-dimethylpicene (IV) was identified among the products obtained both from the dehydrogenation of a mixture of the amyrenols (19) and from α-amyrenol itself (21), he modified his original formulation to (VII) which differed only in the location of certain of the angular methyl groups, and retained the hydropicene nucleus. This formulation is

one of several which offer a reasonable explanation of the formation of the dehydrogenation products of the α-amyrin group, as shown below:-



To account for the formation of 1:2:5:6-tetramethylnaphthalene (II), and 1:2:3:4-tetramethylbenzene (I) Ruzicka postulated that a retropinacolone dehydration, involving the 2-hydroxy group, takes place as a preliminary to the dehydrogenation thus:-



This theory has been confirmed by the dehydrogenation of 2-methyl-α-amyrenol and of β-amyrene (19).

The formulation (VII) for the skeleton of α-amyrenol has been modified several times in the light of more recent work, but the modifications have usually been confined to rings C, D, and E, and in particular to the location of the angular methyl groups shown in (VII) at C_{13} and C_{17}. These two methyl groups are placed at C_{13} and C_{17} although strictly the only indication provided by dehydrogenation as to their nature is that they are probably tertiary, and hence on this evidence, could equally well be located at C_8, C_{10}, C_{18}, or C_{20}. Rings A and B are still regarded as having the structure assigned to them in (VII), and recent work has suggested that this structure is common to both α- and β-amyrenol (24).

The formulation (VII), which takes no account of the double bond of α-amyrenol, derived as it is solely from dehydrogenation evidence, may only be regarded as an approximation to the structure, since the dehydrogenation experiments were performed under conditions in which methyl group migration is possible. In order therefore, to make the formulation of α-amyrenol more complete, it has been necessary to carry out further degradative experiments of a less drastic nature. These, in the main, have been developments of the early

experiments involving the reactive groups present in the molecule, and will now be discussed with particular reference to these functional groups.

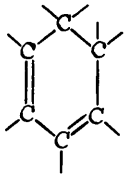
THE UNSATURATED CENTRE AND ITS ENVIRONMENT.

The presence in the α-amyrenol molecule of an unsaturated bond, which although undergoing ozonolysis resisted catalytic hydrogenation, was clearly shown by Ruzicka in 1929 (18). Although the exact location of this double bond is still in doubt, a certain amount of information regarding its environment has been obtained, largely by spectrographic methods.

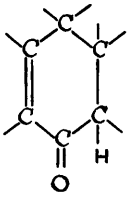
By oxidation, with chromic anhydride, of α-amyrenyl esters, in which the hydroxyl group was protected by esterification, the corresponding esters of α-amyrenonol have been obtained (21). α-Amyrenonol has been proved to be identical with the oxy-α-amyrin obtained under similar conditions by Vesterberg (25), and in addition has been shown spectroscopically to contain an α-β-unsaturated ketone grouping (21). Similar oxidations carried out with ursolic acid esters (26) have resulted in the production of compounds containing this same chromophore. That the original double bond of α-amyrenol constitutes part of the α-β-unsaturated ketone was shown by Ruzicka who obtained α-amyrenol by catalytic reduction of its oxidation product, α-amyrenonol (27). Consequently it follows that α-amyrenol must contain a methylene group adjacent to its double

bond, as suggested by Spring (21).

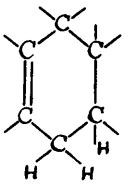
A different product from that already mentioned, however, was obtained when α-amyrenonol was reduced with sodium and amyl alcohol (21). This product α-amyradienol has also been obtained by partial sulphur dehydrogenation of α-amyrenol (28) and by the action of N-bromsuccinimide on α-amyrenyl acetate (29), and has been shown, again by spectroscopic methods to contain a conjugated diene system located in one ring (30,31). It must therefore contain the grouping (VIII), from which it follows that α-amyrenonol contains the fragment IX, and α-amyrenol the fragment (X).



VIII



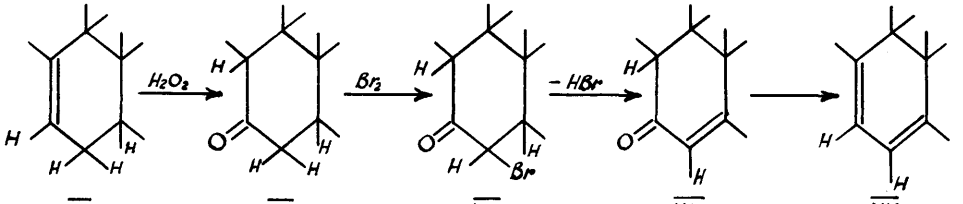
IX



X

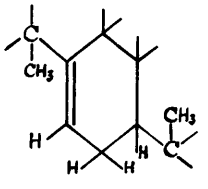
Further information concerning the double bond has been obtained from the oxidation of α-amyrenyl benzoate with hydrogen peroxide. The product on treatment with bromine gave first a halogen derivative (32) which in turn broke down into a substance containing an α-β-unsaturated ketone group (31). This compound although isomeric with α-amyrenonyl benzoate was not identical with it, and was designated iso-α-

amyrenonyl benzoate. It did, however, give α-amyradienol identical with that from α-amyrenonyl acetate, on reduction with sodium and amyl alcohol (31). Consideration of these reactions led Spring (31) to suggest that iso- α-amyrenonyl benzoate contained the fragment (XIV), and α-amyradienol the fragment (XV). On the same basis, the hydrogen peroxide oxidation product, α-amyranonyl benzoate, was supposed to contain the grouping (XII) in which the ketone group has been formed by the direct oxidation of the double bond of α-amyrenol, and the bromo derivative was represented by (XIII). Recently, however, it has been shown that the product from the hydrogen peroxide oxidation of α-amyrenyl benzoate is not the ketone (XII), but in fact, the corresponding oxide, which easily isomerises to the ketone shown.



From this, the formulation (X) for α-amyrenol can now be extended to (XI), which is further substantiated by a study of the products obtained by ozonolysis of α-amyradienyl acetate, and their subsequent reduction (34).

The environment of the double bond has been further elucidated by consideration of the relative inactivity towards mild dehydrogenating agents displayed by α-amyrenyl esters. From a study of the β-amyrin series, in which β-amyrenyl acetate has been partially dehydrogenated with N-bromsuccinimide to β-amyratrienyl acetate, (29) it might have been supposed that α-amyrenyl esters would act similarly. The formation of this conjugated triene system, however, does not occur in the α-amyrin series, α-amyradienyl acetate, containing only a conjugated diene chromophore, being produced from α-amyrenyl acetate by the action of N-bromsuccinimide (29). This, along with the fact that partial dehydrogenation with bromine, successful on β-amyrenonyl esters (35) has no effect on α-amyrenonyl benzoate (36), has been taken to indicate that in the α-amyrin series, the carbon atoms immediately adjacent to the diene system (XV) are either quaternary or in sterically hindered positions. The fragment (XVI) has therefore been suggested as being representative of the environment of the double bond in α-amyrenol.



XVI

Several formulations for α-amyrenol have been put forward by correlating this partial formula (XVI) with the dehydrogenation evidence discussed in the previous section. The incorporation of this fragment (XVI) within a hydropicene skeleton can be accomplished, however, in a less ambiguous manner by consideration of its relative position with respect to the already located hydroxyl group rather than on the basis of dehydrogenation evidence alone.

THE ETHYLENIC LINKAGE AND THE HYDROXYL GROUP.

Attempts to fix accurately the position of the double bond of α-amyrenol with respect to the secondary hydroxyl group located at C_3, have usually involved dehydration reactions first investigated by Vesterberg in 1891 (26). The full interpretation of his and subsequent experiments has however, only been possible by use of modern spectroscopic methods.

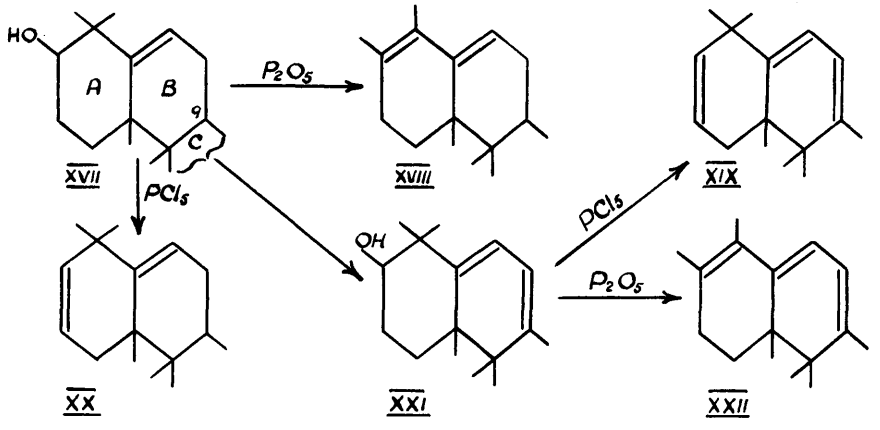
α-Amyrenol when dehydrated, has been shown to give two distinct series of compounds depending largely on the dehydration reagent employed. One series consists of compounds which are similar to the products obtained in the β-amyrin series, in that they preserve the strong dextra-rotation shown by α-amyrenol and show no selective light absorption in the ultra-violet region. The second series, which has no parallel in the β-amyrin group, contains compounds which are strongly laevo-rotatory and which display a characteristic ultra-violet light absorption.

d-α-Amyradiene-I obtained by dehydration of α-amyrenol with phosphorus pentachloride (4,37) and d-α-amyradiene-II obtained by pyrolysis of α-amyrenyl benzoate (38,39,40) both belong to the first group, and it has been shown spectroscopically that the double bond

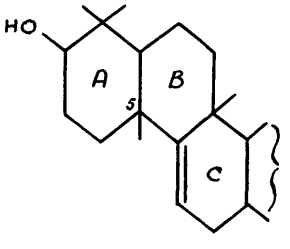
introduced into both compounds is not conjugated with that originally present in α-amyrenol (41). Similarly, it has been shown that d-α-amyratriene from the dehydration of α-amyradienol, does not contain a conjugated triene system, but only the original conjugation of α-amyradienol. When, however, α-amyrenol was dehydrated with phosphorus pentoxide, the product, l-α-amyradiene (26), was found to belong to the second of the groups previously mentioned, and to contain a conjugated diene system located in two rings (41). It has also been shown by Spring (41), that the triene obtained by phosphorus pentoxide dehydration of α-amyradienol differed from that obtained using phosphorus pentachloride, in that the double bond so introduced was conjugated with the diene system already present.

From the production of these conjugated compounds, the same author concluded that the double bond and the hydroxyl group of α-amyrenol were in the same vicinity, and put forward the following tentative partial formulations for α-amyrenol (XVII), l-α-amyradiene (XVIII), d-α-amyratriene (XIX), d-α-amyradiene-I (XX), α-amyradienol (XXI), and l-α-amyratriene (XXII). These account for the greater conjugation produced in the phosphorus pentoxide dehydrations, by assuming retro-

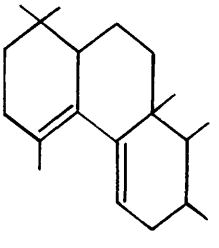
pinacolone dehydration, while those involving phosphorus pentachloride were assumed to take place normally.



It was recognised, however, that this formulation for α-amyrenol was in disagreement with the dehydrogenation evidence postulating an angular methyl group at C_9, and hence the alternative (XXIII) was suggested for α-amyrenol (41). To obtain the conjugated 1-α-amyradiene from this structure it is necessary to assume the simultaneous migration of one double bond, and the methyl group at C_5. It was, therefore, suggested that the double bond produced in the dehydration,



XXIII



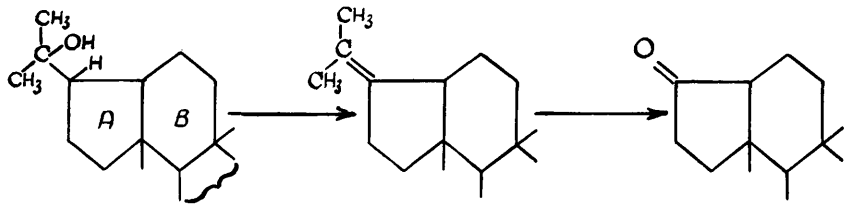
XXIV

migrated to the position C_4-C_5, while the angular methyl group moved to C_4, giving the partial formulation (XXIV), for l-α-amyradiene.

The formulation (XXIII) for α-amyrenol, although at variance with results obtained by Simpson and Williams (42) from a study of certain oxidation products of β-boswellic acid, has been strengthened by a set of experiments, carried out by Ruzicka, on dehydration products of α-amyrenonol.

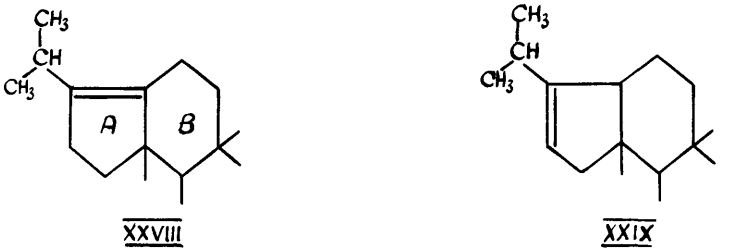
Dehydration of α-amyrenonol with xylene and Fuller's earth has been shown to produce α-amyradienone-II (21,43,44), while dehydration using phosphorus pentachloride yielded an isomeric compound, α-amyradienone-I (21) which was subsequently converted catalytically into α-amyradienone-II, by the action of palladium (44). Both compounds contain only the α-β-unsaturated ketone chromophore of α-amyrenonol and thus show the same lack of conjugation displayed by the d-α-amyradienes. Ruzicka, however, showed that the new double bond introduced into α-amyradienone-I could be oxidised by osmium tetroxide to a diol, and that subsequent treatment of this compound with lead tetracetate caused acetone to be split off from the molecule, leaving a ketonic fragment containing twentyseven carbon atoms. This he represented

by the partial formula (XXVII) from which it follows that α-amyradienone-I has the structure (XXVI) which in turn has been supposed to occur as a result of retropinacolone dehydration of α-amyrenol, through the intermediate (XXV) (48).



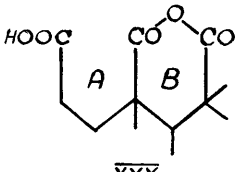
XXV XXVI XXVII

It was later shown by Ruzicka that when α-amyradieneone-II was subjected to the same series of reactions, no acetone was produced, and the final product, a triketone, contained all thirty original carbon atoms (44). He concluded from this that α-amyradienone-II could be formulated as either (XXVIII) or (XXIX), the latter being finally ruled out after consideration of further degradative evidence (44). By adopting this formulation (XXVIII) the possibility of the original double bond of α-amyrenol being located in either ring A or ring B is completely excluded.



XXVIII XXIX

This conclusion was further substantiated by study of the compound obtained from both of the α-amyradienones on reaction with chromic anhydride followed by acetic anhydride. The product which contained only twenty-six of the original carbon atoms, and in which rings A and B had been extensively degraded was formulated by Ruzicka as (XXX) (44). The fact that the ultra-violet



XXX

absorption spectrum of this compound was almost indistinguishable from that of the parent α-amyrenonol, was regarded as conclusive that its chromophore containing the original double bond, was not located in either ring A or ring B.

From the evidence reviewed therefore it would seem that the double bond, excluded from rings A and B must be located in ring C, probably in a similar position to that suggested by Spring in the partial formulation (XXIII) (41).

THE FUNCTIONAL GROUPS OF URSOLIC ACID.

Ursolic acid has been converted into α-amyrenol by the complete reduction of its carboxyl group to a methyl group (12), (p. 2), and consequently with this single exception the structures of both compounds are identical. Ursolic acid has therefore been investigated with a view to fixing the position of its carboxyl group in relation to those of the hydroxyl group and the double bond, and hence obtaining a larger partial structure for α -amyrenol.

The carboxyl group, considered by Jacobs and Fleck (45) to be tertiary on account of the resistance to hydrolysis shown by ursolic esters in general, has been at least approximately related to the double bond. When treated with bromine, ursolic acid, unlike oleanolic acid in the β-amyrin series (46), yielded only a minute amount of neutral brom-compound (47). This absence of brom-lactone formation led Huzii and Osumi to suggest that the carboxyl group was separated from the double bond by more than three carbon atoms (48).

It has, however, since been reported by Spring, that in addition to the formation of ketoacetylursolic acid by the chromic anhydride oxidation of acetylursolic

acid, a small amount of a neutral substance, probably a lactone, was also produced (25). This is assumed to have arisen from the lactonisation of a hydroxyl group, formed during the oxidation, and the carboxyl group. A similar explanation has been put forward by Ruzicka to account for the formation of two lactones obtained by him from the hydrogen peroxide oxidation of acetyl-ursolic acid (49). In addition, in both oxidations there is evidence for supposing that the hydroxyl groups involved in the lactonisations were in the immediate vicinity of the double bond. From this, both authors concluded that the carboxyl group must also be in the neighbourhood of the double bond.

This conclusion has been further substantiated by Spring (25) who has reported that ketoacetylursolic acid, containing the fragment (IX)(p.9), can be decarboxylated by refluxing in quinoline. The decarboxylation was thought to have been facilitated by the proximity of the carboxyl group to the carbonyl group, which in ketoacetylursolic acid is known to be situated immediately adjacent to the double bond (25). Hence, the carboxyl group must also be in the vicinity of the double bond.

Huzii and Osumi (48) have attempted to relate the

position of the carboxyl group to that of the hydroxyl group at C_2. They observed that no lactone formation took place when ursolic acid was treated with zinc chloride and acetic acid, and concluded from this that the carboxyl group and the hydroxyl group do not occupy 1-3 nor 1-4 positions to each other (50). However, Kuwada and Matsukawa (51) have reported the formation of an iso-lactone by the action of phosphorus pentachloride on ursolic acid, a fact which would indicate that the carboxyl group is in the neighbourhood of the hydroxyl group. This is obviously at variance with Huzii and Osumi's findings and consequently it is impossible to derive from it any evidence regarding the relative positions of the carboxyl group, and the hydroxyl group.

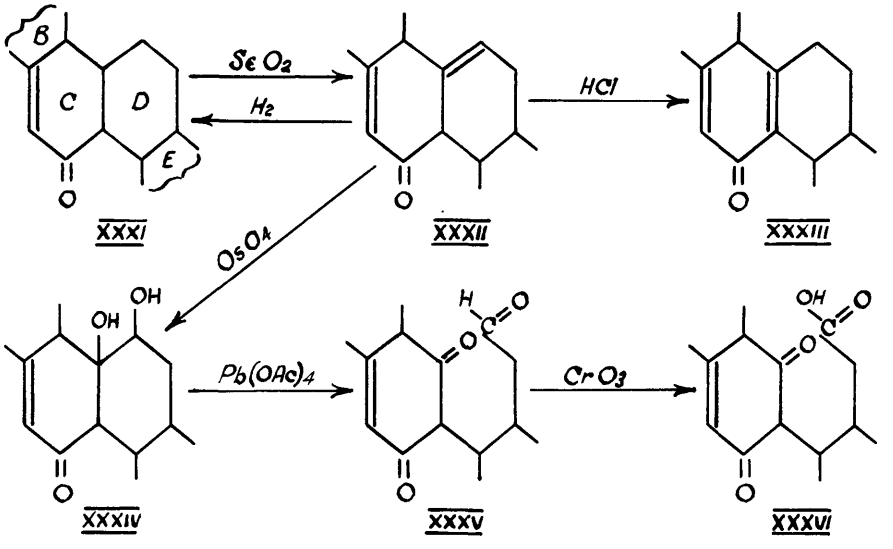
From the foregoing experiments therefore, it will be seen that although the precise location of the carboxyl group in ursolic acid has not yet been established, it has been shown to be situated near the double bond, i.e. on the basis of Ruzicka's formulation (p.29) for amyrenol (LVI), it could be considered as replacing one of the angular methyl groups in the neighbourhood of ring C.

THE OXIDATIVE DEGRADATION OF α-AMYRENOL.

Two series of experiments producing extensive oxidative degradation of the α-amyrenol molecule have recently been carried out by Ruzicka (24,52,53). The first of these involved the treatment of iso- α-amyrenonyl acetate (XXXI) with selenium dioxide and the subsequent degradation of the oxidation product, while the second was brought about by oxidation of α-amyranonyl acetate with nitric acid.

The oxidation of iso- α-amyrenonyl acetate with selenium dioxide was shown by Ruzicka (52), to introduce into the molecule a fresh double bond, which he assumed, on spectroscopic evidence, to be unconjugated with the α-β-unsaturated ketone chromophore originally present. When, however, the product of this oxidation, iso- α-amyradienonyl acetate, was treated with mineral acid an isomeric compound was obtained which was thought to contain the conjugated system $\geq C=\overset{|}{C}-CO-\overset{|}{C}=C\leq$ i.e. the new double bond has been conjugated with the original chromophore of iso- α-amyrenonyl acetate. That this original system had not been affected by the selenium dioxide oxidation was shown by the regeneration of iso- α-amyrenonyl acetate (XXXI) by catalytic hydrogenation of the oxidation product. These reactions, Ruzicka

formulated as follows, on the basis of the partial structure (XXXII) for iso-α-amyradienonyl acetate, and (XXXIII) for its conjugated isomer.

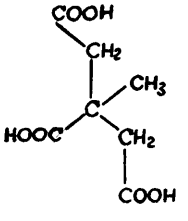


XXXI XXXII XXXIII XXXIV XXXV XXXVI

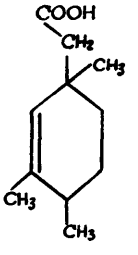
iso-α-Amyradienonyl acetate was next oxidised with osmium tetroxide which converted the isolated double bond into a glycol (XXXIV) treatment of which with lead tetracetate brought about opening of the ring which had originally contained the isolated double bond. Since mild oxidation of this product, with chromic anhydride, gave only a monobasic acid, Ruzicka concluded that one of the carbonyl groups produced must have been secondary while the other existed as an aldehyde. This keto-aldehyde he formulated as (XXXV), and the corresponding keto-acid as (XXXVI) above.

The degradation was continued by pyrolysis of the

keto-acid (XXXVI) when a mixture of products, separable into steam volatile and non-volatile fractions, was obtained. The steam volatile fraction which has been more exactly characterised than the non-volatile product was found to consist of a monobasic acid which on oxidative degradation yielded β-methyl-tricarballylic acid (XXXVII). The identification of this product made possible the unambiguous characterisation of the volatile acid (XXXVIII) which Ruzicka then assumed to result from the breakdown of rings D and E of the α-amyrenol molecule (53).



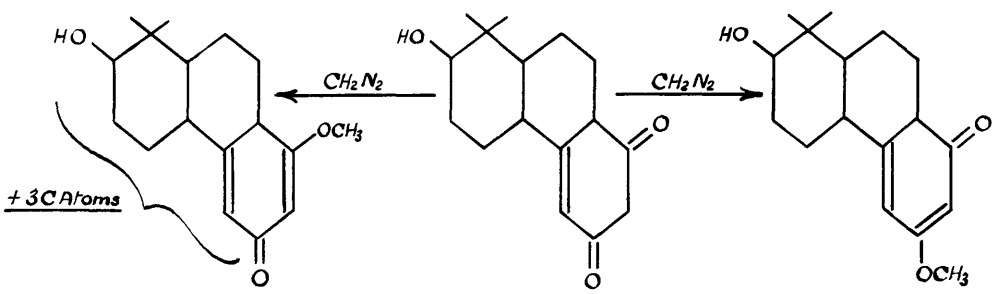
XXXVII



XXXVIII

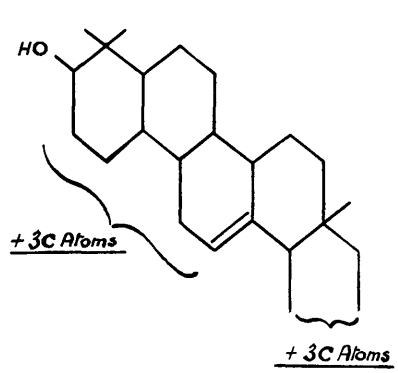
From the non-volatile fraction, after esterification with diazomethane, two main products were isolated. The presence in both of these esters of a hydroxyl group was demonstrated by acetylation, and it was assumed that this hydroxyl group was that originally located at C_2 in α-amyrenol. It was further inferred from analyses and from spectroscopic considerations that both compounds contained rings A, B, and C of the

α-amyrenol structure which led to their formulation as (XXXIX) and (XLI). They can therefore be considered as arising from the same parent (XL) by esterification, in turn, of both enolised ketone groups.



