THE CHEMISTRY

of

SOME NATURALLY OCCURRING COMPOUNDS

CONTAINING CONDENSED ALICYCLIC RING SYSTEMS

An Investigation into the Structure of the Triterpene

α-AMYRIN

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

T H E S I S

presented to

THE UNIVERSITY OF GLASGOW

FOR THE DEGREE OF

DOCTOR of PHILOSOPHY

B Y

STEFAN UDO RUFF

ACKNOWLEDGEMENTS

The author would like to express his most sincere gratitude to Professor F.S. Spring for his friendly and helpful interest and many words of encouragement throughout these investigations. He would also thank Dr. J. MacLean for the liberality with which he gave of his fund of practical experience, and, finally, he would thank Dr. G.T. Newbold for much assistance in the interpretation of spectrographic data.

C O N T E N T S

H I S T O R I C A L

N.B. Arabic numerals in parentheses refer to the Bibliography, Roman numerals to the formulae in the text.

INTRODUCTION

The pentacyclic triterpenes α- and β-amyrin constitute the main neutral, non-volatile fraction of the resin obtained from Manila Elemi, and were first isolated by Rose (1) in 1835. A large number of other triterpenes (alcohols and acids) occur naturally in the saps of a wide variety of plants, and many of these bear a close structural relationship to one or other of the amyrins. Thus α-boswellic acid (2), oleanolic acid (3), glycyrrhetic acid (4), erythrodiol (5), basseol (6), gypsogenin (7) and hederagenin (8) have all been converted to β-amyrin or one of its derivatives, and similarly ursolic acid (9) and β-boswellic acid (10) have been shown to be closely related to α-amyrin.

In 1887 Vesterberg (11) separated the two isomeric amyrins by utilising the difference in solubility of the acetates, and differential solution of various esters has remained the standard method of separation of α- and β-amyrin.

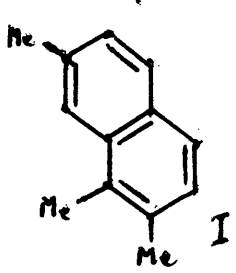
A very complete summary of the chemistry of the triterpenes is given elsewhere (12, 13). In the present dissertation the author will confine himself to a discussion

of the chemistry of α-amyrin, and previous work will be described with particular reference to its bearing on the experimental results obtained by the author.

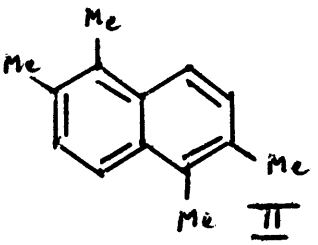
DEHYDROGENATION

The first evidence as to the nature of the carbon skeleton of both the amyrins was obtained by dehydrogenation, using sulphur or selenium at an elevated temperature. Oxidative experiments had already been carried out by Vesterberg (14), but these did not give conclusive results which could be interpreted at the time. Similarly bromination experiments (15) and the oxidation of the bromo-derivatives so formed had not yielded any further information. In 1929 Ruzicka (16) clearly showed the presence in α-amyrin of an unreactive double bond which could not be hydrogenated though it did undergo ozonolysis. The presence of the secondary hydroxyl group had been proved at a very early stage in the investigations (17). Since ordinary methods of degradation, which would allow a step-wise reconstruction of the basic skeleton, had not been successful, Ruzicka (16) studied drastic heat treatment. Dry distillation yielded a number of hydrocarbons which were separated by fractional distillation and some of which could be dehydrogenated with sulphur or selenium to naphthalene type compounds. The amyrins themselves (as a mixture of the two isomers) were therefore subjected to dehydrogenation with the same reagents and

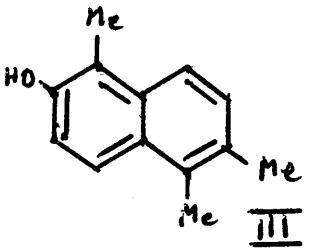
one of the products obtained was finally identified as a trimethylnaphthalene (Sapotalene, I). In 1932 Ruzicka (18) subjected various other triterpenes to the same treatment and identified many of the reaction products by synthesis. It was not, however, till 1937 that α-amyrin was dehydrogenated (19). Among the reaction products was the naphthalene derivative (I) first obtained in 1929, 1:2:5:6-tetramethylnaphthalene (II) and 1:5:6-trimethyl-β-naphthol (III).



I

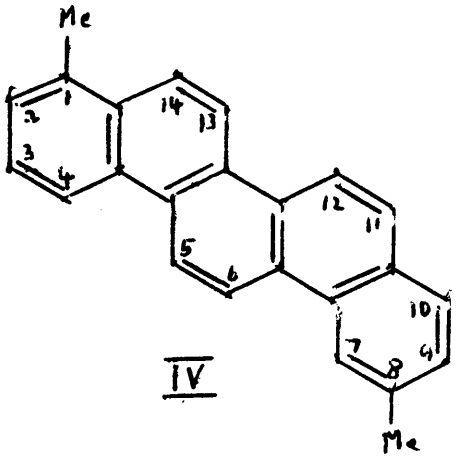


II

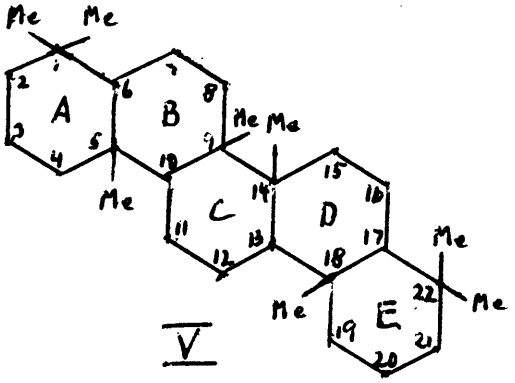


III

The isolation of this last product clearly placed the hydroxyl group at position 2 of the picene skeleton (IV) which had been suggested by Ruzicka (20) who obtained a picene homologue on dehydrogenation of various triterpenes. The isolation of this same hydrocarbon by Spring (19) from α-amyrin showed that this, too, had the basic hydropicene structure. Though allowance had to be made for the possibility of migration of the methyl groups during the process of dehydrogenation, the isolation of the products listed above gave some indication of their possible location.



IV



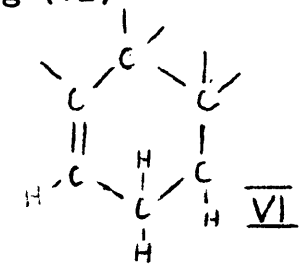
V

It had also to be borne in mind that the compounds should obey the isoprene rule and Spring (21) first suggested the basic structure (V). In the light of further evidence this has had to be modified, and in particular the position of the methyl groups in rings D and E is not at all certain.

The most important of the conclusions about the location of the methyl groups reached on the basis of this dehydrogenation evidence (in particular the isolation of the compounds II and III) is the apparent presence of an angular methyl group at C_9 (V), which would place considerable limitations on the choice of positions for the double bond of α-amyrin.

There is a wealth of evidence to show that the double bond and the hydroxyl group cannot be located in the same

ring of the basic pentacyclic carbon skeleton, and the presence of the double bond in ring A is therefore precluded. The formation of α-amyradienol by a variety of routes (22, 23, 24) necessitates the location of the double bond in a ring (VI)



VI

in which the ready introduction of a double bond in conjugation is permissible in the same ring. The presence of the angular methyl group at C_9 (V) would thus preclude location of the double bond in ring B. Most of the evidence obtained since 1945 (25, 26, 27, 28) has therefore been interpreted with a view to proving the exact location of the double bond in ring C. Much of this evidence, however, is by no means unequivocal, and a rather different interpretation is suggested in the theoretical part of this thesis.

THE ENVIRONMENT OF THE DOUBLE BOND

Vesterberg (14) oxidised α-amyrin esters with chromic acid and thus obtained derivatives which contained one additional atom of oxygen in the molecule. He could not, however, determine the nature of the oxygenated derivative since it did not respond to any of the usual reagents for carbonyl or hydroxyl groups, or, indeed, showed any signs of increased reactivity as compared with α-amyrin esters. It was not until 1937 that the presence of an αβ-unsaturated keto-group was shown by spectrographic examination of the compound (19). Spring (19) therefore suggested that a methylene was present adjacent to the double bond and that this had been oxidised to a carbonyl group. That the original double bond of α-amyrin had not been affected in this oxidation was finally shown by Ruzicka (29) who obtained α-amyrin from the oxidation product by hydrogenation. This evidence proved the presence in α-amyrin of the grouping

$$\rightarrow CH_2\text{-}\overset{|}{C}=C\prec$$

and on this basis the oxidation product was assigned the name α-amyrenonol, indicating the presence of a double bond, a ketonic group and a hydroxyl group.

Further oxidative reactions were carried out by Spring in 1939 (30). α-Amyrin benzoate was oxidised with hydrogen peroxide and the product treated with bromine in hot glacial acetic acid. After an initial bromination period, dehydrohalogenation set in and the final halogen free product was found by spectrographic examination to contain an αβ-unsaturated keto-grouping. Analysis showed that it was isomeric with α-amyrenonyl benzoate and the name iso- α-amyrenonyl benzoate was given to it.