XXXIX XL XLI

The formulations for these non-volatile pyrolysis products have, however, never been satisfactorily substantiated. This will be discussed later, in the appropriate theoretical section, since on the basis of the foregoing experiments, Ruzicka put forward the following formulation (XLII) for α-amyrenol in which the double bond is located between C_{12} and C_{13} (53).

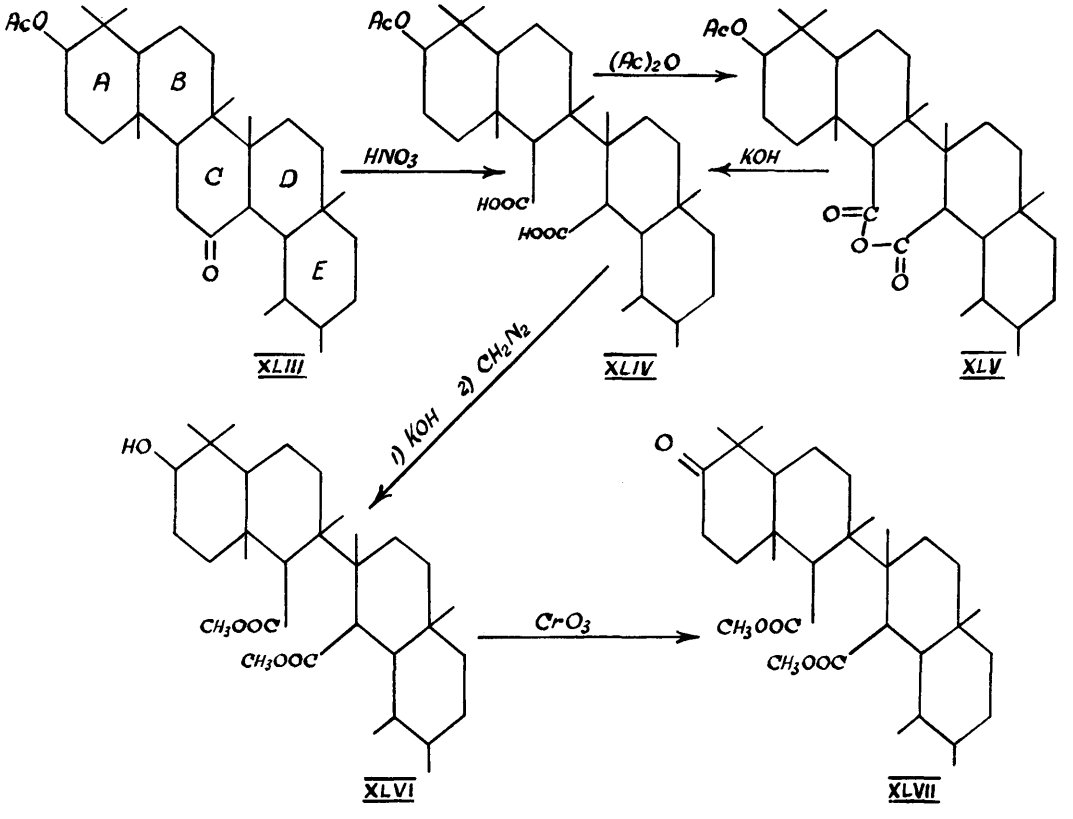


XLII

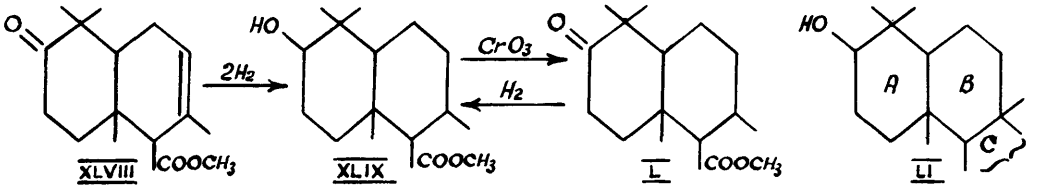
This formulation (XLII) while agreeing well with the foregoing reactions is at variance with the results obtained by the same author, from the nitric acid degradation of α-amyranonyl acetate (24).

α-Amyranonyl acetate (XLIII) on treatment with fuming nitric acid was found by Ruzicka to give an acid (XLIV), which although obtained only in an amorphous state itself, yielded a crystalline anhydride (XLV) on treatment with acetic anhydride (24). The acid, again amorphous, was regenerated by alkaline hydrolysis of this anhydride, and on esterification with diazomethane gave a dimethyl ester (XLVI) which was also amorphous. However, on oxidation of its secondary hydroxyl group to the corresponding ketone, this ester was converted into a crystalline compound (XLVII), pyrolysis of which gave a set of products separable into ketonic and non-ketonic fractions. Ruzicka (24) has formulated these reactions as shown below.

The ketonic fraction, which was thought to arise from rings A and B of the original structure, was found to consist of two keto esters (XLVIII and L) which differed only in that, while one was totally saturated, the other contained a double bond. Catalytic reduction of both esters gave a saturated hydroxy-ester which

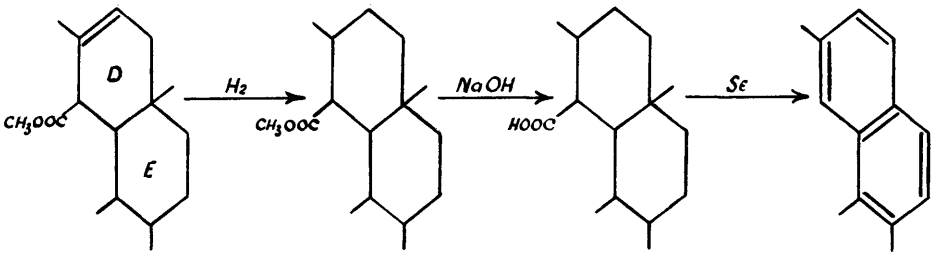


Ruzicka had already obtained, by pyrolysis, from oleanolic acid (54) and which he had supposed to contain, unchanged, the rings A and B of β-amyrenol. Thus he concluded that rings A and B of α- and β-amyrenol were identical, and could be represented by (LI).



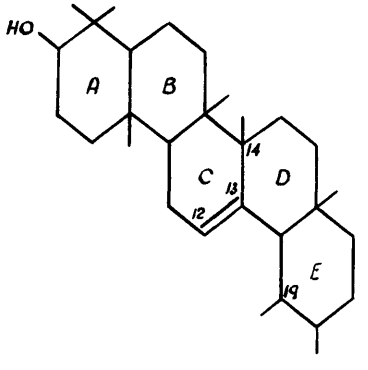
The non-ketonic fraction from the pyrolysis, was found to consist, as in the case of the ketonic fraction, of a mixture of saturated and unsaturated

esters (LII and LIII) but which in this case were believed to have arisen from rings D and E of the original structure. These esters were therefore hydrogenated and then hydrolysed giving a saturated acid (LIV), from which Ruzicka obtained, on selenium dehydrogenation, 1:2:7-trimethylnaphthalene (LV) one of the products supposed to result from the dehydrogenation of rings D and E of α-amyrenol itself. (p.4)

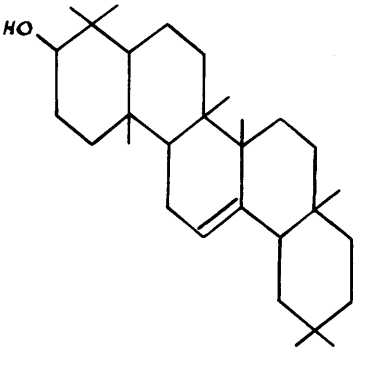


LII LIII LIV LV

Consideration of these degradation products led Ruzicka to formulate α-amyrenol as (LVI), which, while agreeing with his previous formulation (XLII) in the location of the double bond, is at variance with it in postulating an angular methyl group at C_{14}. With a methyl group attached to C_{14} and with the double bond



LVI



LVII

located between C_{12} and C_{13} the formation of the selenium dioxide oxidation products from iso-α-amyrenonyl acetate cannot easily be explained.

In addition, this formulation (LVI) for α-amyrenol differs from that of β-amyrenol (LVII) only in the location of a single methyl group in ring E. Ruzicka has therefore sought to show that the differences in reactivity between the α-amyrin and the β-amyrin series can be attributed entirely to the steric influence of the methyl group at C_{19}, a conclusion scarcely justified, especially on consideration of the dehydration evidence. Further discussion of this formulation and of the oxidative degradations described in this section will be presented in the appropriate theoretical part.

THEORETICAL

INTRODUCTION

From a consideration of the evidence already reviewed in the Historical section, it will be seen that the problem of ascribing a structural formula to α-amyrenol in particular and to the α-amyrin group in general, has been resolved into the locating of eight angular methyl groups about a hydropicene skeleton containing one double bond situated in one of two possible positions.

Of the methyl groups, the positions of three, the pair at C_1 and that at C_{20}, can be considered as having been fixed with a degree of certainty. Attempts at locating the remainder have been made by investigating the part played in various reactions of ursolic acid, by the carboxyl group since this labels a position in the α-amyrenol molecule normally inert.

The exact location of the double bond has been attempted from a study of reactions involving the conjugated double bond of *l*-α-amyradiene, produced by dehydration of α-amyrenol with phosphorus pentoxide.

The evidence gained from these experiments will be discussed in the succeeding sections of this Theoretical part.

l-α-AMYRADIENE AND ITS OXIDATION PRODUCTS.

Reference has previously been made (p.15) to the evidence on the basis of which, *l*-α-amyradiene is regarded as containing a conjugated diene system spread over two rings. Although the existence of this chromophore is well established, its exact location, involving that of the double bond of α-amyrenol, is still in doubt. It was therefore with a view to elucidating this relationship between the double bond and the hydroxyl group, and to establishing the precise location of the double bond in α-amyrenol, that this investigation of *l*-α-amyradiene was initiated.

Earlier work has shown that both *d*-α-amyradiene-I, obtained by the dehydration of α-amyrenol with phosphorus pentachloride (4,37,40), and *d*-α-amyradiene-II from the pyrolysis of α-amyrenyl benzoate (38,40) can be catalytically reduced to their corresponding dihydro compounds (39,40). However, neither by catalytic methods nor by using sodium and amyl alcohol has it been found possible to hydrogenate either of the double bonds of *l*-α-amyradiene (56). In addition, *l*-α-amyradiene has been shown to be inert towards maleic anhydride, and towards such oxidising agents as

potassium permanganate and selenium dioxide (41). Thus it would appear that in ℓ-α-amyradiene both ethylenic linkages occupy sterically hindered positions whereas in the α-α-amyradienes one only of the bonds is thus affected.

However, when treated with chromic anhydride, ℓ-α-amyradiene has been reported by Ewen (36), to yield ℓ-α-amyrenone by oxidation of one of the double bonds, thus replacing the original diene system by an α-β-unsaturated ketone grouping. It has subsequently been reported (56), that a neutral substance containing two oxygen atoms is produced by the chromic anhydride oxidation of ℓ-α-amyradiene. No relationship could be shown to exist between these compounds, however, since although the second product showed no depression in melting point when mixed with ℓ-α-amyrenone it also displayed none of the latter's ultra-violet light absorption.

A further oxidation product, believed to be a monoxide, was obtained by Newbold (56) on treatment of ℓ-α-amyradiene with perbenzoic acid. This differed from the two products already obtained in that it did not contain an α-β-unsaturated ketone chromophore although it did show an ultra-violet light absorption

maximum at 2390Å, ε= 3,100.

Since no conclusions can be satisfactorily drawn from these experiments the oxidation of ℓ-α-amyradiene has been reinvestigated.

When treated with sufficient chromic anhydride to oxidise one of its double bonds, ℓ-α-amyradiene was found to yield a compound, $C_{30}H_{48}O$, of melting point 218-220° which showed no light absorption in the ultra-violet and which gave a yellow coloration with tetra-nitromethane. The substance therefore contained no α-β-unsaturated ketone grouping and hence in this respect differed from the ℓ-α-amyrenone obtained by Ewen (36). It also differed in having a specific rotation in chloroform of -70° in comparison with the value of -220° previously reported.

A second product, of melting point 235-237°, was obtained from the oxidation of ℓ-α-amyradiene, when a large excess of chromic anhydride was employed. This same compound was also obtained by the further oxidation with chromic anhydride of the product $C_{30}H_{48}O$ already described. It was found on analysis to have the molecular formula $C_{30}H_{46}O_2$, and to contain an α-β-unsaturated ketone group since it displayed a maximum light absorption in ethanol at 2500Å, ε= 12,900, and

gave no coloration with tetranitromethane.

With regard to these compounds, it should be stated that they were obtained in a pure state only after prolonged purification involving fractional crystallisation chromatographic methods proving unsuccessful. This process which was complicated by the fact that when mixed with ℓ-α-amyradiene or with each other, both oxidation products gave mixtures whose melting points were intermediate to those of the pure compounds. In addition, both compounds showed a marked tendency to form a series of well defined mixed crystals with ℓ-α-amyradiene. This is illustrated by the fact that during the oxidation of ℓ-α-amyradiene to the product $C_{30}H_{48}O$, two substances later identified as mixed crystals were also formed. Of these, one was identified, by comparison with the synthetic mixed crystal prepared from four parts of ℓ-α-amyradiene and one part of the pure oxidation product. The other, by calculation assumed to be a mixture of three parts of the mono-oxidation product to one part of ℓ-α-amyradiene, had a melting point of 209-211° and showed a light absorption at 2410Å, ε= 3,500. These are in good agreement with the values reported by Newbold (p.33; 56) for the compound obtained from the perbenzoic

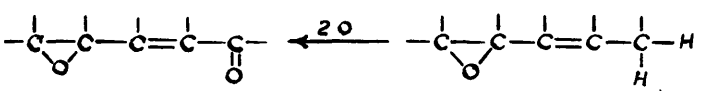
acid oxidation of ℓ-α-amyradiene, namely, a light absorption maximum in ethanol of 2390Å, ε= 3,100, and a melting point of 205-206°. In addition when the substances were mixed, the melting point was 209-211° showing no depression.

The conclusion suggested by these facts that the ℓ-α-amyradiene oxide obtained by Newbold is a mixed crystal and that the compound, $C_{30}H_{48}O$, is in fact the pure ℓ-α-amyradiene oxide has been substantiated by the fact that the latter shows none of the low intensity ultra-violet light absorption characteristic of an isolated carbonyl group (57). The product, $C_{30}H_{48}O$, will therefore be referred to in the future as ℓ-α-amyradiene oxide.

ℓ-α-Amyradiene oxide was found to be unaffected by a solution of hydrogen chloride in chloroform which treatment has been reported by Ruzicka (57) to produce immediate isomerisation of α-α-amyradiene-I oxide, to the corresponding ketone. From this it would appear that the carbon atoms at each end of the oxide link in ℓ-α-amyradiene oxide are both tertiary.

Reference has already been made (p.34) to the fact that ℓ-α-amyradiene oxide when treated with chromic anhydride yielded a compound of molecular formula $C_{30}H_{46}O_2$. The formation of this compound, ℓ-α-amyrenone oxide,

was accompanied by the production of an α-β-unsaturated ketone chromophore which can be accounted for by assuming the oxidation to a ketone of a methylene group adjacent to the double bond in a manner analogous to that occurring in the production of α-amyrenonyl acetate from α-amyrenyl acetate (p.9). Like l-α-amyradiene oxide, l-α-amyrenone oxide was unaffected by hydrogen chloride in chloroform, and can therefore be represented as containing the fragment (LVIII) on the basis of which l-α-amyradiene oxide contains (LIX).

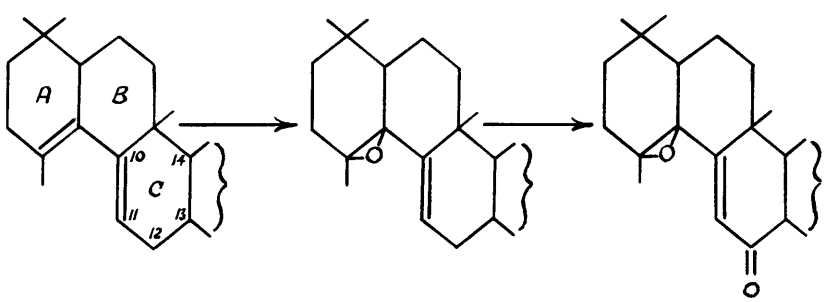


LVIII LIX

The relating of these fragments with the known environment of the double bond of α-amyrenol can be simply accomplished if it is assumed that the position of this double bond remains unaffected throughout the formation of l-α-amyradiene. It then follows that the oxide link cannot have been formed by reaction of the original double bond since this has been shown to contain a secondary carbon atom (p.11), and an oxide thus formed would easily isomerise to the corresponding ketone. Consequently, the double bond in l-α-amyradiene oxide (LIX) can be regarded as occupying the

position of the double bond in α-amyrenol, and the oxide link as marking the position of the double bond which was introduced in the dehydration, and which contains two tertiary carbon atoms.

These findings are in agreement with the formulation (LX) for *l*- α-amyradiene, tentatively put forward by Spring (41), and from which *l*- α-amyradiene oxide can be formulated as (LXI) and *l*- α-amyrenone oxide as (LXII).



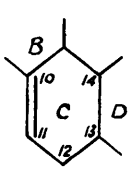
LX LXI LXII

However, these partial structures are based on the assumption that the double bond situated between C_{10} and C_{11} in *l*- α-amyradiene (LX) is the original double bond of α-amyrenol. This is at variance with the formulation recently put forward by Ruzicka (24) for α-amyrenol (LVI, p.29) in which the double bond was located at C_{12}-C_{13}, a situation also postulated for the double bond of *β*-amyrenol. Since, in addition, this formulation assumed that rings A, B, and C were identical

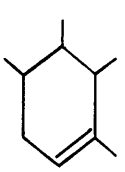
in both amyrenols, it becomes increasingly difficult to account for their dehydration products.

β-Amyrenol when dehydrated under conditions similar to those employed for α-amyrenol gives only compounds in which the diene system produced is not conjugated and which preserve the original dextrarotation shown by β-amyrenol (4,37,40,55). Thus only dehydration products corresponding to the *d*- α-amyradienes are obtained, the counterpart of *l*- α-amyradiene being unknown. This difference in behaviour has hitherto been accounted for by assuming that the unsaturated centre in α-amyrenol differs from that existing in β-amyrenol either by virtue of the location of the double bond itself or by the presence in β-amyrenol of a methyl group differently placed in α-amyrenol.

On the basis of the first of these two alternatives, the unsaturated centres of α- and β-amyrenol can be represented, respectively, by the partial formulations (LXIII) and (LXIV), thus accounting reasonably for the dehydration reactions. Since however it is impossible



LXIII



LXIV

to obtain conclusive evidence either for or against these structures from a purely chemical investigation of derivatives of the amyrin series, attention was turned to their optical properties.

Wallis (59, 60), in an extension of the work initiated by Callow and Young (61) has demonstrated in compounds of the steroid group that optical rotatory power is directly related to molecular structure. These results have been summarised by Barton (62) as showing that "if a certain change be effected in two steroid molecules differing from one another in a portion of the molecule far removed from the reacting centre, then the variation in molecular rotation ($M[\alpha]$) will be approximately the same in each case." If then, α- and β-amyrenol are considered to be two molecules of substantially the same structure, it should be possible to decide by this method, whether or not their double bonds are similarly located, and hence whether or not (LXIII) and (LXIV) are valid representations. Accordingly a comparison, shown below in table (LXV), has been made of the optical properties of certain compounds derived analogously from both amyrenols, by reactions confined in all cases to the immediate vicinity of the double bond.

		$M[\alpha] \times 10^{-2}$		Refs.
		α	β	
A	Amyrenyl acetate.	360	379	63
B	Amyrenonyl acetate.	757	762	30,58
C	Amyradienyl acetate.	1533	1542	36,64,30
D	iso-Amyrenonyl acetate.	328	356	65
E	Amyrenol.	354	375	63
F	Ethyl-amyratriol	234	244	64
	Δ_{AB}	397	383	
	Δ_{AC}	1173	1163	
	Δ_{AD}	-32	-23	
	Δ_{EF}	-120	-131	

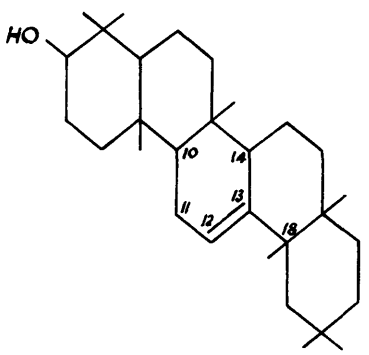
LXV

From the table (LXV) above, in which the molecular rotations, ($M[\alpha]$) have been calculated from specific rotations determined in all cases, in chloroform, it will be seen that the oxidation of α-amyrenyl acetate to α-amyrenonyl acetate has produced a change in molecular rotation, Δ_{AB}, of +397°, while a similar oxidation in the β-series has caused an approximately equal increase of +383°. In addition, the differences in molecular rotation between α-amyrenyl acetate and α-amyradienyl acetate, Δ_{AC}, between α-amyrenol and ethyl-α-amyratriol, Δ_{EF}, and between α-amyrenyl acetate and iso-α-amyrenonyl acetate, Δ_{AD}, are also approximately equal to those occurring between analogous

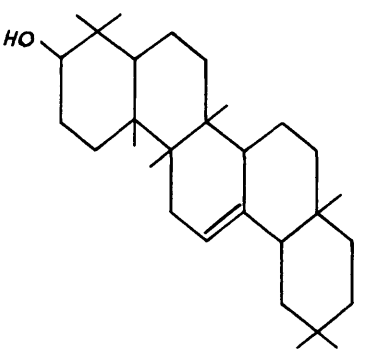
compounds in the β-series. From this parallel behaviour of the two series, which is by no means limited to the compounds listed above, and on the basis of the work of Wallis (59,60) and Barton (62) already mentioned (p.40) it would appear that the double bond of α-amyrenol, and that of β-amyrenol must occupy identical positions in closely related structures. If this is so, it follows that such formulations as (LXIII) and (LXIV) for α- and β-amyrenol respectively, are inadmissable, and hence the first of the two alternatives (p.39) offered by the dehydration evidence is ruled out.

The remaining conclusion is that α- and β-amyrenol having the same location for their double bond must differ by the inclusion in the β-structure of a methyl group in such a position as to prevent the formation of a conjugated diene on dehydration. Regarding this, it is noteworthy that there exists between the two series, one further major difference, namely that bromine which with β-amyrenonyl esters produces partial dehydrogenation, has no effect on α-amyrenonyl esters (p.12). This has been taken as being indicative of the presence in α-amyrenol near the double bond, of an angular methyl group not similarly located in the β-isomer. Thus if the evidence is accepted that the double bonds are similarly

located, the differences between the two series would appear to amount to the fact that α-amyrenol, in addition to containing an angular methyl group where none is present in the β-series, also carries a vacant angular position at which a methyl group is however located in the β-series. This can be most easily explained by assuming that one methyl group is responsible for both differences, as is shown in such formulations as (LXVI) and (LXVII), for α- and β-amyrenol respectively.



LXVI



LXVII

With regard to the dehydration evidence, it will be seen that the formulation previously obtained for α-amyradiene (LX, p.38) can be accounted for by assuming that the double bond at C_{12}-C_{13} in α-amyrenol migrates to C_{10}-C_{11} during the dehydration, an analogous migration in the β-series being impossible.

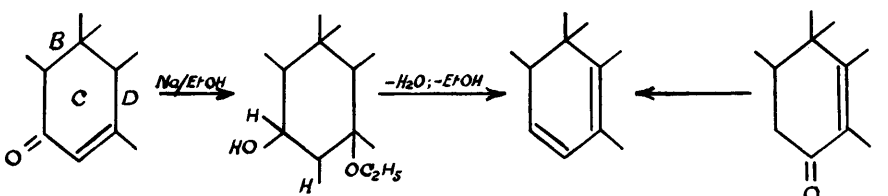
Evidence regarding the possibility of such a

migration can be gathered from the reaction of α-amyrenonol with phosphorus pentoxide. Spring (41) has shown that α-amyrenonol on dehydration with this reagent, which produced *l*- α-amyradiene from α-amyrenol, gave a compound, α-amyradienone-II which displayed none of the characteristic laevorotation or light absorption of *l*- α-amyradiene. This can be explained by supposing the ketone group of α-amyrenonol to be situated at C_{11}, thus preventing migration of the double bond to form a conjugated diene in dehydration.