Because of this reaction with bromine the product obtained by the oxidation of α-amyrin benzoate with hydrogen peroxide was believed to be a saturated keto-ester, the ketonic group having been introduced by oxidation of the double bond of α-amyrin. It was, however, shown later by Spring (45) that this compound was, in fact, an oxide, since it could be isomerised by treatment with mineral acid to a compound which still reacted with bromine in the same manner. The nature of the reaction with bromine was completely clarified by the isolation of an intermediate bromo-derivative and the entire reaction series may be described by the following scheme:

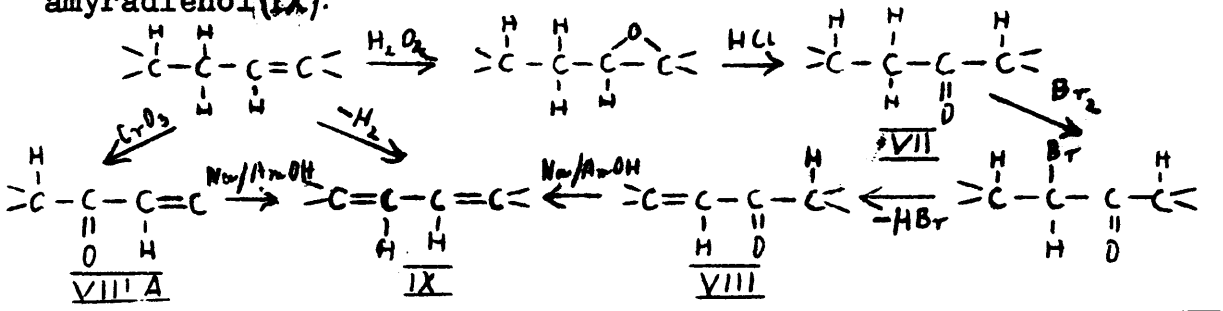
α-Amyrin benzoate $\xrightarrow{H_2O_2}$ oxide $\xrightarrow{HCl}$ saturated ketone

iso- α-amyrenonyl benzoate $\xleftarrow{-HBr}$ bromo-derivative $\swarrow^{Br_2}$

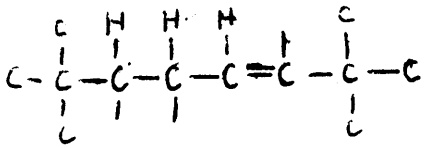
For oxide - ketone tautomerism to be possible at least one of the carbon atoms attached to the double bond of α-amyrin must bear a hydrogen atom. But α-amyrin esters may be partially dehydrogenated to doubly unsaturated derivatives (the α-amyradienyl esters), containing a system of two conjugated double bonds located in one ring (22, 24). This same compound is obtained on reduction of both α-amyrenonol (23) and iso-α-amyrenonol (32) with sodium and amyl alcohol, the intermediate alcohol undergoing dehydration during the reaction, and therefore one of the two carbon - carbon double bonds present in α-amyradienol must correspond to the carbon - carbon double bond of α-amyrenonol, and the other to the double bond of iso-α-amyrenonol. Thus the presence in α-amyrin of the grouping

$$\geq CH - CH_2 - CH = CH -$$

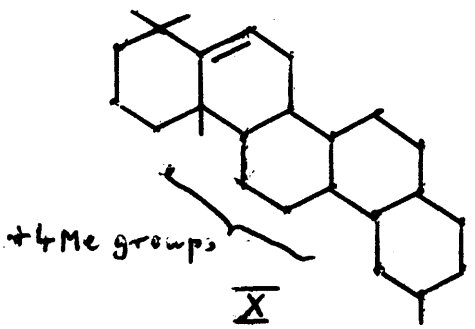
is clearly defined, all four carbon atoms being located in the same ring. This system then undergoes the following changes in the formation of α-amyranonol (VII), iso-α-amyrenonol (VIII), α-amyrenonol (VIIIA) and α-amyradienol (IX):



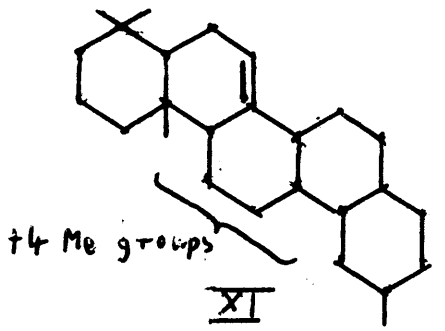
α-Amyradienyl derivatives cannot be further dehydrogenated by treatment with mild reagents such as N-bromosuccinimide nor will it react with bromine, (33), and from this it may be concluded that the carbon atoms immediately adjacent to the conjugated system are quaternary or in sterically very hindered positions. The environment of the double bond of α-amyrin may thus be extended to the grouping



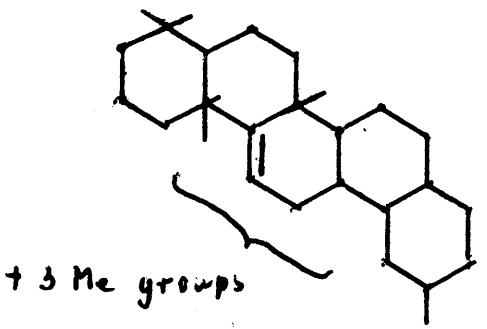
On the basis of this evidence and of the results of dehydrogenation, four alternative formulations for α-amyrin may be suggested (X, XI, XII, XIII).



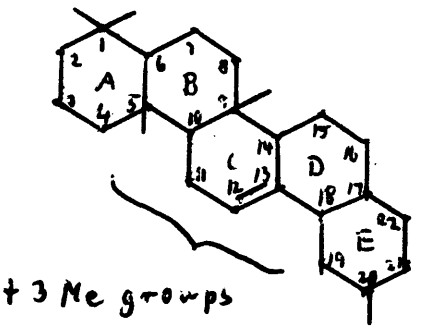
X



XI

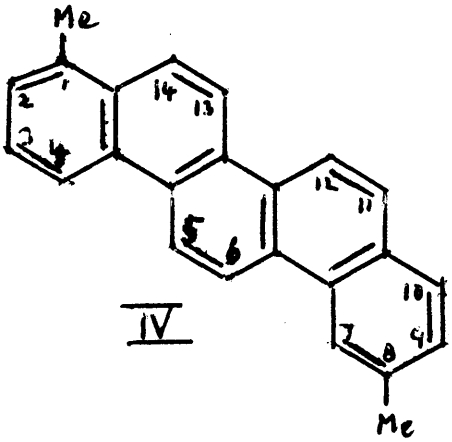


XII

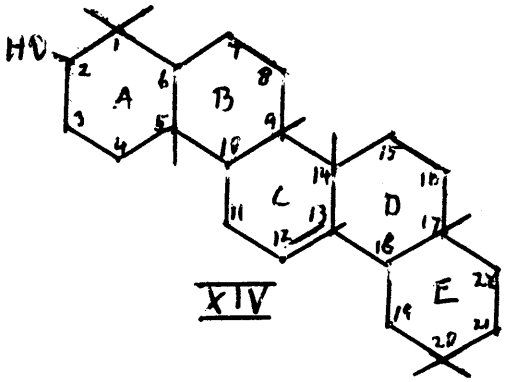


XIII

The position of those substituent methyl groups whose location is indicated in the formulae, has been determined on the basis of dehydrogenation evidence (such as the formation of 1:8-dimethylpicene (IV)



IV



XIV

and of the various naphthalene derivatives, p. 4), of compliance with the isoprene rule and of analogy with β-amyrin (XIV) whose structure has been completely elucidated.

Ruzicka (28) favours formulation (XIII) for α-amyrin (his reasons are discussed in the following pages) and, to avoid unnecessary confusion, all formulae will be based on this throughout the historical sections of this thesis. It must be emphasised that this is an expedient adopted for the sake of clarity and that there are some reactions of α-amyrin which are difficult to explain on this assumption. Particular attention is drawn in this connexion to the existence of the conjugated dehydration products (p. 12).

THE RELATIVE POSITIONS OF THE DOUBLE BOND AND THE HYDROXYL GROUP

α-Amyrin and derivatives in which the secondary hydroxyl group at C_2 (XIII, p. 10) is maintained, may be dehydrated, the nature of the product depending on the reagent employed. Thus on treatment with phosphorus pentachloride, α-amyrin loses the elements of water with the formation of d- α-amyradiene-I (11, 34, 35); α-amyrin benzoate loses benzoic acid at 300° and the reaction product is d- α-amyradiene-II (36). Both these products show no selective absorption in the ultra-violet between λ = 2200A and λ = 2900A and from this it was concluded that the fresh double bond had not been introduced in conjugation with the double bond of α-amyrin. Similar unconjugated products are obtained by the dehydration of α-amyrenonol (p. 7). Treatment of the compound with phosphorus pentachloride yields α-amyradienone-I, treatment with Fuller's earth in xylene yields α-amyradienone-II (19, 37, 38). Isomerisation of α-amyradienone-I to α-amyradienone-II may be effected with a Palladium catalyst (37). These two compounds show approximately the same ultra-violet absorption spectrum as α-amyrenonol. There has, therefore been no increase in conjugation.

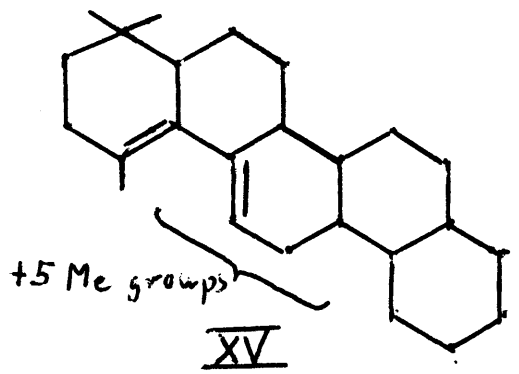
When, however, α-amyrin is dehydrated with phosphoric anhydride (14), the strongly laevo-rotatory product (most α-amyrin derivatives show strong dextro rotation) can be shown to contain a conjugated system of two double bonds distributed between two rings. It shows an absorption maximum in the ultra-violet at λ_{max} = 2480A. This compound, 1- α-amyradiene, is also obtained by treatment of α-amyrin with hydriodic acid (39). This indication of a close relationship between the secondary hydroxyl group and the unsaturated centre of α-amyrin, led Spring (39) to carry out a systematic investigation of the dehydration products of α-amyrin, and of some of its derivatives. Thus α-amyradienol (p. 9) may be similarly dehydrated with the formation of a conjugated triene.