This supposed migration also gives an explanation of the optical properties of the oxidation products of *l*- α-amyradiene. The oxidation of *l*- α-amyradiene oxide to *l*- α-amyrenone oxide is accompanied by an appreciable negative change in rotatory power, whereas the oxidation of α-amyrenyl acetate to α-amyrenonyl acetate produces a substantial positive change. Hence it would seem likely that the α-β-unsaturated ketone grouping in *l*- α-amyrenone oxide differs in location from that in α-amyrenonol. If it is then assumed that the double bond introduced by the dehydration is not affected in the formation of *l*- α-amyrenone oxide from *l*- α-amyradiene oxide, it follows that the original double bond has migrated.

From the foregoing evidence, therefore, it will be seen that the conclusion that the double bond is capable of migration, assumed in the formulations (LXVI) for α-amyrenol, and (LX) for /- α-amyradiene, finds some support in the dehydration reactions.

If, however, α- and β-amyrenol both contain a double bond at C_{12}-C_{13} it should be possible to assign to those compounds showing parallel optical properties between the two series, and which are listed in table (LXV), formulae which will be identical if the differences at C_{10} and C_{18} are excluded. On this basis, the reduction of α-amyrenonol (LXVIII) through its intermediate addition compound, ethyl- α-amyratriol (LXIX) to α-amyradienol (LXX) and the reduction of <u>iso</u>- α-amyrenonol (LXXI) to the same compound can be represented as shown below. Since these partial structures show



LXVIII LXIX LXX LXXI

no changes at positions C_{10} and C_{18} throughout the series of reactions, they can be regarded as applying equally well to the corresponding reactions in the β-series. Thus it is possible to formulate in an identical manner, excluding positions C_{10} and C_{18}, those

compounds of both series already shown by molecular rotation differences to have a common structure.

The structure (LXVI) derived for α-amyrenol from a consideration of its dehydration products and substantiated by a study of the optical properties of certain of its derivatives appears therefore also to give a reasonable account of the general reactions of the α-amyrin series. Further discussion of this formulation (LXVI) in the light of the behaviour of certain ursolic acid derivatives will be presented in succeeding sections.

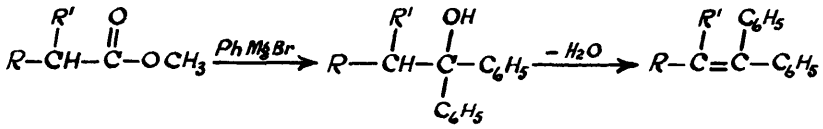
THE NATURE OF THE CARBOXYL GROUP OF URSOLIC ACID.

The investigation of ursolic acid initiated with a view to the elucidation of the structure of the amyrin groups in general, is concerned mainly with those reactions in which the carboxyl group is involved, since it is in this respect only that ursolic acid differs from α-amyrenol. However, before dealing in detail with these reactions, it is convenient to consider first, the nature of the carboxyl group itself.

The only indication as to the nature of the carboxyl group of ursolic acid has been supplied by Jacobs and Fleck (45) who having shown that the esters of ursolic acid and its derivatives could be hydrolysed by alkali, only with extreme difficulty, concluded that the carboxyl group was tertiary. This conclusion was considered reasonable since most formulations suggested for α-amyrenol have represented all the extra-nuclear methyl groups as occupying tertiary positions.

A more conclusive proof of the nature of the carboxyl group, however, should be possible by a method using reactions similar to those employed in the Barbier degradation which although developed for straight chain acids (66) has been shown to be applicable to compounds

of the sterol group, e.g. desoxycholic acid (67). The method consisting of two stages is outlined below. The first stage, involving the formation of a diphenylcarbinol (LXXIII) by the action of phenylmagnesium bromide on the methyl ester of a given acid should take



LXXII LXXIII LXXIV

place whether the carboxyl group is secondary or tertiary, but the second in which the diphenylethylene derivative (LXXIV) is produced by dehydration of this diphenylcarbinol will proceed only if the carbon atom adjacent to the carboxyl group carries a hydrogen atom, i.e. if the carboxyl group is primary or secondary. Thus the failure of a diphenylcarbinol to dehydrate can be taken in general as being indicative of the tertiary nature of the original carboxyl group.

When attempts were made to apply this method to the carboxyl group of ursolic acid it was found that methyl acetylursolate was unaffected by phenylmagnesium bromide under a variety of conditions. The required diphenylcarbinol, $C_{44}H_{60}O_3$, of melting point 242-244°, was finally obtained, however, by the action of phenylmagnesium bromide on the acid chloride of acetylursolic

acid. This compound resisted all attempts at dehydration, and since these involved conditions usually effective in producing dehydration in the sterol series (68), it is reasonable to assume that this was due to the tertiary nature of the carboxyl group, rather than to any extraordinary stability of a compound having a structure of the type (LXXIII).

If its tertiary nature is assumed the carboxyl group can be located at any one of the six positions occupied by a methyl group in the formulation (LXVI, p.43) for α-amyrenol. Attempts at a more precise location will be presented in the following sections.

KETOACETYLURSOLIC ACID AND RELATED OXIDATION PRODUCTS OF URSOLIC ACID.

The experiments to be described in this section have as their aim the relating of the carboxyl group present in ursolic acid with an active group whose position can be accurately determined. This has usually been attempted either by observing the effect on the thermal stability of the carboxyl group caused by the introduction into its neighbourhood of a carbonyl group, or by attempts at lactonisation between the carboxyl group and a hydroxyl group whose location has already been established.

Applying the first of these methods, acetylursolic acid was oxidised with chromic anhydride, yielding as the main product, an acid, $C_{32}H_{48}O_5$, of melting point 323-325°, and $[\alpha]_D$ = +89° which showed a light absorption maximum at 2500Å of intensity 12,500, and which gave no coloration with tetranitromethane. In addition there was obtained a small amount of a lactone, $C_{32}H_{48}O_6$, of melting point 302-304° (α) which appeared to be identical to that previously obtained by Ewen and Spring (25). The acid was further characterised by its ethyl ester of melting point 215-216°, and by its methyl ester of melting point 243-245°. These properties, with the exception

of the specific rotation, are all in close agreement with those reported by Ewen and Spring (25) for ketoacetylursolic acid which the same authors described as having a structure similar to that of α-amyrenonol. The product ketoacetylursolic acid can therefore be assumed to contain a carbonyl group immediately adjacent to the original double bond of ursolic acid.

When refluxed in quinoline, without the exclusion of air, ketoacetylursolic acid was converted into a substance, $C_{31}H_{46}O_3$, of melting point 206-208°, which had a specific rotation in chloroform of +45°. In these respects the substance is identical with the nor-α-amyradienonyl acetate obtained from a similar reaction by Ewen and Spring (25). However, nor-α-amyradienonyl acetate was reported to display a light absorption maximum at 3000Å with a molecular extinction coefficient of 10,000, whereas this substance although showing a light absorption maximum in the same region, 2980Å, has a molecular extinction coefficient of only 5,700, even after repeated recrystallisation. Thus it would appear that the product was in fact a mixture of nor-α-amyradienonyl acetate and a closely related compound showing no selective light absorption whatever.

In this connection it is of interest to note that

the decarboxylation of ketoacetyloleanolic acid under conditions similar to those in the above reaction produced a compound $C_{31}H_{48}O_3$, nor-β-amyradienonyl acetate, which was reported by Ruzicka (69) to have a molecular extinction coefficient of 21,000 at 2975Å. It is therefore possible that the compound obtained by Ewen and Spring having a molecular extinction coefficient of 10,000, may also be a mixture of nor-α-amyradienonyl acetate and a compound showing no light absorption in the ultra-violet region, although in different proportions to the substance having a light absorption maximum at 2980Å, ε = 5,700, obtained above.

The formation of this substance, $C_{31}H_{48}O_3$, was accompanied by the production of much dark resinous material which greatly hindered its purification. Consequently, in an attempt to minimise the formation of these impurities, thought to be caused by the oxidation of either the quinoline or the decarboxylation product itself, the reaction was next carried out in an atmosphere of nitrogen. In this way, a compound, $C_{31}H_{48}O_3$, and which had a melting point of 210-212°, was obtained. This substance, nor-α-amyrenonyl acetate, although similar to nor-α-amyradienonyl acetate in producing a yellow coloration with tetranitromethane,

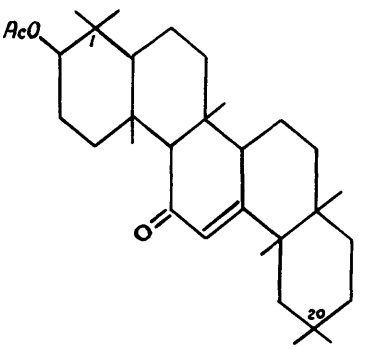
differed by showing no selective light absorption in the ultra-violet region. In addition, the compound was shown to contain only one double bond, since on titration with perbenzoic acid approximately one atom of oxygen was absorbed, and the resulting product gave neither a coloration with tetranitromethane nor showed any selective ultra-violet light absorption. On hydrolysis, nor- α-amyrenonyl acetate, gave nor- α-amyrenonol of melting point 254-256° and specific rotation +52°, which on reacetylation gave nor- α-amyrenonyl acetate identical with that already obtained. It will be seen therefore that the suggestion made above that the substance $C_{31}H_{46}O_3$ obtained by refluxing ketoacetylursolic acid in quinoline in the presence of air, was in fact a mixture, has been at least partly substantiated. In addition it is noteworthy that nor- α-amyrenonyl acetate and the nor- α-amyradienonyl acetate of Ewen and Spring (25) have physical properties, excluding light absorption, which are almost identical, and hence the possibility remains that this latter compound is a mixture.

During the formation of nor- α-amyrenonyl acetate, the evolution was observed of a quantity of carbon dioxide approximately equivalent to one carboxyl group. Since this was the only evidence that the reactions

described above were in fact due to decarboxylation, α-amyrenonyl acetate, having a structure believed to be exactly similar to that of ketoacetylursolic acid excluding the carboxyl group, was subjected to the same conditions as those responsible for the formation of nor- α-amyrenonyl acetate. No carbon dioxide was evolved, however, and the α-amyrenonyl acetate was recovered unchanged, almost quantitatively. Since acetylursolic acid was also recovered unchanged after similar treatment the presence in ketoacetylursolic acid of the carbonyl group has been shown to be necessary for decarboxylation. This being so, the formation of nor- α-amyrenonyl acetate can be assumed to be caused by simple decarboxylation of ketoacetylursolic acid followed by migration of the double bond, while the formation of nor- α-amyradienonyl acetate would appear to involve decarboxylation followed by the introduction of an additional double bond by aerial oxidation.

Ketoacetylursolic acid as has already been mentioned, is assumed to have a structure identical with that of α-amyrenonyl acetate with the exception that it contains a tertiary carboxyl group in place of one of the angular methyl groups of the latter. Hence if α-amyrenonyl acetate is formulated as (LXXV) by derivation from the

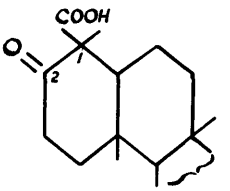
structure (LXVI, p.43) for α-amyrenol, it will be seen that the carboxyl group of ketoacetylursolic acid can be located at any one of six positions. However, consideration of the influence of the ketone group in the decarboxylation would appear to exclude the possibility of the carboxyl group being located at either C_1 or C_{20}.



LXXV

It is possible to support this conclusion at least regarding position C_1 in the following manner.

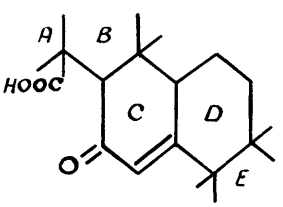
When ursolic acid was treated with chromic anhydride under the conditions described by Jacobs and Fleck (45) the hydroxyl group at C_2 was oxidised to a ketone, giving ursonic acid identical with that obtained by these authors. If the carboxyl group of ursolic acid is located at C_1, ursonic acid can be represented by (LXXVI), and would be expected to decarboxylate readily. However, when subjected to the same treatment as was used for the decarboxylation of ketoacetylursolic acid, ursonic acid



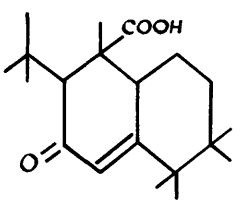
LXXVI

remained unchanged, and hence the possibility of the carboxyl group being at C_1 appears to be excluded.

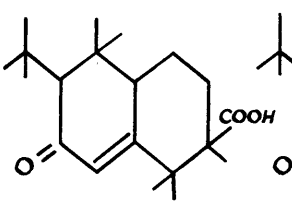
On the basis of the formulation (LXXV) for α-amyrenonyl acetate, therefore, ketoacetylursolic acid can be represented by any of the four structures, shown below, from which it should be possible to derive satisfactory formulations for <u>nor</u>- α-amyrenonyl acetate and for <u>nor</u>- α-amyradienonyl acetate. The latter,



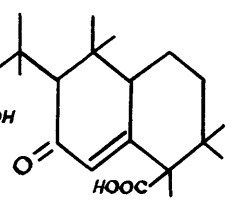
LXXVII



LXXVIII



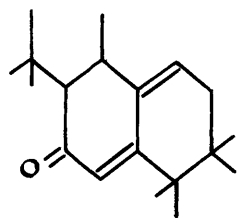
LXXIX



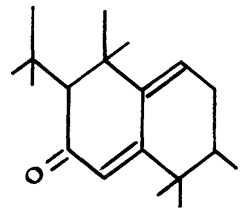
LXXX

however, shown by Spring (25) to contain the system $-CO-\dot{C}=\dot{C}-C=C<$ spread over two rings cannot readily be formulated from (LXVII) for ketoacetylursolic acid, and consequently the presence at C_5 of the carboxyl group of ursolic acid is doubtful. If the structures (LXXVIII) and (LXXIX) for ketoacetylursolic acid are next considered it will be seen that formulations for <u>nor</u>- α-amyradienonyl acetate containing the necessary

chromophore can only be derived by assuming migration of the double bond formed by aerial oxidation after the decarboxylation; nor- α-amyradienonyl acetate would then be represented by (LXXXI) and (LXXXII) respectively.

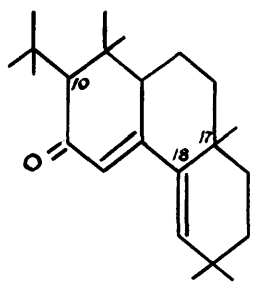


LXXXI

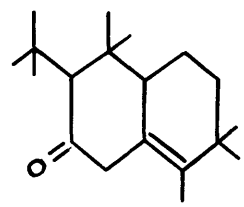


LXXXII

A simpler explanation of the formation of nor- α-amyradienonyl acetate can, however, be gained from a consideration of the structure (LXXX) for ketoacetyl-ursolic acid in which the carboxyl group is located at C_{18}. From this nor- α-amyradienonyl acetate can be formulated as (LXXXIII).



LXXXIII



LXXXIV

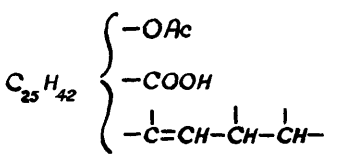
Thus of the four formulations for ketoacetylursolic acid derived from (LXVI, p.43) for α-amyrenol the most reasonable explanation of the formation of nor- α-

amyradienonyl acetate is given by (LXXX), in which the carboxyl group is situated at C_{18}.

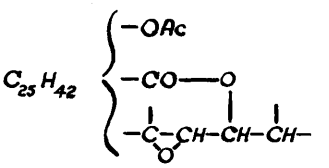
The formation of nor-α-amyrenonyl acetate, during which the α-β-unsaturated ketone grouping of keto-acetylursolic acid has been destroyed, can be satisfactorily accounted for by assuming the migration, after decarboxylation, of the double bond to a position hitherto blocked by the carboxyl group. Hence, of the four locations for the carboxyl group considered above, i.e. C_5, C_9, C_{17} and C_{18}, C_5 and C_{17} would appear to be too far removed from the double bond to exert such an influence, whereas the location of a carboxyl group at C_9 or C_{18} could relatively easily give rise to nor-α-amyrenonyl acetate. If the carboxyl group of ketoacetyl-ursolic acid is located at C_{18}, nor-α-amyrenonyl acetate can be satisfactorily represented by (LXXIV), shown above. The partial structure (LXXX) for ketoacetylursolic acid, can therefore account reasonably for both decarboxylation products.

The location of the carboxyl group of ursolic acid at C_{18}, is also in agreement with the behaviour of acetylursolic acid towards hydrogen peroxide. The main product obtained from this reaction was an acetyl-lactone, $C_{32}H_{48}O_5$, of melting point 275-277°, which was found to

be saturated to tetranitromethane. From the molecular formula of this substance it will be seen that after assuming the presence of an acetoxy group and a lactone ring, one oxygen atom remains to be accounted for. This has been supposed to be present as an oxide, since the compound was unaffected by acetic anhydride in pyridine, and would appear on spectrographic evidence to contain no ketone group, outside of the lactone ring. The substance was therefore identical with the oxido-lactone prepared by Ruzicka (49), and which he represented by (LXXXVI) in terms of (LXXXV) for acetylursolic acid.



LXXXV



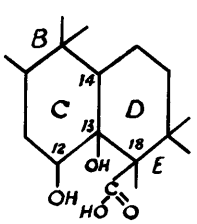
LXXXVI

In the formulation (LXXXVI), Ruzicka has assumed that the lactone has been formed between the carboxyl group, and a >CHOH group produced by oxidation of the methylene group immediately adjacent to the double bond, which has itself been oxidised. While the formation of the oxide across the double bond seems not unlikely, especially in view of the fact that a small amount of oxido-acetylursolic acid (p.65) was also recovered from the reaction mixture, the oxidation of a methylene

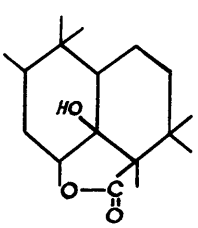
group under these reaction conditions, seems improbable.

In this connection it is noteworthy that when methyl acetylursolate was treated with perbenzoic acid no oxide formation took place. The ester was also stable to oxidation by potassium permanganate, and by selenium dioxide.

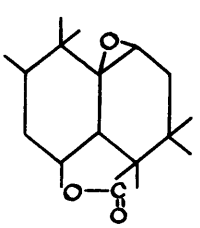
A more probable explanation of the lactone formation can be gained by supposing that the action of hydrogen peroxide on acetylursolic acid, first produces a glycol system at C_{12}-C_{18}, as represented by (LXXXVII), which on dehydration between the hydroxyl group at C_{12}, and the



LXXXVII



LXXXVIII



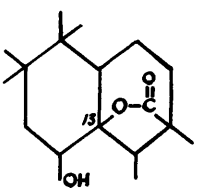
LXXXIX

carboxyl group at C_{18}, yields the saturated hydroxy-lactone (LXXXVIII). If the tertiary hydroxyl group is now supposed to dehydrate with the hydrogen atom adjacent at C_{14}, the double bond is free to migrate, and on oxidation with excess hydrogen peroxide can yield the oxido-lactone (LXXXIX).

The formation of small amounts of oxido-acetylursolic acid can also be accounted for, on the basis of this

formulation, by assuming that the diol (LXXXVII) decomposes with the loss of water forming an oxide bridge. In addition, it will be seen that the introduction of a double bond at C_{18}-C_{14} is similar to the formation from amyl- α-amyratriol, of one of the double bonds of α-amyradienol.

This formulation (LXXXIX) for the lactone, can be supported at least partly by consideration of the hydrogen peroxide oxidation of the β-amyrenol derivative, acetyloleanolic acid, in which the carboxyl group is thought to be located at C_{17}. The product obtained was a saturated hydroxy-lactone (70), which can be formulated as (XC), analogous to the hypothetical hydroxy-lactone (LXXXVIII) from acetylursolic acid.



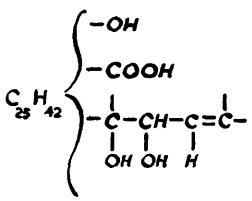
XC

In this case, however, no further dehydration can be expected since the hydroxyl group at C_{18}, supposed to dehydrate in the ursolic acid derivatives has already been incorporated in the lactone ring.

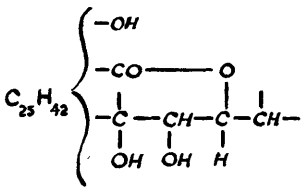
The representation (LXXXIX) for the lactone, can therefore be regarded as showing that the oxidations of acetylursolic acid and acetyloleanolic acid although

resulting in different types of products can be accounted for by the same initial reaction of their respective double bonds, a fact for which Ruzicka's formulation (LXXXVI) makes no allowance.

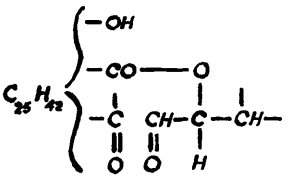
Further degradative work carried out on the lactone by Ruzicka (49) can be explained at least equally as well by the formulation(LXXXIX) as by the partial structure (LXXXVI) of Ruzicka. On treatment with potassium hydroxide, the lactone was reported to yield two products represented by Ruzicka as (XCI) and (XCII). In each of these, the oxide bridge has been replaced by a glycol system, and in one (XCI), the lactone ring has



XCI



XCII

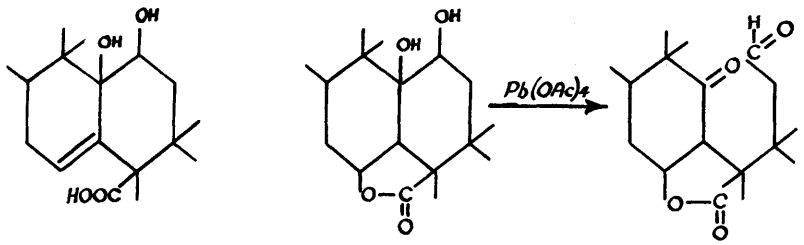


XCIII

also been broken with subsequent dehydration of the freed hydroxyl group, to give a double bond. Ruzicka considers that the glycol system is at the position originally occupied by the double bond of ursolic acid, and has supposed that a new double bond has been formed after the rupture of the lactone ring.

Each of these conclusions is open to criticism on the grounds that such a glycol system would be expected to dehydrate with the formation of a compound similar to α-amyranonol or the corresponding oxide, whereas the hydroxyl group freed from the lactone ring would be expected to be stable.

On the basis of (LXXXIX) for the oxido-lactone, the unsaturated acid formulated as (XCI) by Ruzicka (p.62) and the glycol-lactone formulated as (XCII) can therefore be more satisfactorily represented by (XCIV) and (XCV) respectively.



XCIV XCV XCVI

Ruzicka has also reported (49) that the glycol-lactone, formulated by him as (XCII), on treatment with lead tetracetate yielded a compound (XCIII), in which the glycol system had been replaced by a ketone and an aldehyde group. It will be seen that the formulation (XCV) above, for the glycol-lactone, also permits of the formation of a compound of this type (XCVI), although it should be noted that there is no direct evidence for the

presence of an aldehyde group since Ruzicka has recorded no attempt at oxidising the compound to the corresponding acid.

The evidence presented in this section can be summarised as showing that a reasonable account of certain decarboxylation and lactonisation reactions of ursolic acid derivatives may be obtained by assigning to ursolic acid a structure identical to (LXVI) already derived for α-amyrenol (p. 43), with the exception that the methyl group at C_{18} is replaced by a carboxyl group. This structure for ursolic acid will be discussed further in the remaining sections of this Theoretical part.

METHYL KETODIHYDRO-ACETYLURSOLATE AND ITS OXIDATION PRODUCTS.

Reference has already been made to the oxidation by hydrogen peroxide, of the double bond of α-amyrenyl acetate, and the subsequent conversion of the product to iso- α-amyrenonyl acetate (p.10). This compound has been further partially dehydrogenated by Ruzicka (52) using selenium dioxide, to give two isomeric iso- α-amyradienonyl acetates, whose precise formulation, at present doubtful, is important since one of them has been the basis of a large scale degradation of the α-amyrenol molecule (52, 53). Since the production of these compounds is thought to affect a part of the α-amyrenol molecule near which the carboxyl group of ursolic acid is located, the elucidation of their structure has been attempted by the preparation of the corresponding compounds from ursolic acid.