Dehydration of α-amyrenonol with hydriodic acid yields α-amyradienone-III whose ultra-violet absorption spectrum is indicative of the grouping

$$>CH=CH-CH=CH-C=O$$

This type of conjugated dehydration product cannot be derived from β-amyrin and the differences between the two amyrins were therefore attributed to a difference in

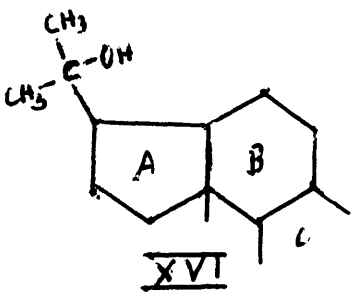
the location of the double bond; this view was supported by several authors until 1945 (39, 40, 41). Thus on the basis of some reactions of β-bowellic acid which can be converted to α-amyrin (10), Simpson (40) placed the double bond at C_6 - C_7 (X, p.10)



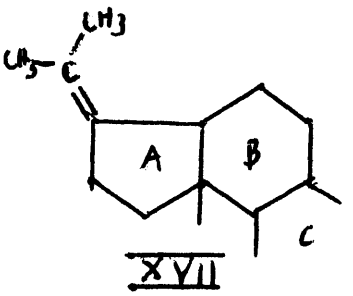
XV

Spring (39) also made the alternative suggestion that the double bond was located in ring C (XII, p. 10) and accounted for the formation of the conjugated dehydration products by assuming migration of the double bond resulting from the removal of the elements of water and a simultaneous move of the C_5 methyl group, resulting in the formation of the compound (XV). At this stage work on the conjugated dehydration products was discontinued. Indeed, the difficulties attending their preparation are considerable, and the compounds themselves are surprisingly unreactive.

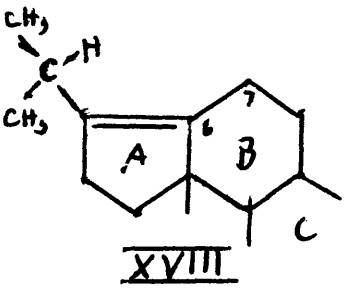
Investigations were therefore continued on the more promising α-amyradienone-I (38). Ruzicka (38) showed that the new double bond introduced in the compound could be oxidised to a diol by means of osmium tetroxide and that, on treatment of this latter compound with a glycol splitting reagent the molecule was disrupted with the formation of acetone and of a fragment containing twenty-seven carbon atoms. This he interpreted as the result of retropinacolin dehydration in the formation of the unsaturated ketone (XVII). This may be assumed to proceed via stage (XVI)



XVI



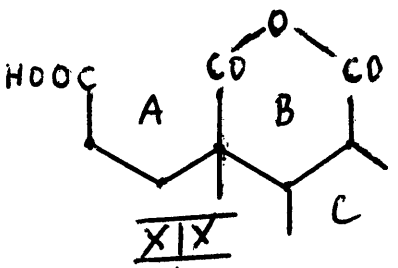
XVII



XVIII

Later (37) the same authors showed that α-amyradienone-II also contained a reactive double bond and by a similar process of oxidation they obtained a triketone which still retained all its thirty carbon atoms. On this basis α-amyradienone-II was formulated as (XVIII); this clearly makes the position of the double bond in ring B between carbon atoms #6 and #7 untenable, and, to account for the

fact that throughout these changes the ultra-violet absorption spectrum characteristic of the $\alpha\beta$-unsaturated ketone α-amyrenonol is changed only slightly, the suggestion is made that the double bond is located in ring C, as had also tentatively been suggested by Spring (39). Further vigorous oxidation of both the α-amyradienones with chromic acid, followed by treatment with acetic anhydride yielded a compound for which the formula (XIX) was suggested.



XIX

It is remarkable, however, that this acid anhydride should not be readily titrateable with alcoholic alkali and should apparently require eight hours' refluxing with alcoholic potash (37) to effect neutralisation. In this compound, too, the absorption spectrum is only slightly altered, suggesting that the original chromophore has been preserved; this is cited as further proof that the double bond of α-amyrin is located in ring C.

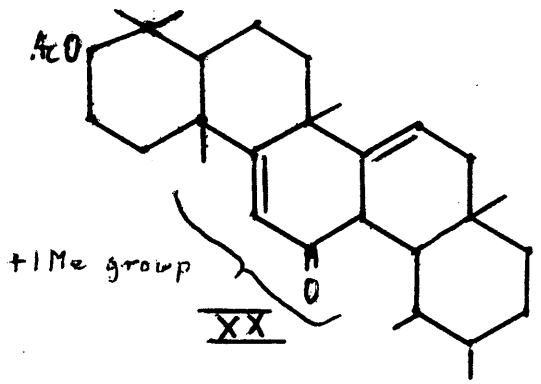
RING FISSIONS IN α-AMYRIN

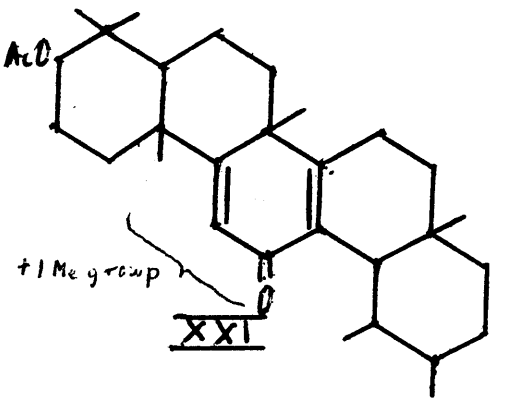
Ruzicka (26) oxidised iso- α-amyrenonyl esters with selenium dioxide, and obtained a compound which gave a yellow colour with the tetranitromethane reagent. Like most αβ-unsaturated ketones, iso- α-amyrenonol (p. 8) does not give a colour reaction with tetranitromethane, and it was therefore concluded that the oxidation product contained a double bond in addition to the conjugated system

$$>C=C-C=O$$

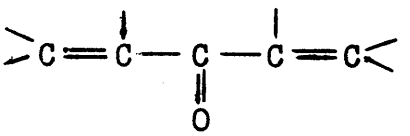
The ultra-violet absorption spectrum of the compound showed maximum absorption at λ_{max} = 2350A, and it was claimed that this represented the absorption spectrum of an ordinary αβ-unsaturated triterpene ketone, identical with that of the starting material, iso- α-amyrenonol. The spectrum of the latter shows, however, maximum absorption at λ_{max} = 2520A (32) and the claim that the selenium dioxide oxidation product still contains the unaltered unsaturated grouping of iso- α-amyrenonol unaffected by the presence of some new chromophore does not appear to be valid. In fact it does suggest the presence of a conjugated system of two carbon - carbon double bonds distributed between two rings.

Nevertheless the formula (XX) (27)





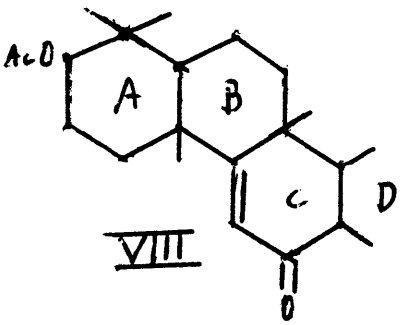
representing iso- -amyradienonol was ascribed to the compound. It could be isomerised by treatment with mineral acid and the product again showed the presence of the same chromophore, which was, however, interpreted this time as the grouping



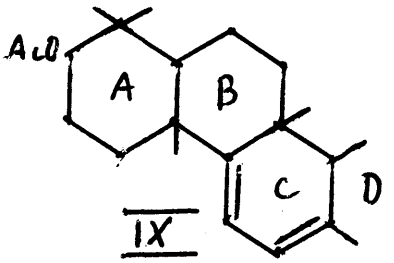
and the formula (XXI) was ascribed to this compound, iso- α-amyradienonol-II.

iso- α-Amyradienonyl acetate could be catalytically hydrogenated and from the reaction mixture both iso- α-amyrenonyl acetate (VIII, p. 9) and α-amyradienyl acetate (IX, p.9) could be isolated. It is interesting to note that iso- α-amyrenonyl derivatives cannot ordinarily be hydrogenated even under the most drastic

conditions (This thesis, theor. section), (42). The fact that partial hydrogenation of this compound would appear to have taken place in this case with the formation of α-amyradienyl acetate is completely ignored. The isolation of iso- α-amyrenonyl acetate (VIII)



VIII

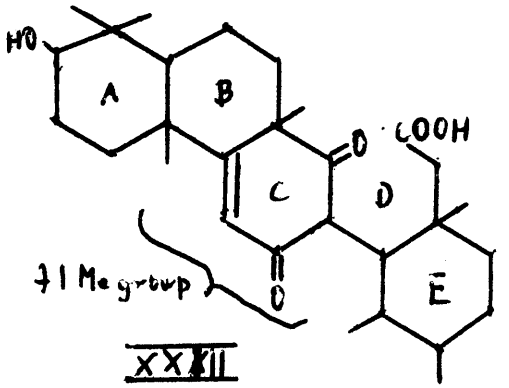


IX

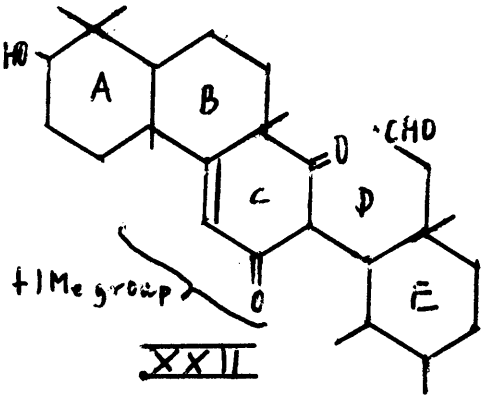
on hydrogenation of iso- α-amyradienonyl acetate (XX, p. 18) indicated that there had been no change in the original carbon skeleton during selenium dioxide oxidation.

Oxidation of the newly introduced double bond (?) with osmium tetroxide and treatment of the resulting glycol with lead tetracetate opened the ring containing the new double bond. Only one of the carbonyl groups formed during the oxidation of the α-glycol can be further oxidised by treatment with mild reagents such as neutral potassium permanganate at room temperature, with the formation of a mono-basic acid. One of the carbon atoms attached to the new double bond must therefore have been

secondary, oxidation of the glycol having yielded a keto-aldehyde. This was formulated as (XXII)

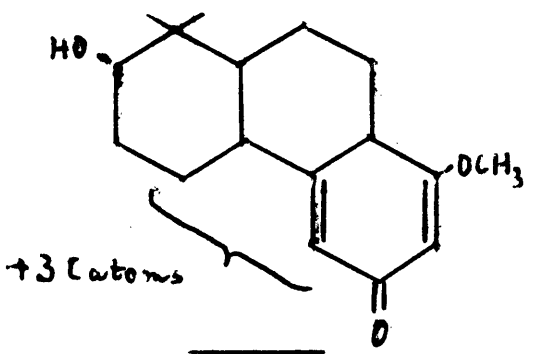


XXII

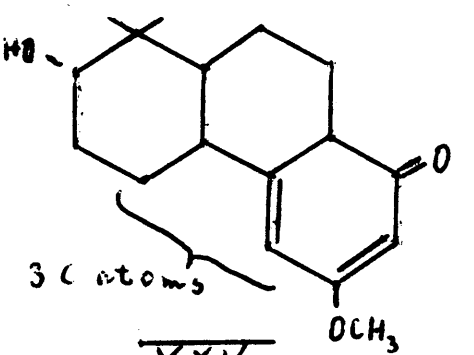


XXII

and the corresponding mono-basic acid as (XXIII). The acid was subjected to pyrolysis, and the reaction product separated into a volatile and a non-volatile fraction; the latter was methylated because it was soluble in alkali and chromatographically separated into three components. The two main products were assigned the formulae (XXIV) and (XXV) on the basis of two analyses, their by no means unequivocal absorption spectra in the ultra-violet, and the fact, that the methyl ethers could be acetylated.

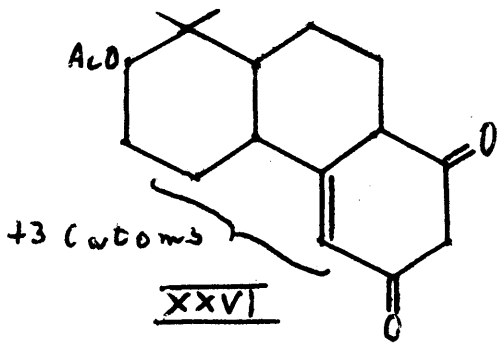


XXIV



XXV

(The acetoxy group at C_2 had been hydrolysed during the isolation of the product of the reaction of osmium tetroxide with iso- α-amyradienonyl acetate (XX)). No molecular weight determinations were carried out, nor was any attempt at further degradation made. Not even any chemical evidence as to the presence of the carbonyl groups was adduced. Neither the tetracyclic derivatives (XXII) and (XXIII) nor the unmethylated amorphous pyrolysis products gave colour reactions with ferric chloride - in spite of the fact that the α-diketone (XXVI)

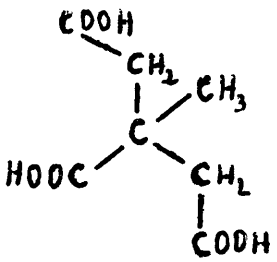


XXVI

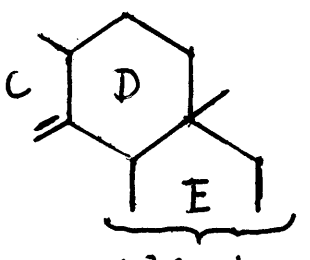
must be very highly enolised to be soluble in alkali and to methylate so readily. In the account of these experiments (27) this absence of colour reaction is dismissed as of no consequence; yet in a discussion of the degradation products of α-amyradienone-I (p. 15) published two years before (38) the same author stated categorically that the fragment containing twenty-seven carbon atoms (p. 15) could not be a β-diketone because it did not give

a colour reaction with ferric chloride.

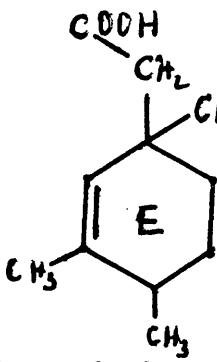
The volatile fragment produced during the pyrolysis was identified as a mono-basic acid $C_{11}H_{18}O_2$ containing one double bond, whose structure was therefore that of a cyclohexene monocarboxylic acid. Degradative chromic acid oxidation gave an identifiable product, β-methyl-tricarballylic acid (XXVII) which must represent a fragment of rings D and E of the α-amyrin skeleton (XXVIII).



XXVII



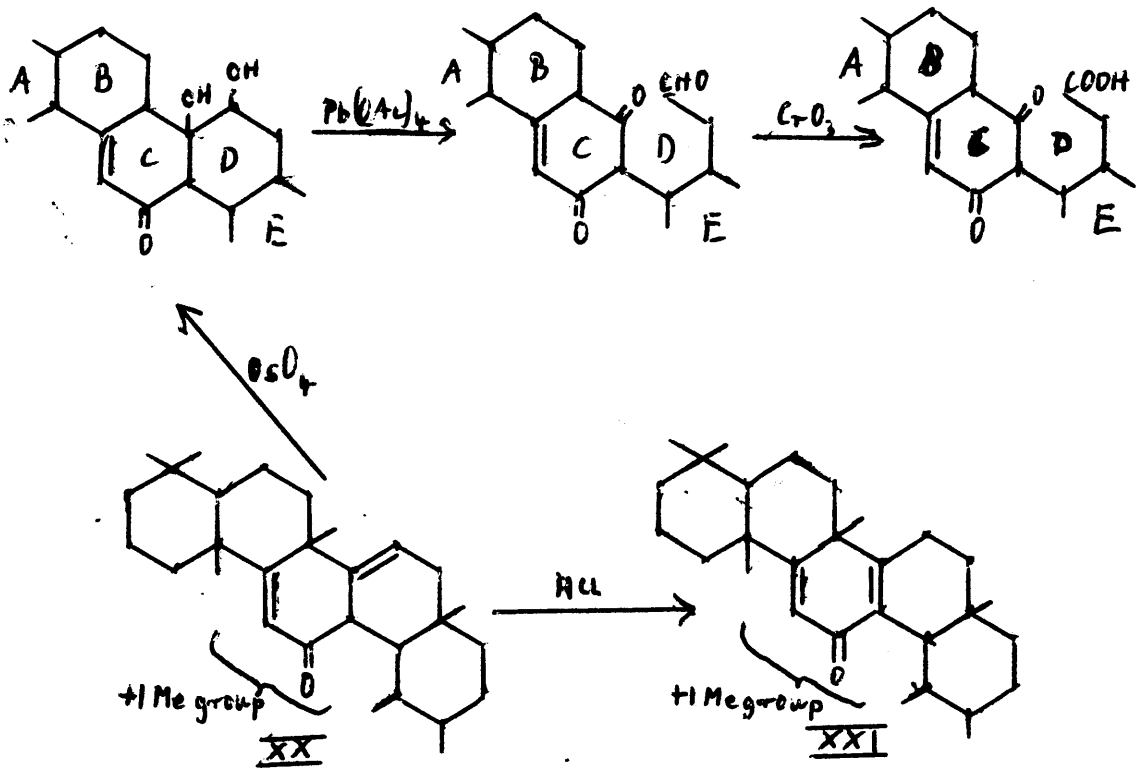
XXVIII



XXIX

Though the evidence for the constitution of the non-volatile fragment in the above fission does not really justify the conclusions drawn from it by Ruzicka (27), the volatile acid is clearly identifiable as (XXIX) by the degradation reactions it undergoes. Any other arrangement of the three carbon atoms about which dubiety is expressed in formula (XXVIII) could not by a simple oxidative degradation lead to the formation of (XXVII). It locates as well the position of the double bond undergoing rupture in ring D and the entire reaction series may be

summarised in the following scheme:



This reaction mechanism, which definitely locates the double bond of α-amyrin in ring C at C_{12} - C_{13}, was, however, devised by Ruzicka on premisses that are not sound. These have been discussed in detail in the preceding pages and may be summarised as follows:

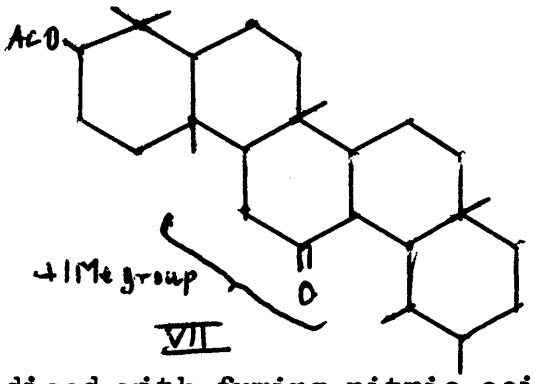
The ultra-violet absorption spectra of <u>iso-</u> α-amyradienonyl acetate (XX) and <u>iso-</u> α-amyradienonyl-II acetate (XXI) exhibit an absorption band at λ = 2350A with a maximum extinction coefficient ϵ_{max} = 16 000. This is stated to be identical with the absorption spectrum of <u>iso-</u> α-amyre-

nonyl acetate (VIII, p. 9) which shows an absorption band at λ_{max} = 2520A with a maximum extinction coefficient of ϵ_{max} = 11 000.

The compounds (XXII), (XXIII) and (XXVI) are stated to be β-diketones, yet they do not give colour reactions with the ferric chloride reagent - but the compound (XXVI) is so highly enolised that it readily forms mono-methyl ethers.