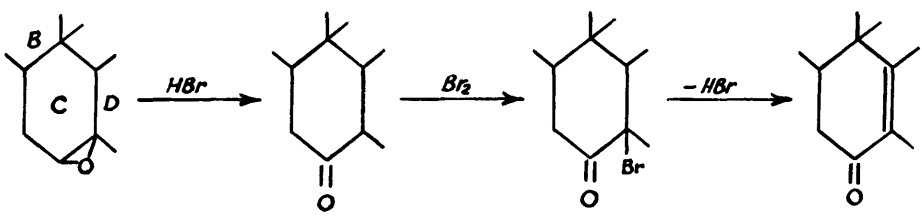
The oxidation of acetylursolic acid with hydrogen peroxide in an attempt to obtain the saturated keto-acid was found to result mainly in the production of a saturated lactone (p.58), although a small amount of a non-crystalline acid was also obtained. On esterification, this acid gave a saturated methyl ester, $C_{33}H_{52}O_5$, of

melting point 246-249°, which had a specific rotation in chloroform of +27°. This ester was undepressed in melting point when mixed with the compound obtained by the oxidation of methyl acetylursolate with hydrogen peroxide, although in this case the maximum melting point of the product was 250-253°. When treated with hydrogen chloride in chloroform this latter compound yielded an isomeric substance of melting point 254-256° which depressed the melting point of the unisomerised ester by 20°. In addition, the isomerisation product showed a light absorption maximum at 2890Å, ε= 84, suggestive of the presence in the molecule of an isolated ketone group. Thus it would seem that the oxidation product obtained directly from acetylursolate was an oxide, methyl oxido-acetylursolate, and that treatment with hydrogen chloride converted it to the saturated ketone, methyl ketodihydro-acetylursolate, although no oxime could be obtained from this product.

Ruzicka has reported (49) that the oxidation with hydrogen peroxide of acetylursolic acid with subsequent methylation of the product yields methyl ketodihydro-acetylursolate. This will be seen to be in disagreement with the results reported above where the product under similar reaction conditions was shown to be methyl oxido-

acetylursolate. Ruzicka has, however, also shown (49) that his product is identical with the substance obtained by the ozonisation of methyl acetylursolate, and consequently its formulation as an oxide as described above would appear to be more reasonable.

Methyl oxido-acetylursolate on treatment with bromine, in glacial acetic acid containing a trace of hydrogen bromide, behaved similarly to α-amyranonyl benzoate (p.11) being smoothly converted at room temperature to a compound, $C_{33}H_{51}O_5Br$, of melting point 196-198°. The same product, which was saturated to tetranitromethane, was obtained by treatment of methyl ketodihydro-acetylursolate with bromine alone, showing that the isomeric oxidation products obtained from acetylursolic acid have in fact the configurations (XCVII) and (XCVIII). From this, the bromo compound, methyl bromketodihydro-acetylursolate, (XCIX) can be supposed to have been formed from methyl oxido-acetylursolate (XCVII) after the latter's isomerisation to methyl ketodihydro-acetylursolate (XCVIII) thus:-

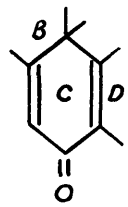


XCVII XCVIII XCIX C

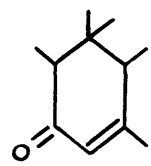
Methyl bromketodihydro-acetylursolate easily underwent dehalogenation yielding methyl iso-ketoacetylursolate, $C_{33}H_{50}O_5$, of melting point 186-188°, which on hydrolysis gave methyl iso-ketoursolate, $C_{31}H_{48}O_4$, of melting point 236-238°. Both compounds gave no coloration with tetranitromethane, and showed a light absorption maximum at 2500Å, ε= 15,500, indicative of the presence of an α-β-unsaturated ketone grouping. Methyl iso-ketoacetylursolate which can also be obtained directly from methyl oxido-acetylursolate by bromination at 70°, can therefore be considered as having a structure analogous to that of iso-α-amyrenonyl acetate, and can be represented by the fragment (C) above.

The treatment of methyl iso-ketoacetylursolate with selenium dioxide, produced a compound, $C_{33}H_{48}O_5$, of melting point 332-334° which on hydrolysis yielded a substance, $C_{31}H_{48}O_4$, of melting point 348-350°. The compound $C_{33}H_{48}O_5$, methyl iso-ketodehydro-acetylursolate gave no coloration with tetranitromethane, and displayed a light absorption maximum at 2380Å, ε= 21,000. This light absorption can be taken as indicative of the presence, either of the chromophore $>C=\acute{C}-CO-\acute{C}=C<$ (52), or of a system of two conjugated double bonds spread over two rings (41) as in Δ-α-amyradiene. However,

the fact that methyl iso-ketodehydro-acetylursolate produces no coloration with tetranitromethane would appear to exclude the latter possibility, and hence the formulation (CI) derived from (C) for methyl iso-keto-acetylursolate, can be regarded as accounting for the light absorption properties of the compound.



CI



CII

If this structure (CI) is assumed to exist in methyl iso-ketodehydro-acetylursolate the stability towards selenium dioxide and bromine shown by methyl ketoacetylursolate (CII) in distinction to methyl iso-ketoacetylursolate can be appreciated.

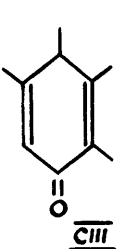
A comparison of the properties of the product obtained by Ruzicka (52) from the selenium dioxide oxidation of iso-α-amyrenonyl acetate, with those of methyl iso-ketodehydro-acetylursolate, shows that although both compounds have similar light absorption properties, they differ from each other in two important respects. Ruzicka's product, iso-α-amyradienonyl acetate-I has been reported to give a yellow coloration with tetranitromethane and to have a specific rotation

of +14°, whereas the ursolic acid derivative gives no coloration whatever and has a specific rotation of -63°. In addition, iso- α-amyradienonyl acetate-I has been isomerised by treatment with mineral acid to iso- α-amyradienonyl acetate-II which, however, displays an almost identical light absorption to that of the unisomerised product. This second product also differs from the ursolic acid derivative by having a specific rotation of +160°, although it gives no coloration with tetranitromethane.

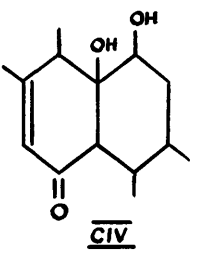
These apparent discrepancies in specific rotation between the α-amyrin derivatives and the ursolic acid derivative cannot be attributed simply to the presence in α-amyrenol of a methyl group where a carboxyl exists in ursolic acid since, acetylursolic acid, acetylursolyl chloride, the corresponding secondary alcohol uvaol and its diacetate, and α-amyrenyl acetate all have approximately the same molecular rotation. Hence it follows that methyl iso-ketodehydro-acetylursolate although containing a chromophore similar to that present in both iso- α-amyradienonyl acetates must differ structurally from them about at least one centre of asymmetry.

Regarding this conclusion, it has also been reported (52) that iso- α-amyradienonyl acetate-I when treated

with osmium tetroxide, yielded by oxidation of a double bond, a diol which however retained the light absorption shown by the iso- α-amyradienonyl acetates and by methyl iso-ketodehydro-acetylursolate. This would seem to support the conclusion, suggested by the isomerisation, that there exists in each of the iso- α-amyradienonyl acetates a double bond outside of the chromophore responsible for their characteristic light absorption. Hence Ruzicka's formulations (CIII, CIV) for iso- α-amyradienonyl acetate-II and for the diol from iso- α-amyradienonyl acetate-I would not appear to be valid.



CIII



CIV

Since methyl iso-ketodehydro-acetylursolate has been shown to differ from the iso- α-amyradienonyl acetates, mainly in specific rotation, it is not unreasonable to suggest that the extra double bond postulated above for the iso- α-amyradienonyl acetates does not exist in the ursolic acid derivative. This supposition is supported by the fact that the diol obtained by destruction of this hypothetical double bond of iso- α-amyradienonyl acetate-I, has a specific

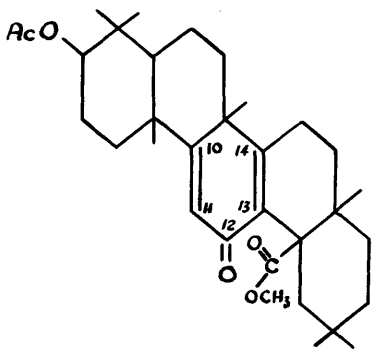
rotation of -51° in comparison with a value of -63° obtained for methyl iso-ketodehydro-acetylursolate. Thus the diol unlike either of the iso- α-amyradienonyl acetates, is in agreement with the ursolic acid derivative, in its behaviour towards tetranitromethane, in its molecular rotation, and in its light absorption properties, i.e. the replacement of the hypothetical double bond by a glycol system, which with regard to the asymmetric centres present could be regarded as being similar to the reduction of the double bond, has removed the differences existing between the products obtained by selenium dioxide oxidation of iso- α-amyrenonyl acetate, and the corresponding derivative of ursolic acid.

This evidence can therefore be summarised as suggesting that, while the iso- α-amyradienonyl acetates-I and -II each have a chromophore identical with that existing in methyl iso-ketodehydro-acetylursolate, each also contains a double bond absent in the ursolic acid derivative.

If, from this, the formation of iso- α-amyradienonyl acetate-I is assumed to take place in two stages, the first of which involves the formation of the system responsible for its light absorbing properties while the second introduces the isolated double bond, it would seem

reasonable to suppose that this second stage necessitates the migration of a methyl group which is not free to migrate in methyl iso-ketoacetylursolate. This is most simply explained by assuming that the methyl group required to migrate is that which is replaced by a carboxyl group in ursolic acid.

Of the possible locations for the carboxyl group considered in previous sections it would appear that the position C_{18} fulfils most of the above requirements. Hence methyl iso-ketodehydro-acetylursolate can be represented by (CV) on the basis of (LXVI, p.43) already derived for α-amyrenol. Similarly, iso- α-amyradienonyl



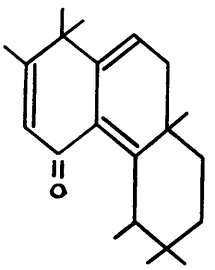
CV

acetate-I could be formulated as (CVI), produced by the migration to C_{19} of the methyl group at C_{18}, and of the double bond at C_{13}-C_{14}, to C_{14}-C_{15}, followed by the introduction of a double bond at C_{13}-C_{18}. In this way, a double bond is located at C_{14}-C_{15}, a position apparently

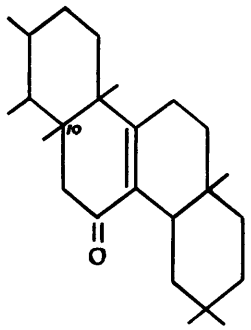
demanded by further degradative work by Ruzicka (53).

Regarding these formulations it should be stated that although all attempts to hydrogenate methyl iso-ketodehydro-acetylursolate proved unsuccessful, Ruzicka (52) has reported that catalytic reduction of iso- α-amyradienonyl acetate yielded iso- α-amyrenonyl acetate and α-amyradienyl acetate. Consequently, the migration of a methyl group during the selenium dioxide oxidation of iso- α-amyrenonyl acetate would appear to be remote, since a return migration would be necessary to account for the formation of the hydrogenation products. However, the formation of α-amyradienyl acetate as a result of hydrogenation is in itself, surprising since all previous attempts to hydrogenate catalytically a ketone group similarly located to that in iso- α-amyradienonyl acetate-I, have failed. The formulation (CVI) for iso- α-amyradienonyl acetate-I cannot therefore be entirely excluded.

If previous deductions concerning the structures of the α- and β-amyrenols are correct, iso- β-amyrenonyl acetate should have the partial structure (CVII) in which the position C_{10} is completely blocked. Consequently, no compound containing a chromophore identical with that present in methyl iso-ketodehydro-acetylursolate would



CVI



CVII

be expected to be produced by the treatment of iso-β-amyrenonyl acetate with selenium dioxide. That this is indeed the case is shown by the fact that iso-β-amyradienonyl acetate obtained by Spring (58) from such an oxidation displayed a light absorption maximum at 2450Å, ε= 10,000, indicating the presence of an α-β-unsaturated ketone grouping only, and not a conjugated dienone system as in the corresponding α-amyrin derivatives.

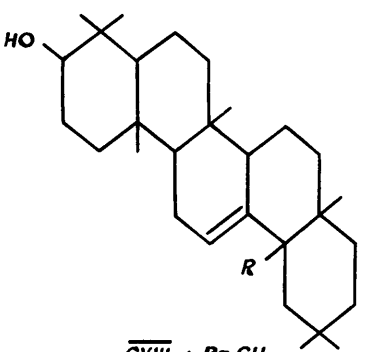
In addition iso-β-amyradienonyl acetate has been shown by its yellow coloration with tetranitromethane, to contain an isolated double bond, and thus shows some agreement with iso-α-amyradienonyl acetate-I. The production of this double bond in the α-amyrin series has however been supposed to depend on the migration of the methyl group occupying the same position as the carboxyl group in ursolic acid. If the formation of

the double bond in iso-β-amyradienonyl acetate is analogous to that of iso-α-amyradienonyl acetate, it therefore follows that the position C_{18} is involved. However, in the structure (LXVII, p.43) derived for β-amyrenol, the position C_{18} unlike the corresponding position in α-amyrenol was assumed to carry no angular methyl group, and hence no methyl group migration is required to account for the formation of the double bond. In these circumstances the introduction of the double bond should be more easily accomplished in the β- than in the α-series. This would in fact appear to be the case, since Spring (58) has reported the formation of iso-β-amyradienonyl acetate by the action of bromine on iso-β-amyrenonyl acetate at 75-80°.

From this comparison of the behaviour towards selenium dioxide of iso-β-amyrenonyl acetate, with that of iso-α-amyrenonyl acetate and methyl iso-ketoacetyl-ursolate it would appear that the structure (CV, p.73) suggested for methyl iso-ketodehydro-acetylursolate in which the carboxymethyl group is located at C_{18}, as well as giving a reasonable explanation for its own properties, also accounts satisfactorily for the differences between it and iso-α- and iso-β-amyradienonyl acetates.

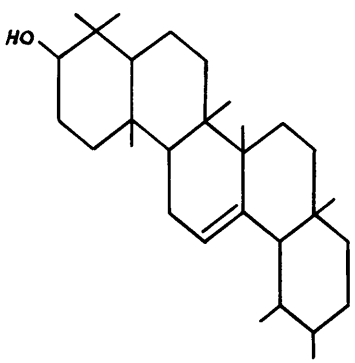
CONCLUSION

Consideration of the evidence, reviewed in the foregoing sections, has led the author to propose the structures (CVIII) and (CIX) for α-amyrenol and ursolic acid respectively, in preference to the formulation (CX) for α-amyrenol, recently put forward by Ruzicka (24, 52, 53).



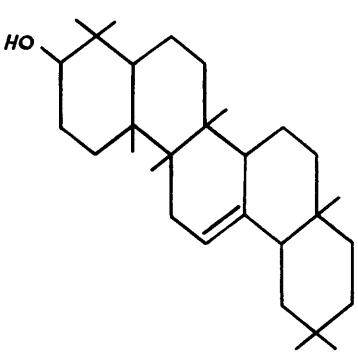
CVIII ; R= CH_3

CIX ; R= COOH

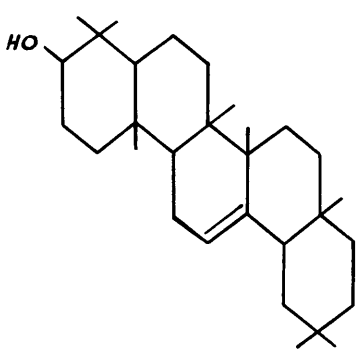


CX

In addition it has been found possible to formulate β-amyrenol as (CXI) in terms of α-amyrenol as (CVIII). It should however be emphasised that this formulation (CXI)



CXI



CXII

for β-amyrenol, being derived from (CVIII) for α-amyrenol is largely dependent on this structure, although it differs from Ruzicka's formulation (CXII) (24), only in the location of one angular methyl group.

In comparing Ruzicka's formulations for α- and β-amyrenol with those proposed by the author, it will be seen that the latter explain satisfactorily, certain differences in behaviour between the two series not accounted for by the former, while at the same time giving a reasonable explanation of the vast majority of the reactions of both series.

Firstly, Ruzicka in formulating α-amyrenol as (CX) and β-amyrenol as (CXII) has made no attempt to account for the existence of two types of dehydration product in the α-series where only one exists in the β-series. These differences can however be appreciated on the basis of the structures (CVIII) and (CXI) proposed above, since the formation of a conjugated diene system by dehydration in the β-series, would appear to be precluded by the presence in β-amyrenol of an angular methyl group at C_{10}.

In addition, Ruzicka has sought to account for the inertness of the double bond of α-amyrenol, by locating an angular methyl group at C_{10}. This inactivity would seem to be more satisfactorily explained by the presence

of an angular methyl group at C_{18}, as in the structure (CVIII) for α-amyrenol.

Thirdly, in formulating α- and β-amyrenol as (CX) and (CXII) respectively, Ruzicka has created in the α-structure, (CX), two asymmetric centres, at C_{18} and C_{20}, not present in the β-structure, (CXII), which does not explain satisfactorily the fact that the specific rotations of the parent amyrenols and the corresponding esters of each series, are practically identical. On the other hand the formulations (CVIII) and (CXI) for α- and β-amyrenol although differing from each other, have each the same number of asymmetric centres, and consequently the similarity in rotatory power existing between the corresponding compounds of the two series is to be expected.

Regarding the dehydrogenation evidence it will be seen that the structures (CVIII) and (CIX) for α-amyrenol and ursolic acid respectively, while accounting for most of the dehydrogenation products cannot account for the formation of 1:2:7-trimethylnaphthalene without assuming methyl group migration. This however cannot be regarded as conclusive evidence against the proposed structures, since under the conditions employed in the dehydrogenation, methyl group migration can be assumed to be possible.

Mention has already been made (p.27) of the degradation of α-amyranonyl acetate with fuming nitric acid recently reported by Ruzicka, from which he concluded that the structures of rings A and B of α- and β-amyrenol are identical. It will be seen that rings A and B of the structures proposed above for α- and β-amyrenol, (CVIII) and (CXI) respectively, are not identical, the β-amyrenol structure containing an angular methyl group at C_{10}, where none exists in the α-structure. This however is not necessarily at variance with Ruzicka's work, since his conclusion was based on experiments involving pyrolysis (24, 54) during which the elimination of the angular methyl group at C_{10} is possible. This being so, rings A and B of the β-structure can be supposed to yield the same degradation products as those obtained from the α-series, which are in agreement with the proposed formulation (CVIII) for α-amyrenol.

From the same oxidative degradation (p.28) Ruzicka has concluded that ring E of α-amyrenol has the configuration shown in the structure (CX). This differs from the structure proposed above in the configuration around C_{20}, but it should be noted that there is no direct evidence to suggest the correct formulation since

all the reactions concerning this part of the molecule have been such as to include the possibility of methyl group migration.

In conclusion, therefore, it can be stated that although the structures, (CVIII) and (CIX), proposed above are at variance with certain dehydrogenation evidence concerning the environment of position C_{10}, they account satisfactorily for the vast majority of the reactions of α-amyrenol and ursolic acid.

EXPERIMENTAL

Micro-analyses were carried out by Drs. Weiler and Strauss, Oxford.

EXPERIMENTAL

All melting points are uncorrected.

Isolation of α-Amyrenyl Benzoate.

The solid material obtained from Manila Elemi resin (350 g.) after steam distillation to remove volatile oils, was roughly dried and dissolved in ether (1000 ml.). This ethereal solution was then washed twice with sodium hydroxide solution (10%; 350 ml.), once with hydrochloric acid (10%; 350 ml.), and finally twice with water (350 ml.), before being dried over sodium sulphate. The solid residue obtained after removal of the ether, was crystallised once from 95% ethanol, dried at 105°, and finally powdered giving a crude mixture of α- and β-amyrenol (200 g.).

The crude mixed amyrins (200 g.) were dissolved in pyridine (120 ml.) and benzoyl chloride (140 ml.) added dropwise to the stirred solution, at 100°. After the addition, the reaction mixture was heated with stirring for a further six hours. The dark red mixture was cooled, diluted with benzene (625 ml.), and washed twice with hydrochloric acid (10%; 400 ml.), once with sodium hydroxide solution (10%; 400 ml.) and twice with salt solution (2%; 400 ml.). The benzene solution was then

dried over sodium sulphate, and concentrated to ca. 300 ml. Hot ethanol was added to the boiling solution until faintly turbid, and on cooling a crystalline mass separated. This was washed with cold absolute ethanol and dried at 105°.

The crude mixed benzoates were then transferred to a wide necked stoppered bottle containing a tap for relief of pressure. Ether (200 ml.) was added and the bottle shaken for ten minutes. The undissolved solid was collected. Since the melting points of the mixed benzoates is indistinct the clearing point was determined. The process was then repeated a further three times after which the clearing point was above 210°. The residue, after repeated crystallisation from benzene-acetone gave pure β-amyrenyl benzoate as colourless plates of m.p.233-235° (30 g.).

The ethereal washings, on evaporation to dryness, gave a solid residue which after six crystallisations from benzene-alcohol gave α-amyrenyl benzoate as colourless prismatic needles of m.p.195-196° (70 g.).

α-Amyrenol.

α-Amyrenyl benzoate (50 g.) was dissolved in boiling benzene (300 ml.) and a solution of potassium hydroxide (50g.

in aqueous ethanol (65 ml. water, and 1150 ml. ethanol refluxed and distilled over sodium hydroxide) added. The solution was refluxed for 24 hours, concentrated until solid began to appear, and finally poured into water. The solid was collected, washed well with water, and dried at 105°, giving α-amyrenol of m.p.170-174°. Yield 40 g. (87%).

α-Amyrenyl Acetate.

α-Amyrenol (10 g.) was dissolved in pyridine (30 ml.) and heated on a steam bath with acetic anhydride (45 ml.) for two hours. The solution was then cooled, and the crystalline material which separated, filtered and washed with a little cold glacial acetic acid. After recrystallisation from chloroform-methanol, α-amyrenyl acetate was obtained as prismatic needles of m.p.224-225°. $[\alpha]_D^{18}$ + 79° (l=1; c=1·01 in chloroform). Yield 9·0 g. (80%).

l- α-Amyradiene.

(cf. Vesterberg, Ber.,1891,24,3834).

α-Amyrenol (41 g.) was dissolved, with warming, in pure dry benzene (1250 ml.) and to the solution phosphorus pentoxide (95 g.) was added, gradually and with shaking. The mixture was then shaken mechanically

at room temperature for 24 hours. The resulting orange coloured viscous liquid was washed twice with water, twice with dilute sodium sulphate solution, and finally dried over sodium sulphate. The dried benzene solution was then passed through a column of activated alumina, to remove any unchanged α-amyrenol, and concentrated under reduced pressure until the volume was approximately 120 ml. On the addition of ether, and after standing for 24 hours at 0°, a crop of semi-crystalline material was obtained. This was separated, and washed free of adhering resin with a little ice-cold ether. After three crystallisations of this solid from benzene-ether, *l*-α-amyradiene was obtained as hard prismatic needles of m.p.193-195°. It gave a reddish-brown coloration with tetranitromethane in chloroform. Further pure material was obtained from the mother liquors. Yield 10·5 g. (27%).

$[\alpha]_D$ -103° (*l* = 1; c = 0·72 in chloroform.)

Light absorption in ethanol:- Maximum at 2400Å, ε=13,500.

Oxidation of *l*-α-Amyradiene with Chromic Anhydride.