The reactions of the iso- α-amyradienonyl derivatives will be discussed in detail in connexion with the present author's experimental investigations.

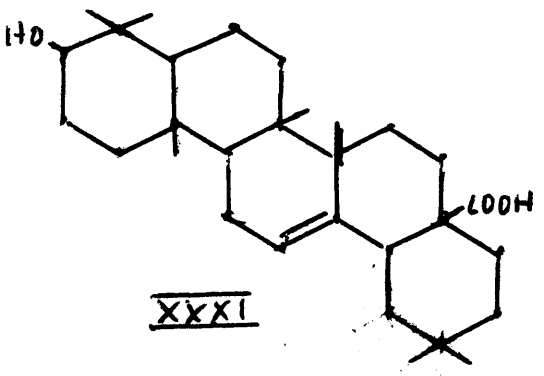
Very recently (28) α-amyranonyl acetate to which Ruzicka ascribes formula (VII, p. 8 & 9)



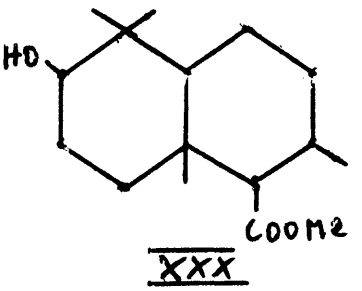
VII

has been oxidised with fuming nitric acid. The acid products could not be crystallised, but treatment with acetic anhydride gave a crystalline "anhydride". Attempts to crystallise the free acid obtained from this by hydrolysis were equally unsuccessful, nor could the dimethyl ester

be obtained crystalline. This last amorphous product was pyrolysed after oxidation of the hydroxyl group in ring A to a carbonyl group, and the product distilled in vacuo. The distillate was then separated into ketonic and non-ketonic fragments. The former was a liquid which showed the presence of unsaturation by the yellow colour which it developed with the tetranitromethane reagent. It was therefore hydrogenated. The resultant saturated hydroxy-ester was identical with the product (XXX) obtained from oleanolic acid (XXXI) by a similar pyrolytic process and subsequent hydrogenation (43).



XXXI



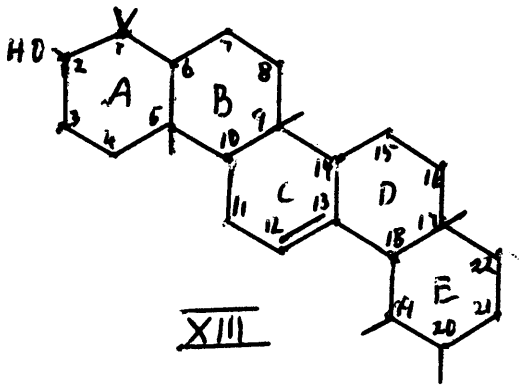
XXX

This would appear to prove that the configuration and structure of rings A and B is identical in the α- and β-amyrin series. This evidence is difficult to reconcile with many of the reactions of α-amyrin, in particular with the formation of the conjugated dehydration products (p. 13 ff) and also with the experimental results

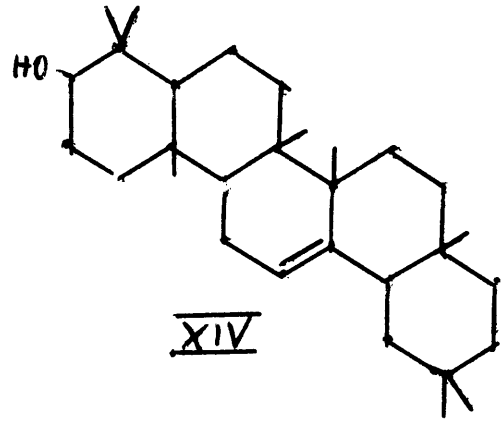
obtained by the present author. These discrepancies are discussed and a different interpretation of the reactions so far described is suggested in the theoretical part of this thesis, but it should be pointed out at this stage that there are doubts as to the identity of the compound used by Ruzicka for the nitric acid oxidation with α-amyranonyl acetate. The material was prepared by the method of Ruzicka, Jeger, Redel and Volli (44). It has, however, been shown by Silverstone (45) that α-amyranonyl acetate does not have the same physical properties as the compound described by Ruzicka (44).

The non-ketonic fraction of the pyrolysis product was found to consist of a mixture of saturated and unsaturated esters. These were converted to the saturated acid by hydrogenation and hydrolysis. On dehydrogenation with selenium, sapotalene (I, p. 4) was formed in good yield. The isolation of this compound locates an angular methyl group at position C_{14} of the α-amyrin skeleton (XIII, p. 10) - which is quite incompatible with the formation of the selenium dioxide oxidation product iso- α-amyradienonyl acetate (XX, p. 20) which, if Ruzicka's formulations (26, 27) are correct, is formed without change in the carbon skeleton of α-amyrin. Using the evi-

dence favourable to his conclusions and entirely omitting that which is not, Ruzicka has ascribed to α-amyrin the formula (XIII)



XIII



XIV

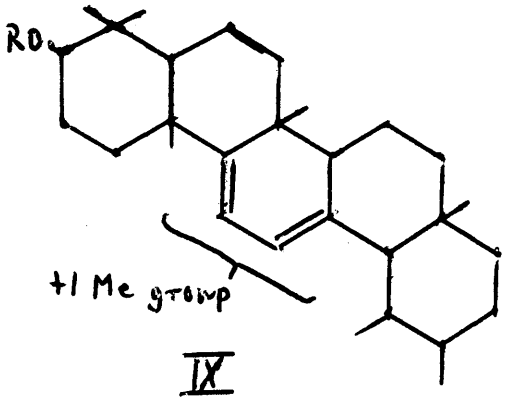
which differs from that of β-amyrin only by the position of a methyl group in ring E, (XIV, p. 11). The differences in reactivity shown by the two compounds are, however, much more fundamental than a mere steric influence due to the location of a methyl group at C_{19} in α-amyrin. They are not limited, as is implied by Ruzicka (28), to a lesser reactivity of the double bond and of the hydrogen atom at C_{18}, and these differences will be discussed in the appropriate sections.

T H E O R E T I C A L

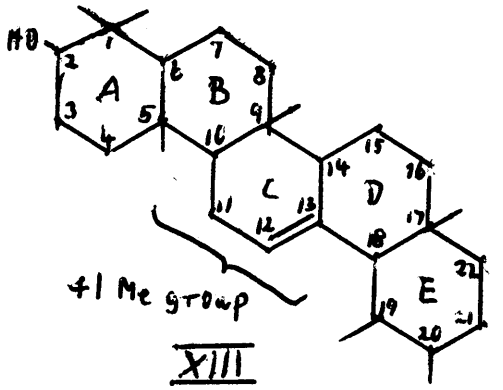
N.B. Arabic numerals in parentheses refer to the Bibliography, Roman numerals to the formulae in the text.

INTRODUCTION

Oxidation reactions carried out on α-amyradienyl Derivatives (IX, p. 9)



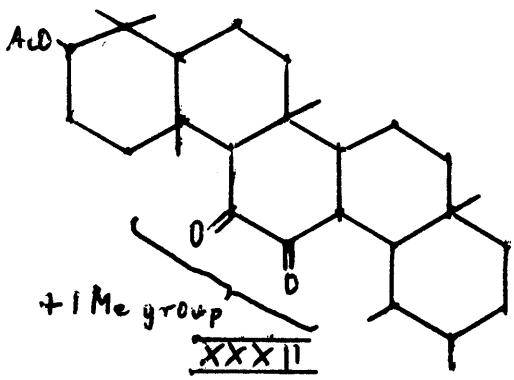
IX



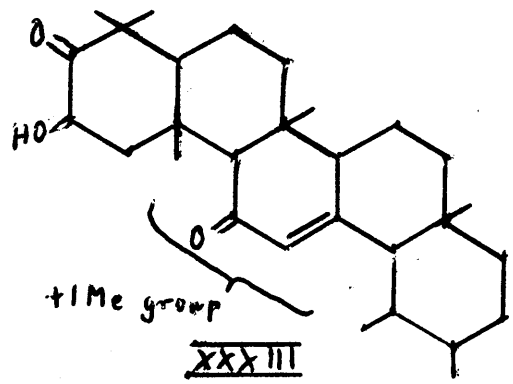
XIII

have given products whose formation should preferably involve an asymmetric centre at the junction of the terminal rather than the central rings of the molecule. The reactivities of the rings containing the secondary hydroxyl group and the unsaturated centre respectively show mutual influences. The evidence adduced by other investigators, which has led to the formulation (XIII) for α-amyrin is critically discussed and an attempt is made to interpret the observed reactions in a different manner reconcileable with the evidence obtained by the present author. The results obtained by Redel (46) in the oxidation of α-amyradienyl acetate with perbenzoic acid have been reconciled with the results obtained by Spring (19) in the same reaction.

α-Hydroxy ketones generally react with the Malaprade reagent with the formation of an aldehydic and an acid fragment (47). Experiments in this direction have, however, only been carried out with aliphatic derivatives (48). The reaction has not been investigated with regard to alicyclic α-ketols and α-diketones, and anomalous results have been obtained when the action of the reagent on this type of α-amyrin derivative was studied. Thus α-amyrandionyl acetate (XXXII, Ruzicka's formulation)



XXXII



XXXIII

on treatment with periodic acid gives a neutral product which has not been identified (45), and in the case of α-amyrenedionol (XXXIII), apart from the expected aldehydo-acid, a neutral product containing iodine was obtained. The comparable reaction with lead tetracetate has also been found to take an anomalous course. The nature of this reaction with periodic acid has been studied, though the investigation was much hampered by preparative difficulties.