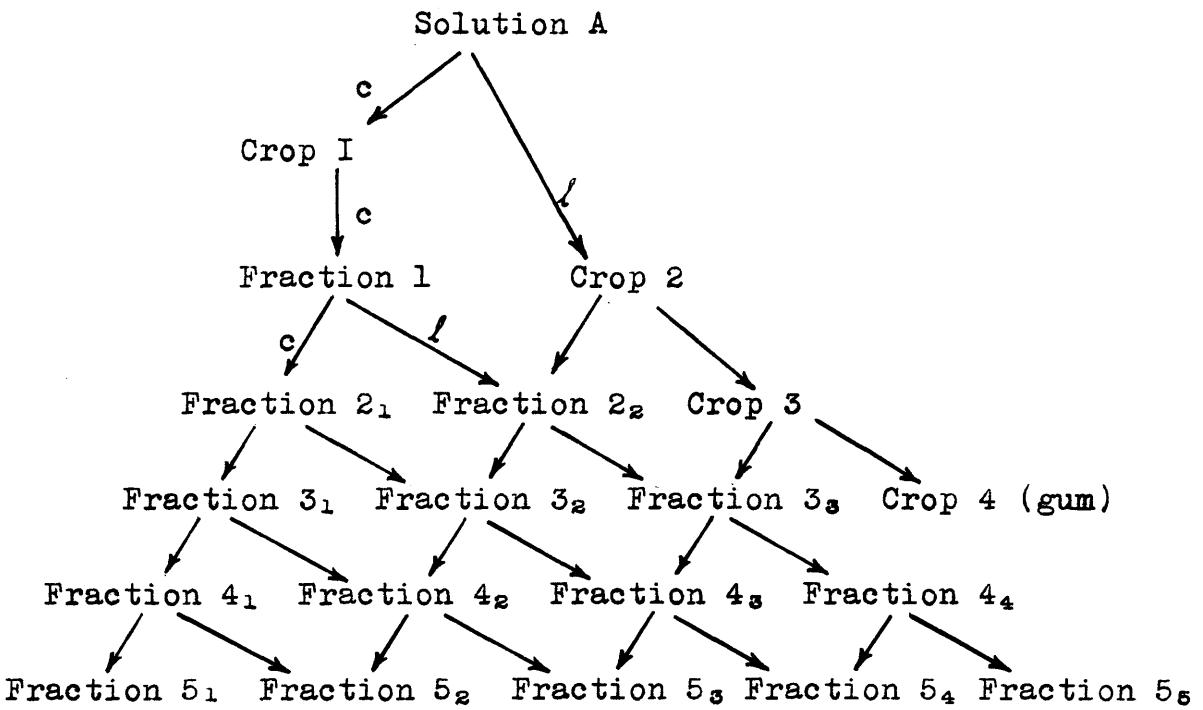
The glacial acetic acid used in these and subsequent oxidations was freed from oxidisable impurity by distillation from chromium trioxide.

Method I.

l-α-Amyradiene (2 g.) in stabilised glacial acetic acid (60 ml.) and benzene (20 ml.) was heated under reflux, and chromic anhydride (1000 mg. ≡ 3 oxygen atoms) in acetic acid (85%; 16 ml.) added to the boiling solution over 15 minutes. After refluxing for a further hour, the solution was concentrated under reduced pressure, poured into water, and extracted with ether. The extract was washed successively with water, with dilute sodium carbonate solution, and finally again with water. The sodium carbonate washings gave no precipitate on acidification. After drying over sodium sulphate, the ethereal solution was evaporated to dryness under reduced pressure, and the residual resin crystallised from chloroform-ethanol (Solution A.) giving small prismatic needles (Crop I) of m.p.214-232°. Repeated recrystallisation, from chloroform-ethanol, produced no pronounced sharpening in the melting point. The substance was then purified by fractional crystallisation, as follows.

The product, m.p.214-232° (Crop I) on recrystallisation, give a crystalline substance, Fraction 1. The mother liquor from the crystallisation of Crop I (Solution A) was concentrated to half-bulk from which a

further quantity of crystalline material was obtained (Crop 2). Crop 2 was then combined with the mother liquor from the crystallisation of Fraction 1, and this solution on crystallisation gave Fraction 2_2. The fractionation continued as shown below until five fractions had been obtained.



m.p.228-235°	226-232°	222-228°	222-226°	218-223°
Yield 380 mg.	430 mg.	240 mg.	130 mg.	50 mg.

Fraction 5_1 after four further crystallisations from ethanol, gave l-α-amyrenone oxide as small colourless needles of m.p.235-237°, which gave no coloration with

tetranitromethane in chloroform.. Yield 110 mg.

Found: C,81·9; H,10·5%

$C_{30}H_{46}O_2$ requires : C,82·2; H,10·5%

$[\alpha]_D^{18}$ -135° (l = 1, c = 0·38 in chloroform)

Light absorption in ethanol:- Maximum at 2500Å, ε=12,900.

Fraction 5_2 after five recrystallisations from ethanol gave colourless needles of m.p.232-235°, undepressed in melting point on admixture with l-α-amyrenone oxide obtained from Fraction 5_1.

Fractions 5_3 and 5_4 after one recrystallisation from ethanol each had m.p.223-227°, and were not further examined.

Fraction 5_5 when recrystallised once from ethanol was obtained as needles of m.p.219-223°, which gave a pale yellow coloration with tetranitromethane in chloroform.

Found: C,84·1; H,11·4%

$C_{30}H_{46}O_2$ requires: C,82·2; H,10·5%

$C_{30}H_{48}O$ requires: C,84·9; H,11·3%

Light absorption in ethanol:- Maximum at 2500Å, ε=2,400.

Method II.

To a solution of l-α-amyradiene (2 g.) in stabilised glacial acetic acid (60 ml.) and benzene (20 ml.) was

added with vigorous mechanical stirring, a solution of chromic anhydride (360 mg. ≡ 1·1 oxygen atoms) in acetic acid (85%; 10 ml.). The mixture was then heated on a boiling water bath for one hour, cooled, poured into water and extracted with ether. The extract after being washed successively with water, with dilute sodium carbonate solution, and again with water was dried over sodium sulphate, and the ether removed under reduced pressure. The residual gum was crystallised from ethanol giving colourless needles of m.p.207-212°. Yield 1·64 g. There was no acid product of the oxidation, as acidification of the alkaline washings of the ether extract gave no precipitate.

The substance of m.p.207-212° was then extracted successively with four portions of boiling ethanol, giving four solutions from which four crystalline fractions were obtained on cooling.

Fraction 1 was obtained as stout prismatic needles of m.p.202-205°. After two further crystallisations from ethanol the melting point was constant at 205-206°, $[\alpha]_D^{18}$ -94° (l = 1, c = 0·82 in chloroform). With tetranitromethane in chloroform, the substance gave a pale orange coloration. Yield 210 mg.

Fraction 2 had m.p.206-209°. Two recrystallisations

of this substance, from ethanol, gave colourless needles of m.p.209-211°. The compound gave an orange coloration with tetranitromethane in chloroform. Yield 170 mg.

Found: C,85·9; H,11·4%

$C_{30}H_{48}O$ requires: C,84·9; H,11·3%

$C_{30}H_{48}$ requires: C,88·2; H,11·8%

$C_{30}H_{48}O:C_{30}H_{48}$; 3:1 requires: C,85·7; H,11·4%

$[\alpha]_D^{18}$ -77° (l = 1, c = 0·82 in chloroform)

Light absorption in ethanol:- Maximum at 2410Å, ε=3,500.

Fraction 3 crystallised from ethanol as prismatic needles, m.p.213-216°, and after three recrystallisations from ethanol gave l-α-amyradiene oxide as needles of m.p.217-219°. With tetranitromethane in chloroform, l-α-amyradiene oxide gave a pale yellow coloration. Yield 260 mg.

Found: C,84·4; H,11·1%

$C_{30}H_{48}O$ requires: C,84·9; H,11·3%

$[\alpha]_D^{18}$ -70° (l = 1, c = 0·46 in chloroform)

Fraction 4 was obtained as stout prisms of m.p.213-215°, which after two recrystallisations from ethanol gave l-α-amyradiene oxide of constant m.p.218-220°. Yield 300 mg.

Found: C,84·6; H,11·4%

$C_{30}H_{48}O$ requires: C,84·9; H,11·3%

$[\alpha]_D^{18}$ -68° (l = 1, c = 0·62 in chloroform)

A mixture of *l*-α-amyradiene, of m.p.193-195°, and *l*-α-amyradiene oxide had a m.p. of 202-209°, and that of *l*-α-amyradiene and *l*-α-amyrenone oxide, of m.p.235-237°, had a m.p. of 224-229°.

Attempted Oxidation of *l*-α-Amyradiene.

1. With potassium permanganate.

l-α-Amyradiene (1500 mg.) was dissolved in carbon tetrachloride (100 ml.) and to the solution was added aqueous potassium permanganate (150 ml. $^{N}/_{10}$ ≡ 2 oxygen atoms) and sulphuric acid (10%; 15 ml.). The mixture was then shaken for 24 hours at room temperature after which time it was filtered and extracted with carbon tetrachloride. The extract was washed with water and sodium hydroxide solution (10%), and finally evaporated to dryness under reduced pressure. The residue was recrystallised three times from benzene-ethanol giving colourless prisms (1100 mg.) of m.p.195-197°. When mixed with *l*-α-amyradiene, of m.p.193-195°, there was no depression in melting point.

Found:	C,87·9;	H,11·5%
$C_{30}H_{48}$ requires:	C,88·2;	H,11·8%
$C_{30}H_{48}O$ requires:	C,84·9;	H,11·3%

2. With osmium tetroxide.

l- α-amyradiene (1250 mg.) was dissolved in dry ether (100 ml.) and osmium tetroxide (1500 mg. ≡ 2 mol.) added to the solution. After standing for 7 days at room temperature, the solution was evaporated to dryness, and the residue dissolved in benzene (25 ml.) and ethanol (15 ml.). This solution was then shaken for 2 hours with a mixture of mannitol (3·8 g.) and potassium hydroxide (2·4 g.) in aqueous ethanol (5·75 ml. water; 12 ml. ethanol) before being extracted with benzene (4 x 25 ml.). The extract after being washed with water, was dried over sodium sulphate, and evaporated to dryness under reduced pressure. The residual solid crystallised from benzene-ethanol as prismatic needles of m.p.193-195°, undepressed on admixture with authentic *l*- α-amyradiene. Yield 1050 mg.

Oxidation of *l*- α-Amyradiene Oxide with Chromic Anhydride.

l- α-Amyradiene oxide (150 mg.) in benzene (1·0 ml.) and glacial acetic acid (0·5 ml.) was heated on a steam bath, and chromic anhydride (50 mg. ≡ 2 oxygen atoms) in acetic acid (85%; 2·5 ml.) added. The mixture was then heated for a further hour, after which time the benzene was removed under reduced pressure. On cooling, the solution deposited a crop of needle-shaped crystals.

After four recrystallisations of this material from nitromethane, *l*- α-amyrenone oxide was obtained as colourless needles of m.p.235-237° undepressed in melting point when mixed with *l*- α-amyrenone oxide obtained by direct oxidation of *l*- α-amyradiene. With tetranitromethane in chloroform, the substance gave no coloration.

Found: C,82·0; H,10·7%

$C_{30}H_{48}O_2$ requires: C,82·2; H,10·5%

Light absorption in ethanol:- Maximum at 2500Å, ε=11,800.

Attempted Isomerisation of *l*- α-Amyrenone Oxide.

l- α-Amyrenone oxide (50 mg.) was dissolved in chloroform (1·5 ml.) and glacial acetic acid (5 ml.), and to the mixture concentrated hydrochloric acid (0·5 ml.) added. The solution was then warmed to 40° and allowed to stand at that temperature for one hour, after which time it was poured into excess water, and extracted with chloroform-ether. The extract after drying over sodium sulphate was evaporated to dryness under reduced pressure, and the residual solid crystallised from ethanol as small colourless needles, m.p.233-235°. When mixed with starting material of m.p.235-237°, the substance showed no depression in melting point, and was assumed to be unchanged *l*- α-amyrenone oxide.

Light absorption in ethanol:- Maximum at 2500Å, ε=12,100.

Attempted Oximation of *l*- α-Amyradiene Oxide.

l- α-Amyradiene oxide (100 mg.) was dissolved in boiling ethanol (35 ml.) and hydroxylamine hydrochloride (100 mg.) and anhydrous sodium acetate (150 mg.) added. After refluxing for 10 hours, the mixture was poured into water and the precipitated solid filtered off. This after two recrystallisations from ethanol had m.p. 216-218° undepressed when mixed with starting material.

Formation of a Mixed Crystal of *l*- α-Amyradiene and *l*- α-Amyradiene Oxide.

l- α-Amyradiene (390 mg.) was dissolved in boiling ethanol (170 ml.) and to the solution *l*- α-amyradiene oxide (98 mg.) in ethanol was added. The solution was then concentrated to approximately 80 ml., and on cooling a crop of stout needles of m.p.202-204° was obtained. After three further crystallisations from ethanol, the substance had a constant m.p. of 202-204°. With tetranitromethane in chloroform the product gave an orange-red coloration.

Found: C,87·0; H,11·5%

$C_{30}H_{48}O$ requires: C,84·8; H,11·3%

$C_{30}H_{48}$ requires: C,88·2; H,11·7%

$C_{30}H_{48}$: $C_{30}H_{48}O$; 4:1 requires: C,87·5; H,11·6%

$[\alpha]_D^{20}$ -98° (*l* = 1, c = 0·87 in chloroform)

Light absorption in ethanol:- Maximum at 2410Å, ε=9,300.

Attempted Isomerisation of *l*- α-Amyradiene Oxide.

l- α-Amyradiene oxide (100 mg.) was dissolved in chloroform (3·0 ml.) and glacial acetic acid (10 ml.), and to the mixture concentrated hydrochloric acid (1·0 ml.) was added. The solution was warmed to 40° and kept at this temperature for one hour, after which time it was poured into excess water, and extracted with chloroform-ether. From this extract, a crop of colourless needles of m.p.218-220° was obtained. When mixed with starting material of m.p.218-220°, the substance showed no depression in melting point, and was assumed to be unchanged *l*- α-amyradiene oxide.

Purification of Ursolic Acid.

Crude ursolic acid (30 g.) was extracted, at room temperature, with chloroform-ether (2:1; 4*l*), and the extract shaken with sodium hydroxide solution (5%). The white granular precipitate of sodium ursolate which formed, was separated, washed with water and dried. Acidification of the alkaline washings, gave no precipitate of ursolic acid. The sodium ursolate, after drying, was dissolved in boiling ethanol, and glacial acetic acid added until the solution was just acid to Congo Red.

On cooling, the solution deposited a crop of fine needles, which after three further crystallisations from aqueous ethanol gave pure ursolic acid as colourless prismatic needles, m.p.283-285°. With tetranitromethane in chloroform, ursolic acid gave a pale yellow coloration. Yield 12 g.

Found: C,78·2, 78·8; H,10·4, 10·6%

Calc. for $C_{30}H_{48}O_3$: C,78·9; H,10·5%

$[\alpha]_D^{18}$ +65° (l = 1, c = 0·72 in chloroform).

Acetylursolic Acid.

Ursolic acid (8·5 g.) in pyridine (22 ml.) and acetic anhydride (22 ml.) was heated for 2 hours on a steam bath. The mixture was then cooled, poured in water, and the precipitated solid filtered. This substance, diacetyl ursolic acid, after being dried was refluxed with aqueous ethanol (90%; 200 ml.) for 2 hours, and the hot solution diluted with water until faintly opalescent. On standing, acetylursolic acid separated as long colourless needles, which after two further recrystallisations from aqueous ethanol attained a constant m.p. of 286-289°. Yield 7·2 g. (80%).

Found: C,77·0; H,10·0%

Calc. for $C_{32}H_{50}O_4$: C,77·1; H,10·0%

$[\alpha]_D^{18}$ +62° (l = 1, c = 1·3 in chloroform).

Ewen and Spring (J.,1943,523) reported a m.p. of 289-290°, and $[\alpha]_D^{20}$ +61·5° in chloroform, for acetylursolic acid.

Methyl Acetylursolate.

To acetylursolic acid (1000 mg.) in ether was added a slight excess of an ethereal solution of diazomethane. When all signs of evolution of nitrogen had ceased, the ethereal solution was washed with dilute hydrochloric acid to decompose the excess diazomethane and then with dilute sodium hydroxide to precipitate any unchanged acetyl ursolic acid. There was no precipitation, and after being dried over sodium sulphate, the ethereal solution was evaporated to dryness under reduced pressure. The residue on crystallisation from methanol, gave methyl acetylursolate as plates, m.p.242-244°. After three recrystallisations from methanol the m.p. was 246-248°. Yield 850 mg.

Found: C,77·4; H,9·8%

Calc. for $C_{33}H_{52}O_4$: C,77·3; H,10·0%

$[\alpha]_D^{18}$ +61° (l = 1, c = 0·93 in chloroform).

Oxidation of Acetylursolic Acid with Chromic Anhydride.

(cf. Ewen and Spring, J.,1943,523)

To a boiling solution of acetylursolic acid (5 g.) in glacial acetic acid (125 ml.), chromic anhydride (3·8 g. ≡ 6 oxygen atoms) in acetic acid (85%; 30 ml.) was added dropwise over 5 minutes. The solution was refluxed for a further 2 hours, cooled, poured into water and the mixture extracted with ether. The extract was washed first with water, and then with potassium hydroxide solution (5%), a resinous potassium salt separating at the interface of the two layers. This gradually became solid and was then separated. After being dried, the potassium salt was dissolved in boiling ethanol (50 ml.), the solution filtered, and made acid to Congo Red with dilute hydrochloric acid. On dilution with water, ketoacetylursolic acid (3·0 g.) separated out as stout prismatic needles, which after four recrystallisations from chloroform-ethanol had a constant m.p.323-325° (decomp.). In chloroform solution the substance gave no coloration with tetranitromethane. Yield 2·7 g. (52%).

Found: C,74·9; H,9·3%

Calc. for $C_{32}H_{48}O_5$: C,75·0; H,9·4%

$[\alpha]_D^{20}$ +89° (l = 1, c = 1·37 in chloroform)

Light absorption in ethanol:-Maximum at 2500Å, ε=12,500.

The ketoacetylursolic acid prepared by Ewen and Spring (J.,1943,523) was reported to have a m.p. of 315-316° (decomp.) and $[\alpha]_D^{20}$ +40·8° in chloroform. Yield 39%.

The ethereal solution, after removal of traces of ketoacetylursolic acid with potassium hydroxide solution, was again washed with alkali, and water, and dried over sodium sulphate. The extract was then evaporated to dryness under reduced pressure, giving a resin which crystallised from chloroform-methanol as fine needles of m.p.302-304° (decomp.). Yield 20 mg. (0·4%).

Found: C,72·7; H,8·8%

Calc. for $C_{32}H_{46}O_6$: C,73·0; H,8·7%

Ethyl Ketoacetylursolate.

Ketoacetylursolic acid (200 mg.) was ethylated with an ethereal solution of diazoethane obtained from nitrosoethylurea, and the product isolated in a similar manner to that described above for methyl acetylursolate. Ethyl ketoacetylursolate separated from aqueous methanol as stout prismatic needles, m.p.215-216°.

Found: C,75·4; H,9·5%

Calc. for $C_{34}H_{52}O_5$: C,75·6; H,9·6%

$[\alpha]_D^{20}$ +85° (l = 1, c = 1·00 in chloroform)

Light absorption in ethanol:- Maximum at 2500Å, ε=10,500.

Methyl Ketoacetylursolate.

Ketoacetylursolic acid (100 mg.) was esterified with an ethereal solution of diazomethane in a manner similar to that described above for the preparation of methyl acetylursolate. Methyl ketoacetylursolate was obtained from aqueous ethanol as stout needles, m.p. 243-245°.

$[\alpha]_D^{20}$ +86° (l = 1, c = 0·91 in chloroform)

Light absorption in ethanol:- Maximum at 2500Å, ε=10,500.

Decarboxylation of Ketoacetylursolic acid.

I. In air.

(cf. Ewen and Spring, J.,1943,523.)

Ketoacetylursolic acid (1000 mg.) was refluxed gently for 8 hours in quinoline, (40 ml.; dried and distilled over sodium hydroxide) on an oil bath at 255-265°. The solution was cooled, poured into excess dilute hydrochloric acid, and extracted with ether. The extract was washed with water, followed by sodium hydroxide solution (5%), when a sodium salt separated. This was filtered off, dissolved in ethanol and the hot solution acidified with hydrochloric acid. On cooling, there was obtained a crop of prismatic needles, identified by mixed melting point as unchanged ketoacetylursolic acid (550 mg.)

The residual ethereal extract after drying over sodium sulphate, was evaporated to dryness under reduced pressure, yielding a dark resinous residue. This was dissolved in ethanol and the hot solution treated with animal charcoal. On cooling, a crop of pale yellow plates was obtained, which after three further crystallisations from ethanol gave plates of m.p.206-208°. Yield 85 mg. (9%). With tetranitromethane in chloroform the substance gave a strong yellow coloration.

Found: C,79·5; H,10·0%

$C_{31}H_{46}O_3$ requires: C,79·8; H,9·9%

$C_{31}H_{48}O_3$ requires: C,79·4; H,10·3%

$[\alpha]_D^{20}$ +45° (l = 1, c = 0·97 in chloroform)

Light absorption in ethanol:- Maximum at 2980Å, ε=5,700.

II. In nitrogen.

(cf. Ewen and Spring, J.,1943,523.)

Ketoacetylursolic acid (690 mg.) was refluxed gently with dry, freshly distilled, quinoline (30 ml.) on an oil bath at 265-275°, and a stream of nitrogen passed through the solution. The nitrogen stream on leaving the reaction mixture was passed, first, through a trap cooled to -10° and then through a standard solution of barium hydroxide (N/3; 25 ml.). In a short

time, precipitation of barium carbonate began, and increased as the reaction proceeded. After 8 hours heating, and again after 18 hours, the amount of carbon dioxide evolved was determined by titration of the excess standard barium hydroxide solution with acid. The results are shown in the table below, along with the titration value obtained from a blank determination.

	Ketoacetylursolic Acid			Blank
	0 hours	8 hours	18 hours	18 hours
$0 \cdot 983 \frac{N}{3} Ba(OH)_2$	25·00ml.	22·40ml.	18·25ml.	24·80ml.
CO_2 evolved	-	18·7 mg.	48·4 mg.	1·5 mg.
% Decarboxylation	-	31%	82%	-

The reaction mixture was then cooled, poured into excess dilute hydrochloric acid, and extracted with ether. The extract was washed with dilute hydrochloric acid, then with water, and finally with dilute sodium hydroxide solution. A sodium salt (120 mg.) separated, which on conversion to the corresponding acid followed by crystallisation from aqueous ethanol gave a substance identified by melting point and mixed melting point as unchanged ketoacetylursolic acid. The remaining ethereal solution, was washed with water, and dried over sodium sulphate. On removal of the ether under reduced

pressure, there was obtained a white crystalline residue (460 mg.) which, after four recrystallisations from aqueous ethanol gave nor-α-amyrenonyl acetate in colourless plates, m.p.210-212°. With tetranitromethane in chloroform, the product gave a yellow coloration. Yield 300 mg. (48%).

Found: C,79·1; H,10·2%

$C_{31}H_{48}O_3$ requires: C,79·4; H,10·3%

$C_{31}H_{46}O_3$ requires: C,79·8; H, 9·9%

$[\alpha]_D^{20}$ +45° (l = 1, c = 0·62 in chloroform).

nor-α-Amyrenonol.

nor-α-Amyrenonyl acetate (70 mg.) was refluxed for 3 hours with ethanolic potassium hydroxide solution (5%; 10 ml.). On cooling, the reaction mixture was poured into excess water, when a flocculent precipitate formed. This was crystallised from ethanol giving nor-α-amyrenonol as colourless silky needles, m.p.253-255°, which attained a constant m.p.254-256° after two further recrystallisations from ethanol. The substance was unsaturated to tetranitromethane.

Found: C,81·9; H,10·3%

$C_{29}H_{46}O_2$ requires: C,81·7; H,10·8%

$C_{29}H_{44}O_2$ requires: C,82·0; H,10·4%

$[\alpha]_D^{20}$ +52° (l = 1, c = 0·63 in chloroform).

Acetylation of nor-α-amyrenonol (30 mg.) with acetic anhydride (1 ml.) and pyridine (1 ml.) gave plates, m.p.210-212° from ethanol, undepressed on admixture with nor-α-amyrenonyl acetate.

Oxidation of nor-α-Amyrenonyl Acetate with Perbenzoic Acid.

nor-α-Amyrenonyl acetate (75 mg.) was dissolved in chloroform (10 ml.) and added to a solution of perbenzoic acid in chloroform (4·25 ml. 0·41N; = 5 oxygen atoms; prepared after Organic Syntheses,1933,13,86). The mixture was then kept at 0° for 120 hours. A blank experiment of chloroform (10 ml.) and perbenzoic acid in chloroform (4·25 ml. 0·41N.) was kept under identical conditions. At intervals, 2 ml. portions were withdrawn from both the reaction and the blank, run into potassium iodide solution, acidified with dilute hydrochloric acid, and the iodine liberated titrated with standard sodium thiosulphate solution:

Time (hours)	Titre (ml.0·983 N/10 $Na_2S_2O_3$)		O atoms absorbed
	Blank	Reaction	
2	2·40	2·24	0·09
120	2·36	1·82	1·18

After 5 days, the reaction mixture was diluted with

chloroform (10 ml.) and washed with sodium hydroxide solution (2%), followed by water. After drying over sodium sulphate, the chloroform was distilled off under reduced pressure and the residue (30 mg.) after two crystallisations from ethanol yielded colourless plates of m.p.219-220°. The substance gave no coloration with tetranitromethane in chloroform, and showed no high intensity light absorption in the ultra-violet.