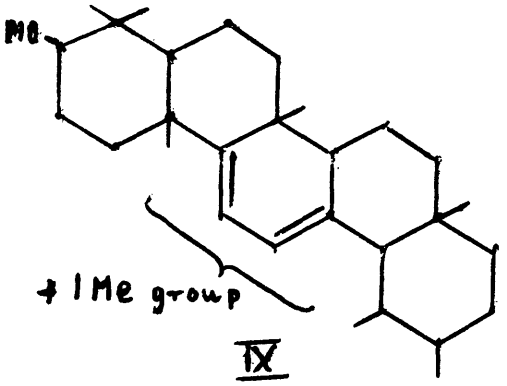
Analogous simple alicyclic compounds have also been prepared and an attempt has been made to duplicate the reaction in the simpler compounds. In general a much closer relationship between the hydroxyl group and the unsaturated centre of α-amyrin has been found to exist than is warranted by Ruzicka's formulation (XIII) of the compound.

The reactions of iso- α-amyradienonyl derivatives (p. 17) have been found to be irreconcileable with the structure of these compounds proposed by Ruzicka (27). In fact, it has been found extremely difficult to suggest a formulation which will account for all the reactions of α-amyrin and closely related compounds such as ursolic acid.

THE OXIDATION OF α-AMYRADIENOL

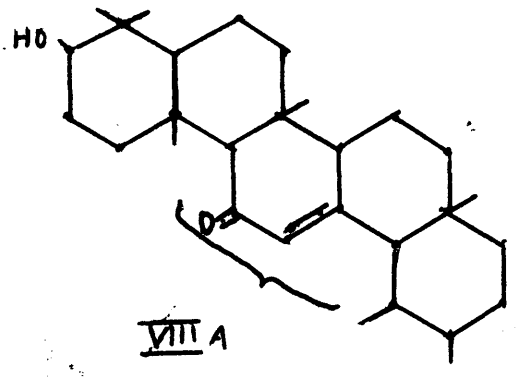
α-Amyradienyl esters (p. 9) are prepared by partial dehydrogenation of the corresponding α-amyrin derivatives. This introduction of an additional unsaturated centre may be achieved by controlled sulphur dehydrogenation and most readily by the use of Ziegler's reagent, N-bromosuccinimide (24). The reaction proceeds by way of bromination of a carbon atom in the position α to a double bond (which need not be carbon-carbon) and subsequent dehydrohalogenation. This gives rise to a conjugated system of two or more double bonds and α-amyradienol contains such a system located in one ring; the ultra-violet absorption spectrum exhibits an absorption band at λ_{max} = 2800A with a maximum extinction coefficient of ε_{max} = 11 000. It is of interest that use of an excess of the reagent will, in the case of β-amyrin derivatives, effect further partial dehydrogenation with the formation of β-amyratrienyl derivatives which contain a conjugated system of three double bonds. No comparable reaction occurs in the case of the α-amyrin derivatives, and this would indicate that the carbon atoms adjacent to both the terminal carbon atoms of the conjugated unsaturated grouping of α-amyradienol are quaternary.

This condition is not fulfilled in Ruzicka's formulation of the compound (IX)

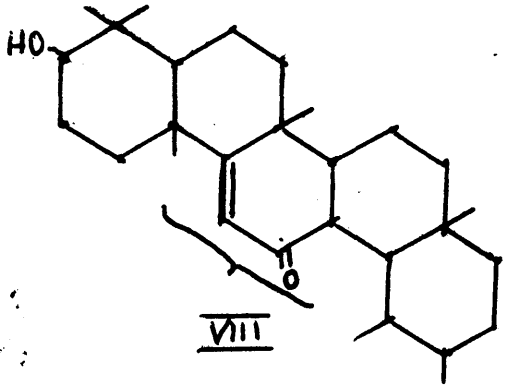


IX

but the unreactivity is attributed (28) to the steric influence of the methyl group at C_{19}. α-Amyradienyl acetate may also be obtained by the reduction of α-amyrenonol (VIIIA, p. 9), (23) and of iso- α-amyrenonol (VIII, p. 9), (32) with sodium and amyl alcohol



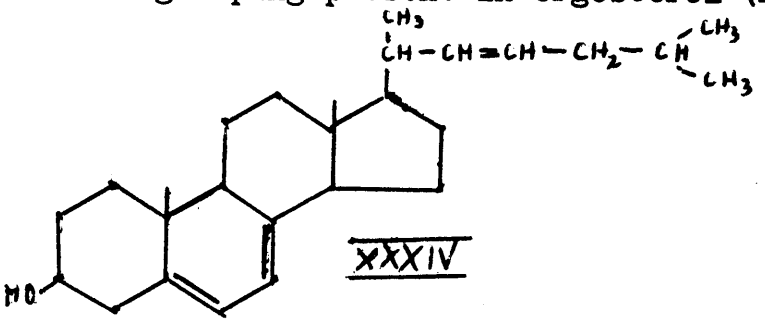
VIII A



VIII

and acetylation of the amyl alcohol complex thus formed. The inter-relationship of these compounds has been discussed in the historical section of this dissertation (p. 7 ff).

The system of two conjugated double bonds located in one ring, which is found in α-amyradienol, is reminiscent of a similar grouping present in ergosterol (XXXIV)



XXXIV

and attempts were therefore made to duplicate reactions which the latter is known to undergo. α-Amyradienyl acetate could not, however, be further dehydrogenated by mercuric acetate nor did irradiation with visible light have any effect. In this connexion it must be pointed out that prolonged irradiation with ultra-violet light does produce changes in the structure (46), though their nature has not yet been elucidated.

The new double bond is not noticeably more reactive than the single bond present in α-amyrin and attempts to oxidise α-amyradienyl acetate with osmium tetroxide and thus obtain an α-glycol as a preliminary to ring fission, were unsuccessful.

The compound is, however, attacked by those reagents

which will effect oxidation in the case of α-amyrin itself. By the use of hydrogen peroxide it has been possible to prepare an entirely new series of derivatives which has led to important conclusions about the structure of α-amyrin.

When α-amyradienyl benzoate is oxidised with hydrogen peroxide in hot glacial acetic acid, the reaction product is a mixture. This can be separated into its components only by prolonged fractional crystallisation from ethanol at the boiling point. No separation could be effected by chromatographic methods. It was thus possible to isolate two pure products, one of which (the more soluble fraction) was identified as α-amyrenonyl benzoate (VIIIA, p. 9). The other product (the less soluble fraction) was a new compound isomeric with α-amyrenonyl benzoate and <u>iso</u>- α-amyrenonyl benzoate (VIII, p. 9). Spectrographic examination of this derivative gave inconclusive results owing to interference by the benzoyloxy group. A similar effect is observed with α-amyrenonyl benzoate and <u>iso</u>- α-amyrenonyl benzoate in which the characteristic ultra-violet absorption spectrum of a triterpene α/β-unsaturated ketone is almost entirely masked by a strong absorption band at λ_{max} = 2320A

with an extinction coefficient of ε_{max} = 18 000, due to the benzoyloxy group. By careful hydrolysis the benzoyloxy group was removed and the resulting alcoholic derivative showed the typical ultra-violet absorption spectrum of a triterpene $\alpha\beta$-unsaturated ketone (λ_{max} = 2500A, ε_{max} = 11 000). The same was found to be true for the acetate which was first prepared by acetylation of the alcohol. The preparation of the pure acetate by direct oxidation of α-amyradienyl acetate with hydrogen peroxide in hot glacial acetic acid cannot be accomplished with such facility. The main product of this reaction (which was isolated as the least soluble fraction) was identified as α-amyrenonyl acetate, and the acetyl derivative of the new, isomeric, product (α-amyrenonol-II) could only be isolated from the reaction mixture uncontaminated by α-amyrenonyl acetate because a criterion of purity was available in a specimen of α-amyrenonyl-II acetate prepared by hydrolysis and acetylation of the benzoate. α-Amyrenonyl acetate and α-amyrenonyl-II acetate form mixed crystals of constant composition which show a constant melting point at 193°C, 9° lower than the melting point of α-amyrenonyl-II acetate. The mixture cannot be separated into its components chromatographically, but it was finally possible to isolate as product of a prolonged

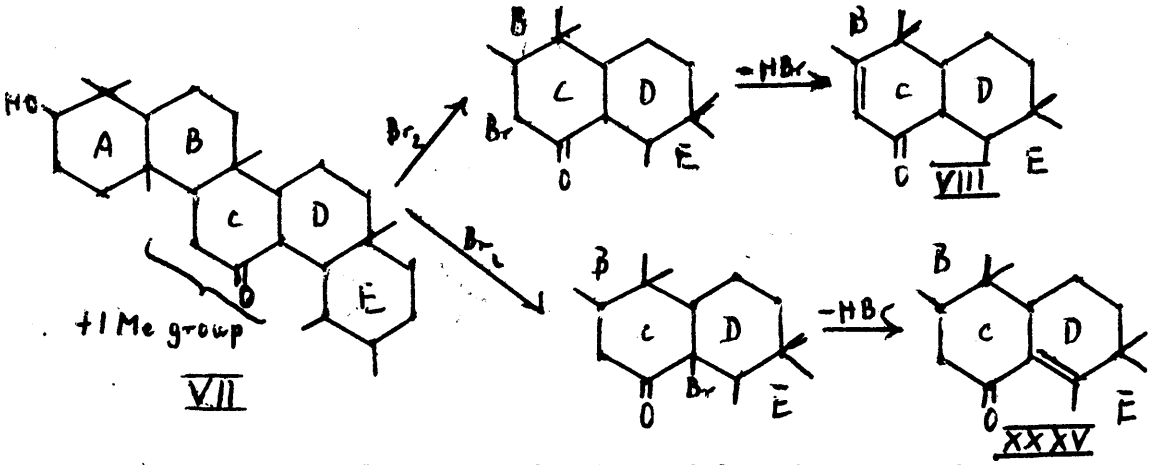
fractional crystallisation from methanol at the boiling point, a pure specimen of α-amyrenonyl-II acetate.

It was not found possible to isomerise any of the α-amyrenonyl-II derivatives to a known compound by means of the usual acid media, nor could the new products - or, indeed, any of the α-amyrenonols - be converted to their enol acetates. Isomerisation of α-amyrenonyl-II derivatives was finally achieved by prolonged treatment with boiling alcoholic alkali. As a result of this reaction iso- α-amyrenonol was isolated quantitatively, clearly identified by its physical constants and by those of its derivatives.

That α-amyrenonol-II was closely related to the two known α-amyrenonols had previously been shown by the fact that all three compounds are reduced under the same experimental conditions by sodium and boiling amyl alcohol to α-amyradienol, (IX, pp. 9, 31). This reduction demonstrates, too, the sterically hindered nature of the carbonyl groups located in the ring containing the double bond of α-amyrin. Generally, reduction of ketones by metal combinations leads to the formation of bimolecular pinacols. Sufficiently close approach of two α-amyrin molecules at the point of reaction being evidently impos-

sible, the reduction proceeds by way of formation of an amyloxide derivative (23) which can be isolated and which readily loses the elements of amyl alcohol and water to form ∝-amyradienol.

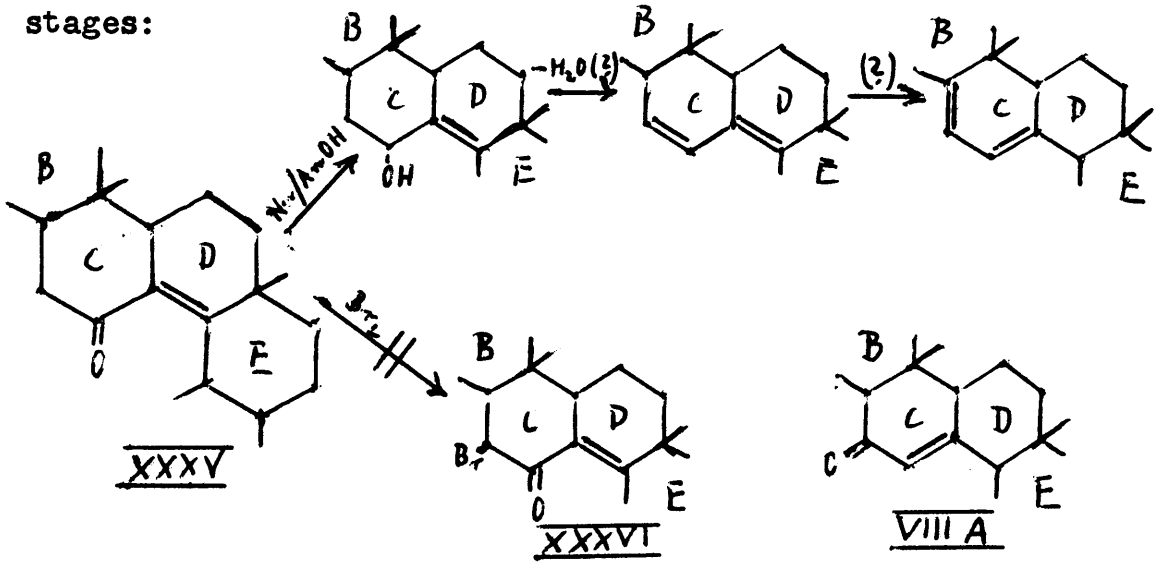
It is not possible for ∝-amyrenonol-II and iso-∝-amyrenonol to be position isomers differing only in the location of the double bond and/or the carbonyl group. (Though Ruzicka's formulations of the compounds is employed in the development of this argument, it is also demonstrably valid for any other possible position of double bond). Since ∝-amyranonol is clearly described by (VII, p. 9)



iso-∝-amyrenonol, which is formed by the introduction of a bromine atom in the position ∝ to the carbonyl group and subsequent dehydrohalogenation, may be formulated as either (VIII) or (XXXV).

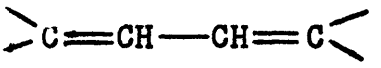
The formation of ∝-amyradienyl derivatives by

sodium and amyl alcohol reduction of iso- α-amyrenonol must, in the case of (XXXV) proceed by the following stages:

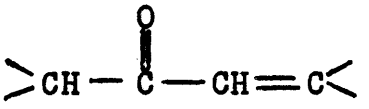


This is in itself an unlikely mechanism. The compound (XXXV) could furthermore be expected to react with bromine with the formation of the derivative (XXXVI) since bromine does substitute in that position in the case of α-amyrin. There is also a clearly defined difference in the absorption maxima in the ultra-violet region between (XXXV) in which the carbon - carbon double bond is located between two tertiary carbon atoms, and (VIII) whose absorption spectrum should be identical with that of α-amyrenonol (VIIIA), the presence of an additional substituent at one of the atoms involved in a conjugated system generally resulting in a move of the absorption maximum by some 80A towards the red end of the spectrum.

The ultra-violet absorption spectra of α-amyrenonol, iso- α-amyrenonol and α-amyrenonol-II are, indeed, found to be identical. For these reasons formula (XXXV) is clearly not tenable. Migration of the keto-group or of the entire conjugated system is equally unlikely. Under these circumstances the isomerism of α-amyrenonol-II and iso- α-amyrenonol cannot be explained by the formulation of the former as (VIII) and of the latter as (XXXV). But α-amyrenonyl derivatives (VIIIA, p. 9) are formed as secondary (or main) products in the oxidation of α-amyradienyl esters with hydrogen peroxide (p, 34). This shows that the oxidation of the system



to



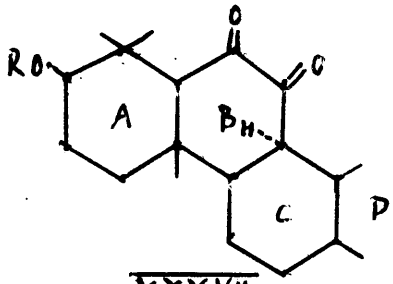
is possible and is therefore probably also responsible for the formation of α-amyrenonol-II; this must in consequence be a diastereoisomeride of either α-amyrenonol or iso- α-amyrenonol. The possibility of alkaline isomerisation of α-amyrenonol-II was not discovered for some time because the compound could be prepared from its esters by alkaline hydrolysis under mild conditions which

did not effect isomerisation. The compound was therefore provisionally formulated as a diastereoisomeride of α-amyrenonol rather than of iso- α-amyrenonol on the basis of the following theoretical considerations:

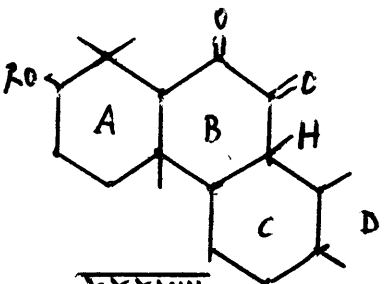
1. The double bond between carbon atoms #12 and #13 is oxidised in the formation of α-amyranonol (VII, p. 9, p. 37). Though the reaction conditions are similar to those employed in the oxidation of α-amyradienol to α-amyrenonol and α-amyrenonol-II, an intermediate oxide is isolated and only one of the two possible diastereoisomerides is formed.

2. In the oxidation of α-amyradienol to α-amyrenonol it is the double bond between carbon atoms #10 and #11 which is involved. It therefore seems probable that the double bond between carbon atoms #10 and #11 was also oxidised in the formation of α-amyrenonol-II, thus making the compound a diastereoisomeride of α-amyrenonol.

Experiments were undertaken to prove this hypothesis. iso- α-Amyrenonyl esters, which contain a double bond between carbon atoms #10 and #11, were oxidised with hydrogen peroxide in the hope that two diastereoisomeric diketones would be formed (XXXVII) and (XXXVIII). The compound was found not to react with hydrogen peroxide.

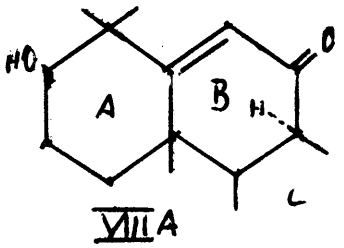


XXXVII

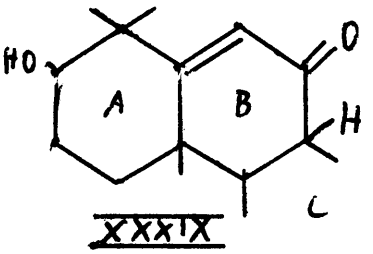


XXXVIII

α-Amyrenonol can be hydrogenated to α-amyrin (49). If α-amyrenonol-II were a diastereoisomeride (XXXIX)

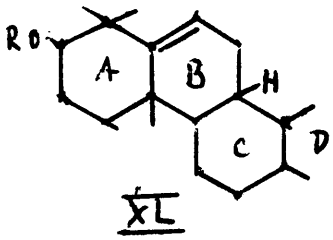


VIIIA

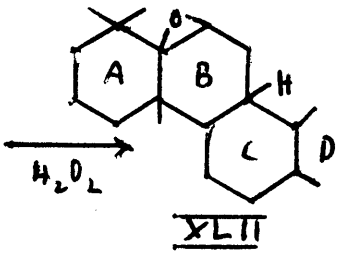


XXXIX

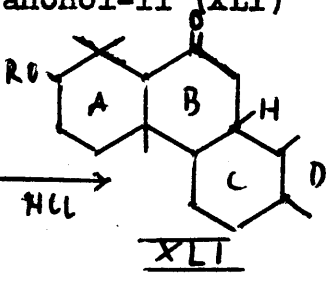
of α-amyrenonol (VIIIA), it should similarly be hydrogenated to α-amyrin-II (XL). Oxidation of this should then lead to the formation of α-amyranonol-II (XLI)