Found: C,76·3; H,10·0%

$C_{31}H_{48}O_4$ requires: C,76·8; H,9·9%.

α-Amyrenonyl Acetate.

(Conditions after Vickerstaff, Ph.D.Thesis, Manchester University, 1935).

α-Amyrenyl acetate (4·5 g.) was refluxed with glacial acetic acid (150 ml.) and to the solution chromic anhydride (3·75 g.) in acetic acid (90%; 50 ml.) was added dropwise over 15 minutes. The solution was refluxed for a further hour, after which time the reaction product was precipitated with water and crystallised from aqueous acetic acid. After three recrystallisations, α-amyrenonyl acetate was obtained as large plates, m.p. 276-278°. Yield 3·9 g.

Found: C,79·2; H,10·2%

Calc. for $C_{32}H_{50}O_3$: C,79·6; H,10·4%

$[\alpha]_D^{20}$ +157° (l = 1, c = 0·96 in chloroform).

Attempted Pyrolysis of α-Amyrenonyl Acetate in Quinoline.

α-Amyrenonyl acetate (1000 mg.) was heated in pure quinoline (45 ml.) for 10 hours in an oil bath at 265-275°. During this time, a stream of nitrogen was passed through the reaction mixture, and into a solution of barium hydroxide exactly as described in the decarboxylation of ketoacetylursolic acid (p.101). After 10 hours heating, only a negligible amount of barium carbonate had been precipitated, and the reaction mixture on cooling was poured into excess dilute hydrochloric acid. The precipitate which formed was worked up in the usual manner, and on crystallisation from aqueous acetic acid, the product was obtained as large colourless plates (900 mg.) m.p.276-278° identified by mixed melting point as unchanged α-amyrenonyl acetate.

Ursonic Acid.

(cf. Jacobs and Fleck, J.Biol.Chem.,1931,92,487)

To ursolic acid (1400 mg.) in stabilised glacial acetic acid (44 ml.), chromic anhydride (300 mg.) in acetic acid (85%; 10 ml.) was added, with mechanical

stirring, and the mixture heated on a steam bath for 30 minutes. The mixture on cooling was poured into water and the precipitated solid separated. This on crystallisation from aqueous ethanol, followed by two crystallisations from acetone gave ursonic acid as needles, m.p.282-284°, which gave a yellow coloration with tetranitromethane in chloroform. Yield 1100 mg. (80%).

Attempted Decarboxylation of Ursonic Acid.

Ursonic acid (1000 mg.) in dry, freshly distilled, quinoline (45 ml.) was heated on an oil bath at 265-275° for 10 hours. The conditions were the same as those already described for the decarboxylation of ketoacetylursolic acid (p.101). After 10 hours there was no evidence of evolution of carbon dioxide, and the reaction mixture was worked up as before. On crystallisation from aqueous ethanol, the product was obtained as needles m.p.276-279° undepressed on admixture with starting material. The recovered yield of ursonic acid was 840 mg.

Attempted Decarboxylation of Acetylursolic Acid.

Acetylursolic acid (750 mg.) was heated in quinoline (30 ml.) at 265-275° in an atmosphere of nitrogen under exactly similar conditions to those used for the

decarboxylation of ketoacetylursolic acid (p.101). After 10 hours, there was no trace of any evolution of carbon dioxide, and the product was worked up in the usual way, giving a crop (650 mg.) of colourless needles, m.p.265-267°. These were undepressed in melting point when mixed with acetylursolic acid, and were unchanged starting material.

Oxidation of Acetylursolic Acid with Hydrogen Peroxide.

(cf. Jeger, Borth, and Ruzicka, Helv.Chim.Acta,1946,29,1999)

Acetylursolic acid (5 g.) in stabilised glacial acetic acid (200 ml.) was heated, with mechanical stirring, on a steam bath, and to the solution hydrogen peroxide (30%; 20 ml.) in glacial acetic acid (20 ml.) was added during 15 minutes. The heating was then continued for two hours, and a further addition of hydrogen peroxide (30%; 20 ml.) in glacial acetic acid (20 ml.) was made. After a further two hours, the reaction mixture was reduced in volume under reduced pressure, to about 50 ml., cooled, and poured into water. The mixture was extracted with ether, and the extract washed successively with water (3 x 40 ml.), with sodium hydroxide solution (5%; 5 x 20ml.) and again with water (2 x 40 ml.), giving a dark red alkaline extract (A) and a neutral ether extract (B).

The alkaline extract (A) was acidified with dilute hydrochloric acid, and the precipitated solid (950 mg.) separated. This could not be obtained crystalline by any of the usual methods, and was finally dissolved in ether and methylated with an ethereal solution of diazomethane. The product obtained (750 mg.) could not be crystallised, and was dissolved in pure dry petroleum ether (60/80°; 200 ml.). The solution was then passed through a column of activated alumina (25 x 1·75 cm.), when complete adsorption of the dissolved material took place. The column was washed with various solvents, and the following fractions obtained.

Fraction	Solvent	Eluate
1.	200ml. petroleum (b.p.60-80°)	Nil
2.	100ml. petrol-benzene; (1:1)	200mg. colourless resin
3.	100ml. " "	130mg. " "
4.	100ml. " "	80mg. " "
5.	100ml. " "	60mg. resin,
6.	100ml. " "	50mg. crystallised
7.	100ml. " "	10mg. from ethanol.
8.	100ml. benzene	80mg. yellow resin
9.	100m. "	30mg. " "
10.	100ml. "	10mg. " "
11.	100ml. methanol	40mg. dark resin.
		690mg.

Fractions 5, 6, and 7 were combined and twice crystallised from ethanol giving needles m.p.242-246°. After two further crystallisations, methyl oxidoacetylursolate was obtained in fine colourless needles, m.p. 246-249°. The substance gave no coloration with tetranitromethane in chloroform, and was undepressed in melting point when mixed with methyl oxidoacetylursolate obtained by direct oxidation of methyl acetylursolate.

Found: C,75·1; H,9·7%

$C_{33}H_{52}O_5$ requires: C,75·0; H,9·9%

$[\alpha]_D^{20}$ +27° (l = 1, c = 0·42 in chloroform).

From the remaining fractions no crystalline material could be obtained.

The neutral ether extract (B) after being dried over sodium sulphate, was evaporated to dryness under reduced pressure, and the residue crystallised from ethanol, as needles, m.p.262-265°. After repeated recrystallisation, a lactone, m.p.275-277° was obtained. This was saturated to tetranitromethane. Yield 2·1 g.(41%)

Found: C,74·7; H,9·3%

$C_{32}H_{50}O_5$ requires: C,74·7; H,9·7%

$C_{32}H_{48}O_5$ requires: C,75·0; H,9·4%

$[\alpha]_D^{18}$ +44° (l = 1, c = 0·68 in chloroform).

Methyl Oxido-acetylursolate.

To methyl acetylursolate (7·5 g.) in boiling glacial acetic acid (150 ml.), hydrogen peroxide (30%; 75 ml.) in glacial acetic acid (75 ml.) was added dropwise over a period of 10 minutes. After refluxing for 2 hours, the solution was diluted with water until slightly turbid. On cooling, a crop of fine needles was deposited, which after five recrystallisations from ethanol, gave methyl oxide-acetylursolate in needles, m.p.250-253° (decomp.). The product was saturated to tetranitromethane. Yield 4·4 g. (58%).

Found: C,75·0; H,9·8%

$C_{33}H_{52}O_5$ requires: C,75·0; H,9·9%

$[\alpha]_D^{20}$ +25° (l = 1, c = 0·92 in chloroform).

Isomerisation of Methyl Oxido-acetylursolate.

Concentrated hydrochloric acid (2 ml.) was added to a solution of methyl oxidoacetylursolate (750 mg.) in chloroform (10 ml.) and glacial acetic acid (40 ml.). The mixture was maintained at 40° for half an hour, after which time the chloroform was removed under reduced pressure. The remaining solution was then poured into water, extracted with ether, and the extract washed with dilute sodium carbonate solution. After being washed with water, the extract was dried over sodium sulphate

and evaporated to dryness under reduced pressure. The residual yellow resin was then crystallised from methanol giving colourless needles, m.p.200-220°, which even after repeated recrystallisation from methanol, retained their diffuse melting point. The substance (520 mg.) was dissolved in petroleum ether (b.p.60-80°; 150 ml.), and the solution passed through a column of activated alumina (13 x 1·75 cm.). There was complete adsorption of the dissolved material on the column which was then washed with various solvents as shown on the following page.

Fractions 2 and 3 after repeated crystallisation from ethanol were obtained as opaque white needles, having respectively the constant m.p.s208-222° and 216-232°. Further purification by crystallisation was however impossible.

Fractions 4 and 7 when mixed gave no depression in melting point and fractions 4, 5, 6, and 7 were therefore combined giving fraction 4/7. After four recrystallisations from ethanol, fraction 4/7 was obtained as small prismatic needles, m.p.253-255°. Yield 30 mg.

Fraction	Solvent	Eluate
1.	150ml. petroleum (b.p.60-80°)	Nil
2.	100ml. petrol-benzene; (1:1)	70mg. m.p.204-216°
3.	100ml. "	40mg. m.p.216-242°
4.	100ml. "	30mg. m.p.242-244°
5.	100ml. "	30mg. m.p.240-242°
6.	100ml. "	10mg. m.p.240-242°
7.	100ml. "	10mg. m.p.246-248°
8.	100ml. benzene	50mg. m.p.252-253°
9.	50ml. "	20mg. m.p.250-251°
10.	150ml. "	30mg. m.p.246-247°
11.	100ml. "	5mg. m.p.230-240°
12.	100ml. benzene-ether; (1:1)	40mg. m.p.222-228°
13.	100ml. "	40mg. m.p.232-238°
14.	100ml. ether	40mg. }
15.	100ml. "	10mg. } m.p.230-240°
16.	100ml. "	5mg. }
17.	50ml. ethanol	20mg. } oil
18.	50ml. "	10mg. }
		455mg.

Fractions 8 and 9, being undepressed in melting point when mixed, were combined and recrystallised three times from ethanol giving fraction 8/9 as needles m.p.255-256°.

Fraction 10 after two recrystallisations from ethanol was obtained in small prismatic needles m.p.253-255°. This was undepressed in melting point on admixture with fraction 8/9, giving fraction 8/10 (50 mg.).

Fractions 4/7 and 8/10 when mixed gave no depression in melting point, and were then combined, giving on crystallisation, from ethanol, methyl ketodihydro-acetyl-ursolate (70 mg.) as small prismatic needles, m.p.254-256°. With tetranitromethane in chloroform, the substance gave no coloration, and when mixed with methyl oxido-acetylursolate the melting point was depressed by 20°.

Found: C,75·3; H,9·8%

$C_{33}H_{52}O_5$ requires: C,75·0; H,9·9%

$[\alpha]_D^{20}$ +32° (l = 1, c = 0·86 in chloroform).

Fraction 12 after two crystallisations from ethanol was obtained in needles m.p.246-250°, which when mixed with fraction 8/10 were depressed in melting point by 15°.

Fractions 13 and 14 after two crystallisations from ethanol gave needles m.p.249-252° and 248-251° respectively, and were combined giving fraction 13/14, (30 mg.) m.p. 249-252° not depressed in melting point on admixture with methyl oxido-acetylursolate. The substance was therefore unchanged starting material.

Found: C,74·5; H,9·8%

Calc. for $C_{33}H_{52}O_5$: C,75·0; H,9·9%

Isomerisation of Methyl Oxido-acetylursolate.

(cf. Picard and Spring, J.,1940,1387.)

Methyl oxido-acetylursolate (500 mg.) in glacial acetic acid (18 ml.) was heated for 6 hours on a steam bath, with hydrochloric acid (10%; 0·5 ml.). After standing at room temperature for a further 24 hours, the mixture was poured into water, and the precipitate dissolved in methanol. On standing for two days, small rosettes of needles appeared, which after three recrystallisations from ethanol gave methyl ketodihydro-acetylursolate as prismatic needles, m.p.254-256°. When mixed with methyl ketodihydro-acetylursolate (p.115) there was no depression in melting point. Yield 85 mg. (17%).

Found: C,74·9; H,10·2%

$C_{33}H_{52}O_5$ requires: C,75·0; H, 9·9%

$[\alpha]_D^{18}$ +35° (l = 1, c = 1·03 in chloroform).

Bromination of Methyl Oxido-acetylursolate.

1. At room temperature.

To a solution of methyl oxido-acetylursolate (500 mg.) in glacial acetic acid (60 ml.) containing a trace of

hydrogen bromide (47% aqueous; 0·2 ml.), bromine (3%; 5·5 ml.) in acetic acid was added dropwise, and with stirring, until the red coloration just failed to be discharged. After standing for a further three hours at room temperature, the mixture was poured into excess water, and the solid which separated, filtered off. This was crystallised from aqueous acetone, and after three recrystallisations from the same solvent, gave needles m.p.126-128°. This compound was heated, in vacuo, at 55° for one hour, after which time the melting point was 196-198°. The product, methyl bromketodihydro-acetylursolate, was saturated to tetranitromethane in chloroform. Yield 350 mg. (61%).

Found: C,64·9; H,8·6%

$C_{33}H_{51}O_5Br$ requires: C,65·2; H,8·4%

$[\alpha]_D^{18}$ +106° (l = 1, c = 0·76 in chloroform).

Methyl bromketodihydro-acetylursolate of m.p.196-198° on recrystallisation from acetone gave again the substance m.p.126-128°.

2. <u>At 70°.</u>

Methyl oxido-acetylursolate (500 mg.) in glacial acetic acid (60 ml.) containing hydrogen bromide (47% aqueous; 0·2 ml.) was heated to 60°, and to the solution

bromine in glacial acetic acid (3%; 5 ml.) was added dropwise and with frequent shaking. The mixture was then heated at 70° for one hour, after which it was poured into excess water. The precipitated solid was crystallised from aqueous ethanol, and after four recrystallisations methyl iso-ketoacetylursolate was obtained as colourless needles, m.p.186-188°, which gave no coloration with tetranitromethane in chloroform. Yield 280 mg. (56%).

Found: C,74·6; H,9·8%

$C_{33}H_{50}O_5$ requires: C,75·2; H,9·5%

$[\alpha]_D^{17}$ +87° (l = 1, c = 0·81 in chloroform).

Light absorption in ethanol: Maximum at 2490Å, ε=15,500.

Debromination of Methyl Bromketodihydro-acetylursolate to Methyl iso-Ketoacetylursolate.

Methyl bromketodihydro-acetylursolate (300 mg.) in glacial acetic acid (15 ml.) containing a trace of hydrogen bromide (47% aqueous; 0·1 ml.) was heated on a steam bath for three hours. The solution was then left at room temperature for 12 hours before being poured into water and extracted with ether. The extract was washed with dilute sodium carbonate solution and water, and dried over sodium sulphate. On evaporation to

dryness under reduced pressure a resinous material which crystallised from acetone in needles was obtained. After two recrystallisations, methyl iso-ketoacetylursolate was obtained in needles m.p.186-188°. When mixed with methyl iso-ketoacetylursolate obtained directly from methyl oxido-acetylursolate, there was no depression in melting point. Yield 200 mg. (77%).

Found: C,74·9; H,9·6%

$C_{35}H_{50}O_5$ requires: C,75·2; H,9·5%

$[\alpha]_D^{18}$ +85° (l = 1, c = 0·93 in chloroform)

Light absorption in ethanol:- Maximum at 2490Å, ε=15,000.

Methyl iso-Ketoursolate.

Methyl iso-ketoacetylursolate (100 mg.) was refluxed with ethanolic potassium hydroxide (0·5%; 20 ml.) for 2 hours. The reaction mixture was then poured into excess water, and extracted with ether. The extract was washed with water, with dilute hydrochloric acid, again with water, and finally dried over sodium sulphate. The residue obtained, after evaporation of the extract to dryness, was crystallised from aqueous acetone, and after two further crystallisations gave methyl iso-ketoursolate as plates, m.p.236-238°. Yield 75 mg.(81%).

Found: C,76·5; H,9·7%

$C_{31}H_{48}O_4$ requires: C,76·8; H,9·9%

$[\alpha]_D^{22°}$ +82° (l = 1, c = 0·61 in chloroform).

Light absorption in ethanol:- Maximum at 2500Å, ε=15,500.

Acetylation of methyl iso-ketoursolate with acetic anhydride in pyridine gave methyl-iso-ketoacetylursolate m.p.186-188° identical with that already obtained (p.119).

Bromination of Methyl Ketodihydro-acetylursolate.

Methyl ketodihydroacetylursolate (40 mg.) was dissolved in glacial acetic acid (5 ml.), and to the solution bromine in acetic acid (3%; 0·45 ml.) was added dropwise and with stirring, until a permanent colour just remained. The mixture was then allowed to stand for three hours at room temperature before being poured into water. The precipitate was separated, roughly dried, and dissolved in acetone. On standing, methyl bromketodihydro-acetylursolate separated out as needles m.p.118-122°, undepressed when mixed with the product, of m.p.126-128°, obtained by the direct bromination of methyl oxido-acetylursolate.

Methyl iso-Ketodehydro-acetylursolate.

(cf. Green, Mower, and Spring, J.,1944,527)

Methyl iso-ketoacetylursolate (500 mg.) was refluxed for 24 hours in glacial acetic acid (15 ml.) with powdered

selenium dioxide (500 mg.). The mixture was then filtered free from selenium, poured into water, and extracted with ether. The extract was washed successively with dilute sodium hydroxide solution, with potassium cyanide solution (3%) and finally with water. After drying over sodium sulphate, the extract was evaporated to dryness under reduced pressure, and the residue crystallised from aqueous acetone. After two further recrystallisations, methyl iso-ketodehydro-acetylursolate was obtained as plates, m.p.332-334°. The substance gave no coloration with tetranitromethane in chloroform. Yield 300 mg. (60%).

Found: C,75·1; H,9·4%

$C_{33}H_{48}O_5$ requires: C,75·6; H,9·2%

$[\alpha]_D^{20}$ -63° (l = 1, c = 1·03 in chloroform).

Light absorption in ethanol:- Maximum 2380Å, ε=21,000.

Methyl iso-Ketodehydro-ursolate.

Methyl iso-ketodehydro-acetylursolate (100 mg.) was refluxed with ethanolic potassium hydroxide (0·5%; 20 ml.) for two hours. The solution was then poured into dilute hydrochloric acid solution, and extracted with ether. The extract was washed first with dilute sodium hydroxide solution, then with water, and dried over sodium sulphate,

giving a residual gum which crystallised from acetone as rectangular plates. After a recrystallisation from aqueous acetone, methyl iso-ketodehydro-ursolate was obtained as plates m.p.348-350° (decomp.). Yield 55 mg. (60%).

Found: C,77·4; H,9·4%

$C_{31}H_{46}O_4$ requires: C,77·2; H,9·6%

$[\alpha]_D^{20}$ -69° (l = 1, c = 0·77 in chloroform).

Light absorption in ethanol:- Maximum at 2400Å, ε=23,000.

Methyl iso-ketodehydro-ursolate (30 mg.) when acetylated in pyridine with acetic anhydride gave methyl iso-ketodehydro-acetylursolate, m.p.332-334° identical with that already obtained (p.121).

Attempted Catalytic Reduction of Methyl iso-Ketodehydro-acetylursolate.

1. In ethanol.

Methyl iso-ketodehydro-acetylursolate (150 mg.) was dissolved in absolute ethanol (50 ml.) and the solution added to a suspension of platinum (from Adams platinum oxide catalyst (40 mg.), prepared after Organic Syntheses, 1928,8,92) in ethanol (20 ml.). The mixture was then shaken with hydrogen at room temperature and atmospheric pressure for 24 hours. During this time

there was no apparent uptake of hydrogen. The catalyst was removed by filtration, and on concentration of the ethanolic solution under reduced pressure, plates m.p.332-334° (120 mg.; 80%) separated out. These were undepressed in melting point when mixed with methyl iso-ketodehydro-acetylursolate.

2. In glacial acetic acid.

Methyl iso-ketodehydro-acetylursolate (200 mg.) was dissolved in glacial acetic acid (50 ml.) and Adam's platinum oxide catalyst (30 mg.) added. The mixture was shaken with hydrogen at room temperature and atmospheric pressure for 24 hours. The catalyst was then removed by filtration and a further quantity (30 mg.) added. This mixture was shaken for a further 24 hours, after which time the catalyst was removed as before, and the product precipitated with water. On crystallisation from ethanol this gave plates m.p.332-334° (180 mg.; 90%), undepressed on admixture with methyl iso-ketodehydro-acetylursolate.

Uvaol.

A solution of methyl acetylursolate (1500 mg.) in dry ether (100 ml.) was added dropwise to a suspension of lithium aluminium hydride (500 mg.) in dry ether (100ml.)

at room temperature. The mixture was then refluxed for three hours, after which time, excess water was cautiously added to decompose the reduction adduct and the excess lithium aluminium hydride. Dilute sulphuric acid (10%) was then added until the mixture was just acid when it was extracted with ether. The extract was washed with water until neutral to litmus, dried over sodium sulphate, and the ether removed under reduced pressure. The residual solid, after two crystallisations from ethanol gave uvaol in plates, m.p.226-228°. With tetranitromethane in chloroform, uvaol gave a pale yellow coloration. Yield 980 mg. (76%).

Found: C,81·1; H,11·2%

Calc. for $C_{30}H_{50}O_2$: C,81·4; H,11·3%

$[\alpha]_D^{20}$ +80° (l = 1, c = 0·62 in chloroform).

Huzii and Osumi (J.Pharm.Soc.,Japan,1939,59,176) reported uvaol to have m.p.233°, and $[\alpha]_D$ +74° in chloroform.

Uvaol Diacetate.

Uvaol (500 mg.) in pyridine (25 ml.) was heated for two hours on a steam bath with acetic anhydride (30 ml.). The mixture was then poured into water, and extracted with ether. The extract, after being washed with dilute hydrochloric acid, and with water was dried over sodium

sulphate, and evaporated to dryness under reduced pressure. On crystallisation of the residue, followed by two recrystallisations from ethanol, uvaol diacetate (300 mg.; 59%) was obtained in prisms of m.p.152-153°. The product gave a pale yellow coloration with tetranitromethane in chloroform.

Found: C,77·8; H,10·5%

Calc. for $C_{34}H_{54}O_4$: C,77·6; H,10·3%

$[\alpha]_D^{18}$ +65° (l = 1, c = 0·68 in chloroform).

Huzii and Osumi (J.Pharm.Soc.,Japan,1940,60,178) reported uvaol diacetate to have m.p. of 157-159°.

Attempted Partial Dehydrogenation of Methyl Ketoacetylursolate with Bromine.

Methyl ketoacetylursolate (200 mg.) was heated on a steam bath with glacial acetic acid (10 ml.), and bromine in glacial acetic acid (3%; 3·5 ml.) added dropwise and with stirring. Hydrogen bromide (47% aqueous; 0·10 ml.) was added to the solution and heating continued for one hour. After this time, the mixture was cooled, poured into water and extracted with ether. The extract was washed successively with water, with dilute sodium carbonate solution, and again with water, before being dried over sodium sulphate. On evaporation of the ether,

a crystalline residue was obtained which after two recrystallisations from aqueous acetone gave needles m.p.241-243° undepressed in melting point on admixture with methyl ketoacetylursolate. The substance (150 mg.; 75%) was therefore unchanged starting material.

Attempted Oxidation of Methyl Ketoacetylursolate with Selenium Dioxide.

To methyl ketoacetylursolate (500 mg.) in stabilised glacial acetic acid (30 ml.), selenium dioxide (500 mg.) was added, and the solution refluxed for 24 hours. After this time, the mixture was poured into water, extracted with ether and the extract washed with dilute sodium hydroxide solution, followed by water before being finally dried over sodium sulphate. The ether was then removed under reduced pressure and the residue crystallised from ethanol giving colourless prisms, m.p.239-242°, which were undepressed in melting point when mixed with methyl ketoacetylursolate. The recovered starting material was 450 mg. (90%).

Attempted Oxidation of Methyl Acetylursolate.

1. With potassium permanganate.

Methyl acetylursolate (1500 mg.) was refluxed with acetone (100 ml.) containing dilute sulphuric acid

(10%; 100 ml.), and to the hot solution a saturated solution of potassium permanganate in acetone was added dropwise. The heating was continued for two hours after which time the greater part of the acetone was distilled off under reduced pressure, and the remaining mixture decolourised with a slight excess of a solution of sodium bisulphite. When colourless the mixture was extracted with ether, and the extract washed with water and with dilute sodium hydroxide solution (5%). Acidification of the alkaline washings gave no precipitate. The ether solution was then washed again with water, dried over sodium sulphate, and evaporated to dryness under reduced pressure, giving a residue which crystallised from ethanol. After two further crystallisations from ethanol, the substance was obtained as plates m.p.242-244° undepressed in melting point when mixed with methyl acetylursolate and was therefore unchanged starting material. Recovered yield 950 mg. (63%).

2. With selenium dioxide.

Methyl acetylursolate (500 mg.) in stabilised glacial acetic acid (40 ml.) was refluxed with selenium dioxide (300 mg.) for 24 hours. The mixture, on cooling, was poured into water, extracted with ether, and the

extract washed with dilute sodium hydroxide solution, and with water. After drying over sodium sulphate the extract was evaporated to dryness under reduced pressure, and the residue crystallised from aqueous ethanol as plates, m.p.240-242°. Since the substance on admixture with methyl acetylursolate showed no depression in melting point, it was assumed to be unchanged starting material. Recovered yield 450 mg. (90%).

3. With perbenzoic acid.

Methyl acetylursolate (250 mg.) was dissolved in chloroform (20 ml.) and added to a solution of perbenzoic acid in chloroform (25 ml. 0·41N containing sufficient reagent to introduce 10 oxygen atoms; prepared after Organic Syntheses,13,86). The mixture was then kept at 0° for 144 hours. A blank experiment using chloroform (20 ml.) and perbenzoic acid (25 ml.; 0·41N.) was kept under identical conditions. 1 ml. portions were taken at intervals from both the reaction and the blank, run into potassium iodide solution, acidified with dilute hydrochloric acid, and the liberated iodine titrated with standard sodium thiosulphate solution:

Time (hours)	Titre (ml. 0·878 $\frac{N}{10}$ $Na_2S_2O_3$) Blank	Reaction	O atoms absorbed
1	2·02	2·01	—
24	2·01	2·00	—
48	2·01	2·00	—
72	2·00	2·00	—
96	2·00	1·98	—
120	1·99	1·98	—
144	1·98	1·97	—

After 6 days, the reaction mixture was washed with dilute sodium hydroxide solution (5%) and then with water. After drying over sodium sulphate, the chloroform was removed under reduced pressure, and the residue crystallised from ethanol in plates, m.p.240-242° undepressed in melting point when mixed with starting material. The recovered yield of methyl acetylursolate was 170 mg. (68%).

Attempted Oximation of Methyl Ketodihydro-acetylursolate.

Methyl ketodihydro-acetylursolate (100 mg.) was dissolved in boiling ethanol (75 ml.), and hydroxylamine hydrochloride (100 mg.) and anhydrous sodium acetate (200 mg.) added. After refluxing for 10 hours, the mixture was poured into water, and the precipitated solid filtered off. On crystallisation from ethanol, prisms

m.p.250-252° were obtained, which were not depressed in melting point when mixed with methyl ketodihydro-acetyl-ursolate. Recovered yield 85 mg. (85%).

Attempted Formation of Acetylursolyl Diphenylcarbinol from Methyl Acetylursolate.

1. In boiling benzene.

Methyl acetylursolate (5 g.) in dry benzene (30 ml.) was added dropwise to an ethereal solution of phenyl magnesium bromide (prepared from bromobenzene (16 g.) and magnesium (25 g.)). The mixture was refluxed for 3 hours, after which time, the benzene and ether were removed under reduced pressure, and pure dry benzene (150 ml.) added. The refluxing was then continued for a further 20 hours. The solution was evaporated to dryness under reduced pressure, and the residue, after being dissolved in ether was poured on to ice (80 g.) and dilute hydrochloric acid (10%; 140 ml.). The ether layer from this mixture was washed with dilute hydrochloric acid (5%), and with water before being dried over sodium sulphate. After removal of the ether under reduced pressure, the oily residue was steam distilled to remove any diphenyl present, and finally again extracted with ether. The extract, after drying over sodium sulphate was evaporated

to dryness, and attempts made to crystallise the residual resin. These were unsuccessful, and the substance was then acetylated with acetic anhydride (75 ml.) in pyridine (75 ml.). The product, purified through ether in the usual manner, was crystallised from ethanol in plates, m.p.242-244°, (4·2 g.; 84%) undepressed in melting point when mixed with methyl acetylursolate.

2. In boiling xylene.

The reaction was carried out exactly as before, using xylene in place of benzene. The methyl acetylursolate remained unchanged, giving a recovered yield of 4·6 g.(92%).

3. In boiling N-methyl morpholine.

The attempted diphenylcarbinol formation was carried out exactly as described above, except that N-methyl morpholine (prepared after Atherton, Openshaw, and Todd, J.,1945,660) was used as the solvent in place of benzene. The methyl acetylursolate was again recovered unchanged. (4·5 g.; 90%).

Acetylursolyl Chloride.

(cf. Goodson, J.,1938,999)

Acetyl ursolic acid (2·5 g.) was refluxed with thionyl chloride (15 ml.) for 30 minutes. The excess thionyl

chloride was then removed by distillation under reduced pressure, and the residual solid, after drying over potassium hydroxide in vacuo, crystallised from benzene-petroleum ether (b.p.40-60°). After two further crystallisations from the same solvent, acetyl ursolyl chloride was obtained in prismatic needles m.p.222-224°. Yield 2·1 g. (81%).

Found: C,74·6; H,9·7%

Calc. for $C_{32}H_{49}O_3Cl$: C,74·3; H,9·5%

$[\alpha]_D^{20}$ +52° (l = 1, c = 0·87 in chloroform)

Acetylursolyl Diphenylcarbinol.

Acetylursolyl chloride (2·0 g.) in dry benzene was added gradually to a solution of phenylmagnesium bromide in ether (prepared from bromobenzene (6·2 g.) and magnesium (1·0 g.)). The mixture after refluxing for one hour was poured into dilute sulphuric acid (10%; 80 ml.) and ice (50 g.), and extracted with ether. The extract was washed with dilute sulphuric acid (10%), with dilute sodium hydroxide solution (5%) and finally with water, before being dried over sodium sulphate. Removal of the solvent, under reduced pressure, gave a resinous residue which was freed from diphenyl by steam distillation, and then extracted with ether. The extract

was dried over sodium sulphate, and evaporated to dryness under reduced pressure. The resin obtained was then acetylated by heating on a steam bath with acetic anhydride (40 ml.) and pyridine (40 ml.) for 12 hours. The mixture was poured into water, extracted with ether, and the extract washed successively with dilute hydrochloric acid (5%), with dilute sodium hydroxide solution (5%) and with water. After drying over sodium sulphate, the extract was evaporated to dryness, and the residue obtained, crystallised from ethanol. After three further crystallisations, acetylursolyl diphenylcarbinol was obtained as colourless plates, m.p.242-244°. Yield 1300mg. (52%).

Found: C,82·6; H,9·5%

$C_{44}H_{60}O_3$ requires: C,83·0; H,9·4%

$C_{38}H_{54}O_4$ requires: C,79·4; H,9·4%

$[\alpha]_D^{20}$ +45° (l = 1, c = 1·08 in chloroform).

Attempted Dehydration of Acetylursolyl Diphenylcarbinol. Method 1.

Acetylursolyl diphenylcarbinol (500 mg.) was refluxed for two hours, with dry benzene (20 ml.), containing a crystal of iodine. After this time, the benzene was evaporated off under reduced pressure, and

the residue crystallised from ethanol giving plates m.p.240-242° undepressed in melting point when mixed with starting material. Recovered yield 420 mg. (84%).

Method 2.

Acetylursolyl diphenylcarbinol (500 mg.) was refluxed for two hours with dry benzene (20 ml.) containing hydrochloric acid (35%; 0·05 ml.). On evaporation of the solvent, and crystallisation of the residue from ethanol, only unchanged starting material was obtained. Recovered yield 450 mg. (90%).

Method 3.

Acetylursolyl diphenylcarbinol (500 mg.) was refluxed for 15 hours with glacial acetic acid (20 ml.), after which time the mixture was poured into water and extracted with ether. The ethereal extract, after washing with water, and drying over sodium sulphate, was evaporated to dryness. The residue on crystallisation from ethanol, gave plates m.p.240-242° undepressed in melting point when mixed with acetylursolyl diphenylcarbinol. Recovered yield 370 mg. (74%).

Tetranitromethane.

(cf. Chattaway, J.,1910,2099; Organic Syntheses,21,105)

Anhydrous nitric acid (31·5 g.) prepared by distillation of fuming nitric acid from its own volume of concentrated sulphuric acid, was cooled to below 10° and to it was added acetic anhydride (51 g.) dropwise so that the temperature never rose above 10°. After the addition was complete the mixture was allowed to stand at room temperature for 7 days, before being poured into water. On steam distillation of the mixture, tetranitromethane was obtained as a pale yellow oil which was washed with water, followed by dilute sodium carbonate solution, before finally being dried over sodium sulphate. Yield 15·2 g. (61%).

Nitrosomethylurea.

Nitrosomethylurea was prepared by the method described in Organic Syntheses,1935,15,38.

Nitrosoethylurea.

Nitrosoethylurea was prepared from ethylamine hydrochloride and potassium cyanate using the procedure described for nitrosomethylurea in Organic Syntheses, 1935,15,38.

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PART II

THE RESIN FROM CANARIUM SCHWEINFURTHII.

HISTORICAL

INTRODUCTION.

The vegetable resins are exudations from the trunks of certain species of trees and shrubs which are most commonly found in the tropics. In general, the resins have been found to consist of mixtures of acid and neutral substances of fairly high molecular weight (ca. 500) together with one or more essential oils. Their physical state may vary from hard and brittle to soft and sticky depending largely on the proportion of essential oil present, although all are easily fusible.

The resins although at present limited to their use in the preparation of varnishes, cements, and printing inks, have in the past been used medicinally, and it was with a view to the possible isolation of a constituent of medicinal value that the present analytical study of Canarium Schweinfurthii was initiated.

Previous work on the resin, which was obtained by incision of the trunk of the tree Canarium Schweinfurthii, has indicated the presence of a pale yellow oil reported to be rich in phellandrene (1), along with a non-volatile substance similar in crystalline form to the mixture of α - and β-amyrin obtained from Manila Elemi resin (2).

No further information was available regarding the resin, whose investigation will now be reported in the following sections.

THEORETICAL

INTRODUCTION.

The Canarium Schweinfurthii resin was separated first into a volatile and a non-volatile fraction by steam distillation. The residual non-volatile fraction was then dissolved in ether, and the solution extracted with aqueous alkali thus separating the resin acids from neutral components of the resin.

The three fractions obtained in this way were then examined separately as described in the succeeding sections.

THE STEAM VOLATILE FRACTION.

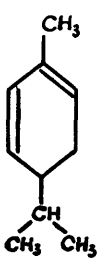
The volatile oil obtained from the resin, after fractional distillation yielded the following three fractions, of which fraction 2 was the largest:-

1) b.p.57-62°/16 mm.

2) b.p.62-64°/16 mm.

3) b.p.64-69°/16 mm.

Fraction 2, after four further fractionations yielded a colourless oil, $C_{10}H_{16}$, of b.p.62-62·5°/15 mm., $[\alpha]_D^{20}$ + 111·4°, η_0^{20} 1·4755, and d^{20} 0·846. In addition the oil was shown by perbenzoic acid titration, to contain two double bonds which were assumed to be unconjugated since the oil showed no light absorption in the ultra-violet region, and was unaffected by maleic anhydride. The main fraction of the volatile oil cannot therefore consist of a mixture of the phellandrenes as previously reported (1) since both the α- (I) and β-phellandrenes (II) exhibit a characteristic light absorption in the ultra-violet (3,4,5), and react readily with maleic anhydride (6).



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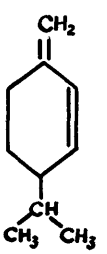

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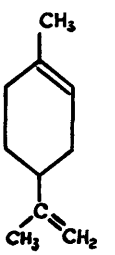

III

The oil, $C_{10}H_{16}$, appeared to be a monocyclic terpene containing two isolated double bonds, and a comparison of its physical constants with those of similar terpenes revealed the greatest similarity between it and d-limonene (III) for which Braun and Lemke (7) have recorded a b.p. of 176-176·4°, d^{20} 0·8411, and $[\alpha]_D^{20}$ + 126·84. d-Limonene has been reported by Bruhl (8) to have a b.p. of 175·5-176°/763 mm., d^{21} 0·8402, $[\alpha]_D^{21}$: 123·8°, while Richter and Wolff (9) have recorded a b.p. of 64·4°/15 mm., η_D^{17} 1·473, and $[\alpha]_D$ + 124°. It will be seen therefore that the main bulk of the oil from Canarium Schweinfurthii resin has physical constants in agreement with those of d-limonene. The slight differences occurring among the values quoted for d-limonene have been attributed to the difficulty of preparing the hydrocarbon absolutely free from its isomers (10).

On treatment with bromine by the method described by Baeyer and Villiger (11) for the bromination of d-limonene, the oil, $C_{10}H_{16}$, yielded a crystalline tetrabromide, $C_{10}H_{16}Br_4$, of m.p.104-106°, and $[\alpha]_D^{20}$ + 72°. This is identical in melting point and specific rotation with the isomeric d-limonene tetrabromide obtained by Baeyer and Villiger (11) who accounted for the relatively poor yield (37%) by suggesting the simultaneous formation of liquid

bromides due to cis-trans-isomerism.

From this it would appear that fraction 2 from the volatile oil consists of d-limonene.

Fraction 1 from the first fractionation on treatment with maleic anhydride gave in very low yield (ca. 0·5%) a compound, $C_{14}H_{18}O_3$, of m.p.125-127°. This substance was identified as the adduct obtained by treatment of both α- and β-phellandrene with maleic anhydride (12). It is clear however from the yield of this product that the amount of phellandrene present in the oil is extremely small (6) especially as the oil itself shows none of the characteristic ultra-violet light absorption of the α- and β-phellandrenes. Apart from this difference fraction 1 appeared to be identical with fraction 2, described above, and can therefore be considered as consisting of d-limonene containing a trace of one of the isomeric phellandrenes.

The third fraction from the original fractionation was obtained only in small yield and is thought to consist of a mixture of d-limonene, and one of its oxidation products, since after each of the four fractional distillations of fraction 2, small amounts of a similar liquid were obtained along with a dark oil of b.p. 70°/16 mm.

From the foregoing discussion therefore it will be seen that the steam volatile oil from Canarium Schweinfurthii resin consists of d-limonene containing a trace of one or more of the phellandrenes.

THE NON-VOLATILE ACID FRACTION.

The non-volatile acid fraction from the Canarium Schweinfurthii resin was obtained as a resinous material which resisted all attempts at crystallisation, as did the product obtained on esterification with diazomethane. On treatment with acetic anhydride in pyridine, however, the acid fraction yielded a product which after repeated crystallisation from nitromethane had a m.p. of 216-222° and analysed for $C_{32}H_{50}O_4$. This on alkaline hydrolysis gave the parent hydroxy-acid, $C_{30}H_{48}O_3$, of m.p.212-216°. Esterification of the acetylated acid, $C_{32}H_{50}O_4$, with diazomethane gave a methyl ester, $C_{33}H_{52}O_4$, of m.p.112-114° and $[\alpha]_D^{20}$ - 41°, which on titration with perbenzoic acid was found to contain two double bonds, and hence appeared identical with methyl acetyl-α-elemolate for which Ruzicka has reported a m.p. of 113·5-114°, and $[\alpha]_D$ - 43°.

In view of the difficulty encountered initially in crystallising the acid fraction, the presence of β-elemonic acid in addition to α-elemolic acid was suspected. That this was in fact the case was shown when a ketonic and a non-ketonic fraction were obtained on treatment of the crude acid fraction with Girard's reagent P.

The ketonic fraction yielded β-elemonic acid, $C_{30}H_{46}O_3$, of m.p.224-225° and $[\alpha]_D^{20}$ + 44° in agreement with the β-elemonic acid reported by Ruzicka (14) having a m.p. of 224-225° and $[\alpha]_D$+ 46·2°, + 44·9°, + 47·6°. Lieb and Mladenovic (15) have recorded a m.p. of 220° and $[\alpha]_D$+ 42·8° for β-elemonic acid. Methylation of the β-elemonic acid from Canarium Schweinfurthii resin yielded methyl β-elemonate of m.p.104-105°, and $[\alpha]_D^{20}$ + 36° in good agreement with the m.p. of 104-105° and $[\alpha]_D$+ 35° obtained by Ruzicka (14), and the m.p. of 108-109°, $[\alpha]_D$+ 30° recorded for the ester by Bilham and Kon (16).

The non-ketonic acid fraction obtained after the removal of the β-elemonic acid, gave on acetylation, acetyl-α-elemolic acid, $C_{32}H_{50}O_4$, of m.p.238-240° and $[\alpha]_D^{20}$ -40°. This melting point will be seen to agree more closely with the value of 241-242° reported by Ruzicka (13) than the figures of 225° reported by Lieb and Mladenovic (15), and 218-220° recorded by Bilham and Kon (16). The specific rotation value is however identical with those reported by Ruzicka (13) and Mladenovic (15).

On esterification, the acetyl-α-elemolic acid obtained above yielded a methyl ester of m.p.113-114°,

and $[\alpha]_D^{20}$ -43° identical with the ester previously obtained directly from the acetylated crude acid fraction.

No further crystalline material was obtained from the acid fraction which can therefore be considered to consist of α-elemolic acid and β-elemonic acid.

THE NON-VOLATILE NEUTRAL FRACTION.

The neutral non-volatile fraction from the Canarium Schweinfurthii resin, itself resinous, on treatment with acetic anhydride in pyridine, yielded a substance which after repeated recrystallisation had a molecular formula of $C_{32}H_{52}O_2$, m.p.208-213°, and $[\alpha]_D^{20}$ + 77°. On account of these properties the substance was suspected of being a mixture of α- and β-amyrenyl acetates, and was next hydrolysed and benzoylated. The resulting product on treatment with ether was separated into a readily ether-soluble fraction and a sparingly ether-soluble fraction.

The soluble fraction yielded a product of m.p.193-195° undepressed in melting point when mixed with a specimen of α-amyrenyl benzoate of m.p.195-196°, obtained from Manila Elemi resin. On hydrolysis followed by acetylation the benzoate yielded α-amyrenyl acetate, $C_{32}H_{52}O_2$, of m.p.219-221°, and $[\alpha]_D^{20}$ + 79°, identical with a specimen of α-amyrenyl acetate of m.p.223-225°, from Manila Elemi resin.

The sparingly soluble benzoate yielded a product of m.p.230-232° undepressed in melting point on admixture with β-amyrenyl benzoate of m.p.233-235° obtained from Manila Elemi resin. On hydrolysis followed by acetylation, the benzoate gave β-amyrenyl acetate of m.p.232-

234° and $[\alpha]_D^{20}$ + 82°, identical with a specimen of β-amyrenyl acetate from Manila Elemi resin.

When all the crystalline amyrenyl acetates had been removed from the crude acetylated neutral fraction there remained a quantity of a dark resinous material, which after hydrolysis was subjected to chromatographic purification. In this way, there was obtained in small yield, a substance $C_{30}H_{50}O_2$, of m.p.293-295° (evacuated tube) and $[\alpha]_D^{22}$ + 54°, + 52°. It should be stated at this point, that the only solvent from which the compound could be readily crystallised was nitromethane, and that in general this solvent has been found to be effective in crystallising triterpenoid substances contaminated with substantial amounts of resin.

The compound $C_{30}H_{50}O_2$, on treatment with acetic anhydride and with benzoyl chloride yielded only non-crystalline products thus showing a similarity to certain triterpene diols, e.g. hederadiol (17). Hydrolysis of these resinous products gave again the original compound, $C_{30}H_{50}O_2$, showing that at least one of the oxygen atoms present, existed in a hydroxyl group. Treatment of the compound with hydroxylamine acetate produced no change, from which it was concluded tentatively that no carbonyl group was present, and that both oxygen atoms existed in

hydroxyl groups. This was confirmed by the presence in the molecule of two active hydrogen atoms determined by the Zerewitinoff method. In addition, on treatment with perbenzoic acid the compound was found to absorb two oxygen atoms per molecule indicating the presence of two double bonds which must be unconjugated since the diol displays no light absorption in the ultra-violet.

All attempts to identify this diol $C_{30}H_{50}O_2$ with a known compound have been unsuccessful. Since the diol occurred along with β-elemonic acid and α-elemolic acid it was not unreasonable to suppose that it was related to the diols obtained from these acids by replacement of their respective carboxyl groups by $-CH_2OH$ groupings, and by the reduction of the ketone group of β-elemonic acid. Neither of these compounds, α-tritelemidiol and β-tritelemidiol, prepared respectively from acetyl- α-elemolic acid and β-elemonic acid by treatment of their methyl esters with lithium aluminium hydride, were identical with the isomeric diol from Canarium Schweinfurthii resin although they were similar in that they each contained two double bonds and yielded non-crystalline products on acetylation.

Regarding the tritelemidiols it should be stated that while the β-tritelemidiol obtained above is

identical with that obtained by Bouveault-Blanc reduction of methyl β-elemonate by Ruzicka (14), the α-tritelemidiol obtained above differs from Ruzicka's product (18) resulting from the Bouveault-Blanc reduction of methyl acetyl-α-elemolate. Ruzicka's product is amorphous having a m.p. of 255° and in this respect bears a superficial resemblance to the diol from the Canarium Schweinfurthii resin.

Since the diol appeared to be different from each of the α- and β-tritelemidiols, a comparison between each of the dihydro compounds seemed desirable, to find out whether the diol $C_{30}H_{50}O_2$, differed from either of the tritelemidiols by the location of one of its double bonds. It has however been found impossible to catalytically hydrogenate either of the double bonds of the diol from Canarium Schweinfurthii resin.

In conclusion, the results presented in this section can be taken as showing that the non-volatile neutral constituents of Canarium Schweinfurthii resin are mainly α- and β-amyrenol together with a small amount of a hitherto unknown diol of molecular formula, $C_{30}H_{50}O_2$.

CONCLUSION.

The present investigation has shown the presence in the resin from the tree Canarium Schweinfurthii of a volatile oil consisting largely of d-limonene but also containing a trace of phellandrene. In addition it has been shown that the acid fraction of the resin consists of β-elemonic and α-elemolic acids while the neutral constituents are α- and β-amyrenol along with a small amount of a hitherto undescribed diol of molecular formula, $C_{30}H_{50}O_2$.

It has also been found that the difficulty originally encountered in the isolation and purification of the constituents of the resin particularly those of the acid fraction can be partly reduced by the use of nitromethane as a crystallising solvent.

EXPERIMENTAL

EXPERIMENTAL.

All melting points are uncorrected.

The resin from Canarium Schweinfurthii (500 g.) was initially separated into two fractions, (a) volatile in steam and (b) non-volatile in steam, by steam distillation for 20 hours. The volatile oil obtained in this way, after separation from the aqueous distillate, was dried over anhydrous sodium sulphate. Yield 70 g.

The remaining non-volatile fraction was extracted with ether, and the extract after being filtered free from vegetable debris was washed with potassium hydroxide solution (18%; 3 x 250 ml.) followed by water (2 x 250ml.) before being dried over sodium sulphate. On removal of the ether under reduced pressure, the neutral fraction was obtained as a yellow gum which resisted all attempts at crystallisation. Yield 250 g.

The alkaline extract of the non-volatile fraction was acidified with dilute hydrochloric acid, and the free acids produced, extracted with ether. This extract after being dried over sodium sulphate was evaporated to dryness leaving the acid fraction as a dark red tar from which no crystalline material could be obtained. Yield 80g.

I. THE STEAM VOLATILE FRACTION.

The oil (70 g.) obtained by steam distillation of the resin after being dried over sodium sulphate was distilled under reduced pressure, giving the following three fractions:-

1) b.p.57-62°/16 mm. Yield 13 g.

2) b.p.62-64°/16 mm. Yield 40 g.

3) b.p.64-69°/16 mm. Yield 4 g.

In addition, there was also obtained a dark viscous oil of b.p. 100°/16 mm. Yield 7 g.

Fraction 1.

Treatment with Maleic Anhydride.

The oil (10 g.) was dissolved in ether (40 ml.) and maleic anhydride (5 g.) added to the solution. The mixture was then refluxed for 30 minutes after which time the ether and unreacted oil were removed by distillation under reduced pressure. The solid residue after four crystallisations from methanol yielded colourless needles of m.p.125-127°. Yield 47 mg.

Found: C,71·4; H,7·8%

$C_{14}H_{18}O_3$ requires: C,71·9; H,7·7%.

Fraction 2.

After four further fractionations, fraction 2 yielded a colourless oil (24 g.) of b.p.62-62·5°/15 mm., having a

lemon-like odour. The oil gave a bright yellow coloration with tetranitromethane in chloroform, and showed no selective light absorption in the ultra-violet region.

Found: C,88·2; H,11·6%

$C_{10}H_{16}$ requires: C,88·2; H,11·8%

$[\alpha]_D^{20}$ + 111·4° (l = 1, c = 100)

η_D^{20} 1·4755

d^{20} 0·846 g./ml.

In addition to this oil, there was also obtained from each of the four distillations, a dark viscous fraction, b.p. 70°/16 mm., similar to that obtained in the first fractionation.

Perbenzoic Acid Titration of the Oil, $C_{10}H_{16}$.

The oil (298 mg.) was dissolved in chloroform (25ml.) and a solution of perbenzoic acid in chloroform (5%; 36 ml.) added. A control consisting of chloroform (25 ml.) and the chloroformic solution of perbenzoic acid (5%; 36 ml.) was also prepared. Both solutions were kept at 0°, and the perbenzoic acid content in each, estimated iodometrically using 1 ml. portions every twenty-four hours. This procedure was repeated until oxygen ceased to be absorbed by the oil. At this stage the oxygen absorption amounted to 2.05 oxygen atoms per molecule.

Time (hours)	Titre (ml.s 1·059 N/10 $Na_2S_2O_3$) Blank	Reaction	O Atoms Absorbed
24	4·49	3·36	1·81
48	4·50	3·24	1·86
72	4·45	3·16	1·90
96	4·40	3·09	1·93
120	4·40	3·05	1·99
144	4·40	3·04	2·05
168	4·40	3·04	2·05

Maleic Anhydride Evaluation of the Oil, $C_{10}H_{16}$.

(cf. Birch, J.Proc.Roy.Soc.N.S.Wales,1938,71,261)

To a solution in acetone (10 ml.) of the oil (7 g.) was added maleic anhydride (6 g.) and the mixture allowed to stand for 1 hour at room temperature. The acetone was then removed under reduced pressure on a water bath, and sodium hydroxide solution (10%; 40 ml.) added to the residue. The mixture was heated, with intermittent shaking, for 15 minutes on a steam bath, cooled, and extracted with ether. On evaporation to dryness under reduced pressure, the ether extract gave a colourless oil (6·4 g.) of b.p.62-63°/15 mm., $[\alpha]_D^{18}$ + 108·5 (l = 1, c = 0·96 in chloroform). The oil (6·4 g.; 91%) was therefore unchanged starting material.

Bromination of the Oil, $C_{10}H_{16}$.

(cf. Baeyer and Villiger, Ber.,1894,27,448)

Bromine (1·55 ml. = 2 mols.) was added dropwise and with shaking to a solution of the oil, $C_{10}H_{16}$ (2·5 g.) in amyl alcohol (3 ml.) and ether (6 ml.). After standing at 0° for 2 days the solution gradually deposited a crop of resinous crystals. These were separated and crystallised from nitromethane as colourless plates of m.p.102-104°. After two further recrystallisations from nitromethane, d-limonene tetrabromide was obtained as plates of m.p.104-106°. Yield 3·1 g. (37%).

Found: C,26·1; H,3·38; Br,69·8%

Calc. for $C_{10}H_{16}Br_4$: C,26·3; H,3·50; Br,70·2%

$[\alpha]_D^{20°}$ + 72° (l = 1, c = 0·72 in chloroform).

II. THE ACID NON-VOLATILE FRACTION.

Acetylation of Non-Volatile Acid Fraction.

The acid fraction (30 g.) was dissolved in pyridine (90 ml.) and acetic anhydride (210 ml.) added to the solution. The mixture was then heated for 2 hours on a steam bath, cooled, and poured into ice water. A resinous material which gradually solidified, separated and was filtered off. After drying, it was dissolved in ethanol and the solution allowed to stand at room temperature for 10 days when a crop of a semi-crystalline

solid had separated. This after eight crystallisations from nitromethane yielded long, colourless needles of m.p.216-222°. Yield 3·5 g. The substance in chloroform solution gave a bright yellow coloration with tetranitromethane.

Found: C,76·9; H,10·2%

$C_{32}H_{50}O_4$ requires: C,77·1; H,10·0%.

Hydrolysis of the Acid Fraction Acetate.

The acetylated acid fraction (m.p.216-222, 750 mg.) was dissolved in methanol (20 ml.) and methanolic potassium hydroxide (5%; 25 ml.) added to the solution. The mixture was then refluxed for 3 hours, cooled, and poured into ice water, giving a clear solution which on acidification yielded an amorphous precipitate. Recrystallisation of this material from nitromethane gave small hard cubes of m.p.212-216° which on admixture with starting material of m.p.216-222° were depressed in melting point to 198-206°.

Found: C,78·6; H,10·4%

$C_{30}H_{48}O_3$ requires: C,78·9; H,10·5%.

Methylation of the Acid Fraction Acetate.

To a solution in ether of the acetylated acid fraction (m.p.216-222°; 500 mg.) was added an ethereal

solution of diazomethane. When the evolution of nitrogen had ceased, the ethereal solution was washed with dilute hydrochloric acid, with dilute sodium hydroxide solution, and finally with water, before being dried over sodium sulphate. On evaporation of the ethereal solution to dryness a solid was obtained which was crystallised three times from acetone-methanol giving the methyl ester of the acetylated acid fraction as needles of m.p.112-114°. Yield 320 mg. With tetranitromethane in chloroform, the product gave a bright yellow coloration.

Found: C,77·5; H,10·3%

$C_{33}H_{52}O_4$ requires: C,77·3; H,10·2%

$[\alpha]_D^{20}$ - 41° (l = 1, c = 0·91 in chloroform).

Perbenzoic Acid Titration of the Methyl Ester from the Acetylated Acid Fraction.

The methyl ester, of m.p.112-114°, (100 mg.) in chloroform (25 ml.) was treated with a solution of perbenzoic acid in chloroform (2·5%; 25 ml.) and the mixture allowed to stand at 0°. A control consisting of a mixture of chloroform (25 ml.) and the chloroformic solution of perbenzoic acid (2·5%; 25 ml.) was also prepared and kept at 0°. The perbenzoic acid content of both the reaction mixture and the blank was determined iodometrically every twentyfour hours using 1 ml. samples, until there was no further absorption of oxygen by the

reaction mixture. After 8 days, the oxygen absorption by the ester was complete and was found to correspond to 1·93 atoms of oxygen per molecule of ester.

Time (hours)	Titre (ml.s of 0·716 N/10 $Na_2S_2O_3$) Blank	Reaction	O Atoms Absorbed
24	1·37	1·26	1·01
48	1·35	1·20	1·35
72	1·34	1·17	1·56
96	1·34	1·16	1·65
120	1·33	1·14	1·74
144	1·32	1·12	1·84
168	1·32	1·11	1·93
192	1·31	1·10	1·93

Isolation of α-Elemolic Acid and β-Elemonic Acid from the Acid Fraction.

The crude acid fraction (50 g.) was dried and powdered, before being dissolved in methanol (500 ml.). To this solution was added Girard's reagent P (6 g.; prepared after Girard and Sandulesco, Helv.Chim.Acta, 1936,19,1095) and glacial acetic acid (3·0 ml.) and the mixture refluxed for one hour. The dark red solution obtained was allowed to stand for 17 hours at room temperature before being diluted with ice (ca.500 g.). Sodium carbonate solution (2·8 g. in 50 ml. water) was

then added, and the mixture extracted with cold ether (2·5 litres) in four successive portions, care being taken to keep the temperature below 5° by the regular addition of ice. The ethereal solution (Solution A) containing the non-ketonic fraction, was then washed with ice water (4 x 250 ml.), dried over sodium sulphate, and reserved for further treatment.

The aqueous extract, together with the aqueous washings of the ether extract, was acidified with dilute hydrochloric acid giving a white oily precipitate. After standing for 2 hours at room temperature, the mixture was extracted with ether (1500 ml.) and the extract washed with water until neutral. The extract was then dried, and evaporated to dryness under reduced pressure giving a residual colourless gum (8 g.) which after three recrystallisations from methanol yielded β-elemonic acid as colourless needles of m.p.224-225°. With tetranitromethane in chloroform, β-elemonic acid gave a bright yellow coloration.

Found: C,79·4; H,10·1%

Calc. for $C_{30}H_{46}O_3$: C,79·3; H,10·1%

$[\alpha]_D^{22}$ + 44° (l = 1, c = 1·41 in chloroform).

The dried ethereal solution (Solution A) was evaporated to dryness under reduced pressure giving a

residual tar which could not be crystallised. This was then acetylated with acetic anhydride and pyridine in the usual manner, giving a product which crystallised from nitromethane as needles of m.p.218-222°. After five further recrystallisations from methanol, acetyl-α-elemolic acid was obtained as stout needles of m.p.238-240°. Yield 20 g. In chloroform solution the substance gave a deep yellow coloration with tetranitromethane.

Found: C,77·4; H,10·0%

Calc. for $C_{32}H_{50}O_4$: C,77·1; H,10·0%

$[\alpha]_D^{20}$ - 40° (l = 1, c = 1·25)

Methyl Acetyl-α-Elemolate.

Acetyl-α-elemolic acid (100 mg.) was esterified with diazomethane giving methyl acetyl-α-elemolate as needles of m.p.113-114° undepressed in melting point on admixture with the ester already obtained from the unresolved acid fraction acetate. Yield 70 mg. (68%).

$[\alpha]_D^{20}$ - 43° (l = 1, c = 0·52 in chloroform).

α-Elemolic Acid.

Acetyl-α-elemolic acid (250 mg.) was hydrolysed with methanolic potassium hydroxide in the usual manner, giving α-elemolic acid which crystallised from methanol as small hard cubes of m.p.222-224°. The product gave a bright yellow coloration with tetranitromethane in

chloroform. Yield 77%.

Found: C,79·0; H,10·3%

Calc. for $C_{30}H_{48}O_3$: C,78·9; H,10·5%

$[\alpha]_D^{20}$ - 29° (l = 1, c = 0·92 in chloroform).

Methyl β-Elemonate.

On esterification of β-elemonic acid (100 mg.), of m.p.224-225° with diazomethane methyl β-elemonate was obtained as needles from nitromethane, of m.p.104-105°. Yield 85 mg. (83%).

$[\alpha]_D^{20}$ + 36° (l = 1, c = 0·76 in chloroform).

β-Tritelemidiol.

To a suspension of lithium aluminium hydride (600 mg.) in dry ether (100 ml.) was added slowly and at room temperature methyl-β-elemonate (2000 mg.) in ether (75 ml.). The mixture was then refluxed for 3 hours, cooled, and excess water added cautiously to decompose the excess lithium aluminium hydride, and the reduction adduct. When this reaction had ceased, the mixture was made slightly acid by the addition of dilute sulphuric acid (2N.), and extracted with ether. The extract was washed free from acid with water, dried over sodium sulphate, and evaporated to dryness under reduced pressure. The residue on crystallisation from aqueous ethanol gave β-tritelemidiol as needles of m.p.177-179°.

Yield 465 mg. (82%).

Found: C,81·2; H,11·2%

Calc. for $C_{30}H_{50}O_2$: C,81·4; H,11·3%

$[\alpha]_D^{20}$ - 6·4° (l = 1, c = 1·89 in chloroform).

α-Tritelemidiol.

A solution of methyl acetyl-α-elemolate (1700 mg.) in dry ether (75 ml.) was added to a suspension of lithium aluminium hydride (500 mg.) in ether (100 ml.). The mixture was refluxed for 3 hours, cooled, and treated in exactly the same manner as described above in the preparation of β-tritelemidiol. The product after two crystallisations from aqueous ethanol gave α-tritelemidiol as stout prismatic needles of m.p.169-170° which when mixed with β-tritelemidiol was depressed to 160-163°. With tetranitromethane in chloroform the product gave a bright yellow coloration.

Found: C,81·1; H,11·5%

$C_{30}H_{50}O_2$ requires: C,81·4; H,11·3%

$[\alpha]_D^{20}$ - 28° (l = 1, c = 1·02 in chloroform).

III. THE NEUTRAL NON-VOLATILE FRACTION.

Identification of α- and β-Amyrenol.

(a) Acetylation of Neutral Non-Volatile Fraction.

The dry powdered neutral fraction (50 g.) in pyridine (150 ml.) was heated with acetic anhydride

(350 ml.) for 2 hours on a steam bath. On cooling, the reaction mixture was poured into water, and the oil which separated extracted with ether. The extract was washed successively with water, with dilute hydrochloric acid, with dilute sodium carbonate solution and finally again with water before being dried over sodium sulphate. On evaporation of the ether, a resinous material was obtained. This was dissolved in acetone, and on standing, crystalline material gradually appeared. Concentration of the mother liquor of this crop, gave two further crops of crystalline material, yielding in all 32 g. The acetone mother liquor from the third crystallisation (Filtrate A) was examined as described below (p.169).

Part of the crystalline material (5 g.) after repeated recrystallisation from intromethane gave colourless swords of m.p.208-213°. With tetranitromethane in chloroform the substance gave a yellow coloration.

Found: C,81·6; H,10·8%

$C_{32}H_{52}O_2$ requires: C,82·1; H,11·1%

$[\alpha]_D^{20}$ + 77° (l = 1, c = 1·27 in chloroform).

The remainder of the crystalline material was hydrolysed, as follows.

(b) Hydrolysis of Acetate from Non-Volatile Fraction.

The crystalline acetate (25 g.) was dissolved in

benzene (200 ml.) and a solution of potassium hydroxide (30 g.) in 95% ethanol (600 ml.) added. The mixture was refluxed for 24 hours, concentrated to about 350 ml., and poured into water. The solid which separated was collected, and dried, before being benzoylated as described in (c) below.

(c) Benzoylation of Purified Non-Volatile Fraction.

The product from the hydrolysis of the acetate (19 g.) was dissolved in pyridine (30 ml.) and benzoyl chloride (30 ml.) added. The mixture was then heated on a steam bath for 6 hours, after which time it was diluted by the addition of benzene (50 ml.). On cooling, the mixture was washed with dilute hydrochloric acid (10%; 2 x 50 ml.), with sodium carbonate solution (5%; 2 x 50 ml.), and finally with water (2 x 50 ml.), before being dried over sodium sulphate. On removal of the benzene under reduced pressure, a dark resin was obtained, which on crystallisation from nitromethane yielded a crop of needles of m.p.176-186°. Yield 16 g.

The benzoylated product (16 g.; m.p.176-186°) was powdered and placed in a wide mouthed stoppered bottle, fitted with a tap in the stopper. Ether (30 ml.) was added, and the mixture shaken vigorously for 10 minutes, after which time the undissolved solid was filtered off,

and its clearing point determined. The process was then repeated twice after which the clearing point of the residual solid was 214°. This substance after four crystallisations from benzene-acetone yielded β-amyrenyl benzoate as colourless plates of m.p.230-232°, undepressed on admixture with authentic β-amyrenyl benzoate obtained from Manila Elemi resin (p.83). Yield 2·1 g.

Hydrolysis of the benzoate (1000 ml.) followed by direct acetylation of the product with acetic anhydride (3 ml.) in pyridine (3 ml.) gave β-amyrenyl acetate which after two recrystallisations from acetone attained a m.p. of 232-234°, undepressed when mixed with authentic β-amyrenyl acetate, of m.p.233-235°, obtained from Manila Elemi resin.

Found: C,82·0; H,10·9%

Calc. for $C_{32}H_{52}O_2$: C,82·1; H,11·1%

$[\alpha]_D^{20}$ + 82° (l = 1, c = 0·73 in chloroform).

Evaporation of the combined ether washings obtained from the purification of β-amyrenyl benzoate, gave a crystalline material from which, after six recrystallisations from benzene-ethanol, α-amyrenyl benzoate was obtained as prisms of m.p.193-195°, undepressed in melting point on admixture with authentic α-amyrenyl benzoate, of m.p.195-196°, obtained from Manila Elemi resin.

Hydrolysis of the benzoate (1000 mg.), followed by direct acetylation of the product with acetic anhydride (3 ml.) in pyridine (3 ml.) gave α-amyrenyl acetate which on crystallisation from acetone yielded plates of m.p.219-221°, undepressed when mixed with authentic α-amyrenyl acetate of m.p.223-225°, obtained from Manila Elemi resin (p.83).

Found: C,82·3; H,11·4%

Calc. for $C_{32}H_{52}O_2$: C,82·1; H,11·1%

$[\alpha]_D^{20}$ + 79° (l = 1, c = 0·46 in benzene).

Isolation of a Compound, $C_{30}H_{50}O_2$, from the Neutral Non-Volatile Fraction.

Filtrate A, obtained after the crystallisation of the acetylated neutral fraction, gave no further crystalline material, on concentration. The mixture was then evaporated to dryness under reduced pressure, and the residual dark resin (16 g.) refluxed for 3 hours with ethanolic potassium hydroxide solution (5%; 200 ml.). After this time, the reaction mixture was poured into water, and the product isolated by means of ether. All attempts to crystallise this product failed, and it was finally dissolved (15 g.) in petroleum ether (b.p.60-80°; 230 ml.) and benzene (690 ml.). This solution was passed through a column of activated alumina (60 x 6 cm.)

when complete adsorption of the dissolved material took place. The following fractions were then eluted from the column:-

Fraction	Solvent		Eluate
1	920 ml.	petrol-benzene (1:3)	800 mg. oil
2	500 ml.	" " (1:2)	600 mg. oil
3	500 ml.	" " (1:1)	700 mg. oil
4 - 6	1500 ml.	benzene	700 mg. oil
7	500 ml.	benzene-ether (4:1)	600 mg. resin
8 - 9	1000 ml.	ether	4000 mg. resin
10 - 11	1000 ml.	"	3800 mg. resin
12 - 14	1500 ml.	"	1600 mg. resin
15 - 17	1500 ml.	ether-ethanol (4:1)	900 mg. tar
18	500 ml.	ethanol	200 mg. oil

Fractions 1 to 7 could not be obtained in a solid state although crystallisation from many solvents and sublimation in high vacuum were attempted.

Fractions 8 and 9 were extracted with successive small amounts of cold ethyl acetate. The residual solid (50 mg.), only slightly soluble in ethyl acetate, crystallised from nitromethane in colourless plates of m.p.247-265° (decomp.). After two further crystallisations from nitromethane, the m.p. was 260-279° (decomp.) and when determined in an evacuated tube was 293-295°(decomp.

There was no depression in melting point when mixed with the product obtained below from fractions 10 and 11.

Fractions 10 and 11 were dissolved in ethyl acetate and after standing for 3 days the solution deposited a micro-crystalline solid, which after 3 recrystallisations from nitromethane gave colourless plates of m.p.292-295° (decomp.; in an evacuated tube), undepressed in melting point on admixture with the substance obtained from fractions 8 and 9. With tetranitromethane in chloroform, the substance gave a yellow coloration. Yield 400 mg.

Found: C,81·3,81·7; H,11·4,11·3%

$C_{30}H_{50}O_2$ requires: C,81·4; H,11·3%

$C_{30}H_{48}O_2$ requires: C,81·8; H,10·9%.

Nitrogen was found to be absent by the Lassaigne test.

$[\alpha]_D^{22}$ + 54°, + 52° (l = 1, c = 0·44, 0·64 in chloroform.

Active hydrogen determination (Zerewitinoff): 11·065 mg. evolved 1·11 ml. of methane at 758 mm. and 18° corresponding to 0·85 atoms of active hydrogen.

The remaining fractions from the chromatogram could not be obtained in a solid state.

Attempted Acetylation of the Compound, $C_{30}H_{50}O_2$.

1. At 100°.

The compound $C_{30}H_{50}O_2$, (150 mg.) was dissolved in pyridine (2 ml.), and acetic anhydride (5 ml.) added.

The mixture was allowed to stand at room temperature for 2 hours, before being heated for a further 2 hours on a steam bath. The solution was then poured into water, and extracted with ether. The extract after being washed successively with water, with sodium carbonate solution (5%), and again with water was dried over sodium sulphate, and the ether removed under reduced pressure. It was found impossible to crystallise the resinous material thus obtained, which was then dissolved in petroleum ether (b.p.60-80°; 15 ml.) and the solution passed through a column of activated alumina (7·5 x 0·7 cm.). No adsorption took place, and evaporation of the petroleum ether solution gave only a resin which again could not be crystallised.

2. <u>At room temperature.</u>

The compound, $C_{30}H_{50}O_2$, (75 mg.) was dissolved in acetic anhydride (20 ml.), and the solution allowed to stand at room temperature for 5 days. After this time, the mixture was poured into excess water, and extracted with ether. After neutralising the ethereal extract by washing with sodium carbonate solution (5%) as before, the ether was removed under reduced pressure. The residual gum (60 mg.) could not be obtained crystalline either by sublimation or by crystallisation, and was finally refluxed for 3 hours with methanolic

potassium hydroxide solution (5%; 15 ml.). The mixture was then poured into water, and extracted with ether. The ethereal solution after being washed with dilute hydrochloric acid (5%) was evaporated to dryness under reduced pressure. Crystallisation of the residual gum from nitromethane, followed by two recrystallisations from the same solvent, gave plates (20 mg.) of m.p.287-290° (in an evacuated tube) undepressed in melting point when mixed with the compound, $C_{30}H_{50}O_2$.

Attempted Benzoylation of the Compound, $C_{30}H_{50}O_2$.

The compound, $C_{30}H_{50}O_2$, (40 mg.) in pyridine (4 ml.) was heated with benzoyl chloride (2 ml.) for 2 hours on a steam bath. The reaction mixture was then evaporated to dryness under reduced pressure and the residue dissolved in chloroform-ether mixture. This extract after washing successively with dilute hydrochloric acid, with water, with dilute sodium carbonate solution, and finally again with water, was evaporated to dryness, and attempts made to crystallise the dark resinous residue. All attempts at crystallisation failed, and the gum was then dissolved in methanolic potassium hydroxide (5%; 30 ml.). After refluxing for 3 hours, the reaction mixture was poured into water and extracted with ether. From the ethereal extract a substance was obtained which after two

recrystallisations from nitromethane yielded colourless plates (10 mg.) of m.p.288-290° (in an evacuated tube) undepressed in melting point on admixture with the compound, $C_{30}H_{50}O_2$.

Attempted Oximation of the Compound, $C_{30}H_{50}O_2$.

The compound, $C_{30}H_{50}O_2$, (50 mg.) was dissolved in absolute ethanol (25 ml.), and a mixture of hydroxylamine hydrochloride (50 mg.) and anhydrous sodium acetate (70 mg.) added. After refluxing for 6 hours, the mixture was poured into water and the precipitated solid filtered off. This after two recrystallisations from nitromethane had a m.p. of 290°-292° (in an evacuated tube) and was undepressed in melting point when mixed with the starting material.

Perbenzoic Acid Titration of the Compound, $C_{30}H_{50}O_2$.

The compound, $C_{30}H_{50}O_2$, (100 mg.) in chloroform (25 ml.) was treated with a chloroformic solution of perbenzoic acid (5%; 25 ml.) and kept at 0° together with a control containing only chloroform (25 ml.) and perbenzoic acid in chloroform (5%; 25 ml.). The perbenzoic acid content of 1 ml. samples from both the reaction mixture and the blank, was determined iodometrically at twentyfour hourly intervals, until the oxygen absorption in the reaction mixed had ceased. After 6 days the compound, $C_{30}H_{50}O_2$, had absorbed two atoms of oxygen,

per molecule.

Time (hours)	Titre (ml.s of 1·059 N/10 $Na_2S_2O_3$) Blank	Reaction	O Atoms Absorbed
24	3·31	3·20	1·29
48	3·25	3·11	1·64
72	3·21	3·06	1·76
96	3·18	3·02	1·87
120	3·18	3·01	1·99
144	3·18	3·01	1·99

Attempted Hydrogenation of the Compound, $C_{30}H_{50}O_2$.

The compound, $C_{30}H_{50}O_2$, (75 mg.) was dissolved in dry ethanol (20 ml.) and platinum oxide (20 mg.; prepared after Adams, Org.Synth.,Coll.Vol.I,463) added. The mixture was then shaken at room temperature and at atmospheric pressure, with hydrogen for 36 hours, after which time the mixture was filtered free from catalyst and evaporated to dryness under reduced pressure. The residual resin on crystallisation from nitromethane yielded a crop (60 mg.) of crystals of m.p.291-293°, undepressed in melting point on admixture with starting material.

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