XL



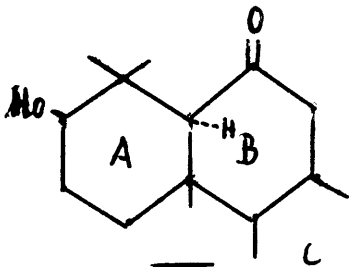
XLII



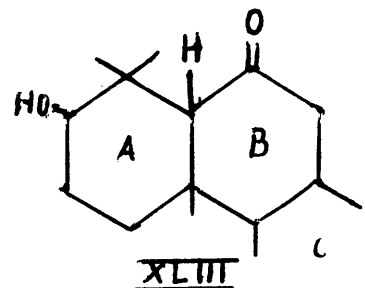
XLI

by way of the intermediate oxide (XLII). On treatment with bromine and subsequent dehydrohalogenation, the asymmetric centre involved would be destroyed and <u>iso</u>-α-amyrenonol formed. On hydrogenation of α-amyrenonol-II it was found that the carbon - carbon double bond was reduced in preference to the carbonyl group. The product

was isomeric with α-amyranonol (VII)



VII



XLIII

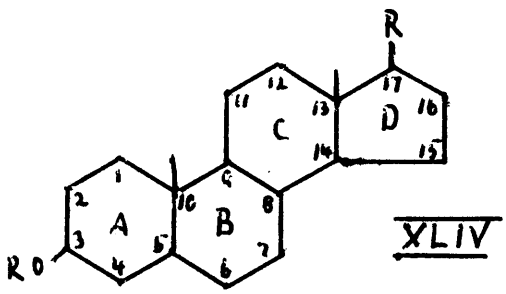
showed no high intensity absorption in the ultra-violet between 2300A and 3300A, and formed a mono-acetate on treatment with pyridine and acetic anhydride at 100°. The same mono-acetate was obtained on catalytic hydrogenation of α-amyrenonyl-II acetate. The formula (XLIII) has therefore been ascribed to this compound, representing a diastereoisomeride of α-amyranonol. It was thus not possible to prepare α-amyrin-II and the projected reaction series could not be carried out.

The discovery that prolonged treatment with alcoholic alkali isomerised α-amyrenonol-II to iso- α-amyrenonol immediately clarified the situation. The possibility of this alkaline isomerisation throws considerable doubt on the validity of Ruzicka's formulation. The isomerisation is from a cis- α-decalone system to the more stable trans- α-decalone system. (Stereochemical changes at an angular carbon atom necessarily involve cis-trans isomerism

of this type). In a classic paper (50), Hueckel proved Mohr's theory of non-planar alicyclic rings in which the carbon atoms retained their tetrahedal valency angles. He was able to isolate two isomeric decalins of virtually identical stability, whereas a planar ring structure would require that the cis-form should be much more stable than the trans-form, since intra-molecular strain is much larger in the latter. By oxidative degradation and by synthetic methods he was able to assign the correct structures to the cis and trans-decalones. He showed that, while the β-decalones were not interconvertible by isomerisation reactions, cis-α-decalone isomerised to trans-α-decalone on subjection to high temperature, drastic treatment with mineral acid or prolonged refluxing with alcoholic sodium hydroxide. The equilibrium mixture contained more than 95% of the trans-isomer and may thus be considered to be the product of an irreversible isomerisation. This type of isomerisation is thus also most probably responsible for the isomerisation of α-amyrenonol-II to iso-α-amyrenonol and this contention is supported by the fact that the melting points of the α-amyrenonyl-II derivatives are lower than those of the corresponding iso-α-amyrenonyl derivatives. Hueckel showed that, among other characteristic differences in physical properties

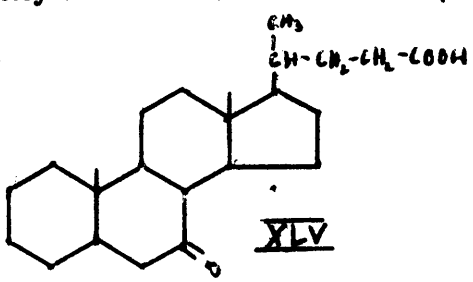
the melting points of cis isomers were always lower than those of trans isomers.

This type of isomerisation is only probable if one of the terminal rings of the α-amyrin nucleus is involved. Thus in the steroid molecule (XLIV)

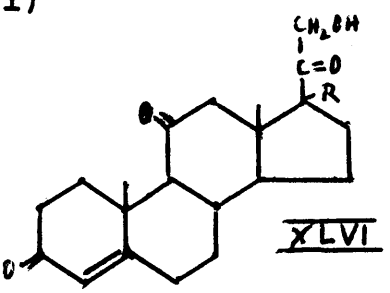


XLIV

cis-trans isomerisation has never been effected at the junction of rings B/C, which are invariably in the trans-configuration. 7-keto-cholanic acids (XLV) for instance, or dehydro-corticosterones (XLVI)



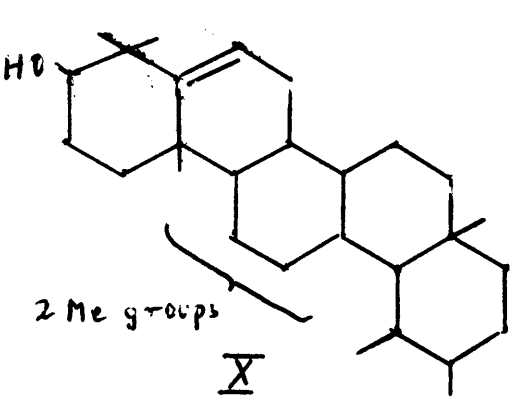
XLV



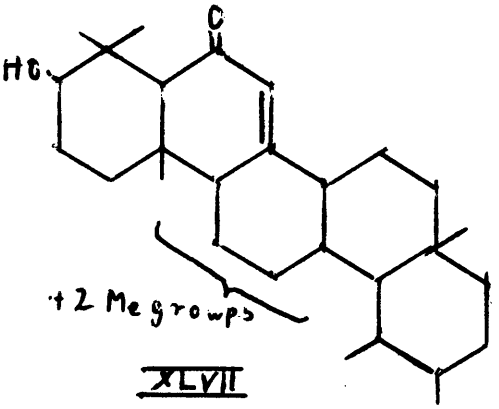
XLVI

in which carbonyl groups are located at C_{11} cannot be isomerised by treatment with the reagents which effect isomerisation of cis-decalones. At the junction of rings B/C this type of change would imply a steric rearrangement of

the entire molecule. Similar considerations can be applied to the possibility of cis-trans isomerism at the junctions of rings B/C and C/D of the triterpene molecule. This evidence suggests formulation (X, p. 10) for α-amyrin and (XLVII) for iso-α-amyrenonol.



X



XLVII

This formulation is the only one in which rings A/B will be involved in the stereochemical changes. Since this is incompatible with the conclusions drawn from the results of oxidative degradation of α-amyradienone-I (p. 15) a complete re-interpretation of these results was necessary and this is discussed in connexion with the chemistry of 1-α-amyradiene. Because the experimental results obtained by the present author all tend to substantiate the choice of formula (X) for α-amyrin, this formulation will be employed in preference to formula (XIII) throughout the further theoretical discussion embodied in this dissertation, but it must again be emphasised that the

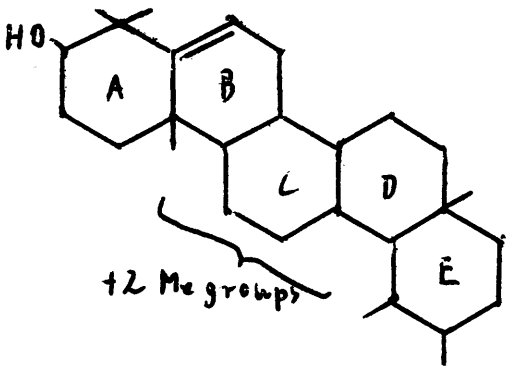
use of any one formula for α-amyrin in preference to another is a convention employed for the sake of clarity and must not be taken to imply that there is conclusive proof for its adoption. Thus it is difficult to explain the evidence obtained in the oxidation of α-amyranonyl acetate with nitric acid (p. 24) on the basis of formula (X) for α-amyrin.

In connexion with the study of the oxidation of α-amyradienyl esters with hydrogen peroxide, the action of perbenzoic acid on α-amyradienyl acetate was reinvestigated. Spring (51) had reported the isolation of a compound, melting at 193°C to which the structure of an oxide was assigned. The same compound would also appear to have been isolated on ozonolysis of α-amyradienyl acetate (52), but this time the structure of an αβ-unsaturated ketone was assigned to it, together with the name epi(iso)- α-amyrenonyl acetate. Redel (46) also oxidised α-amyradienyl acetate with perbenzoic acid, but could not isolate a pure product by the crystallisation methods employed by Spring (51). The reaction product was therefore subjected to chromatography and was thus separated into α-amyrenonyl acetate and epi(iso)- α-amyrenonyl acetate (m.p. 193°C). In the course of the

present investigations, α-amyradienyl acetate was oxidised with perbenzoic acid under conditions similar to those employed by the previous workers. The reaction product could not be crystallised, but by a prolonged chromatography it was finally possible to separate it into α-amyrenonyl acetate and iso- α-amyrenonyl acetate. This evidence is readily interpreted by comparison with the results of the oxidation of α-amyradienyl esters with hydrogen peroxide. The epi(iso)- α-amyrenonyl acetate reported by the previous workers is clearly the constant composition mixture of α-amyrenonyl acetate and α-amyrenonyl-II acetate (p. 35) which cannot be separated into its components by simple crystallisation or by chromatography. In the present case isomerisation of α-amyrenonyl-II acetate to iso- α-amyrenonyl acetate would appear to have taken place during chromatography; this type of reaction is known to occur very readily during the adsorption on alumina.

It has thus been shown that those reagents which oxidise double bonds with the intermediate formation of oxides, react with both double bonds of the -amyradienyl esters to an approximately equal extent. The double bond originally present in α-amyrin gives rise to a ketone in which a cis-decalone structure is present which can

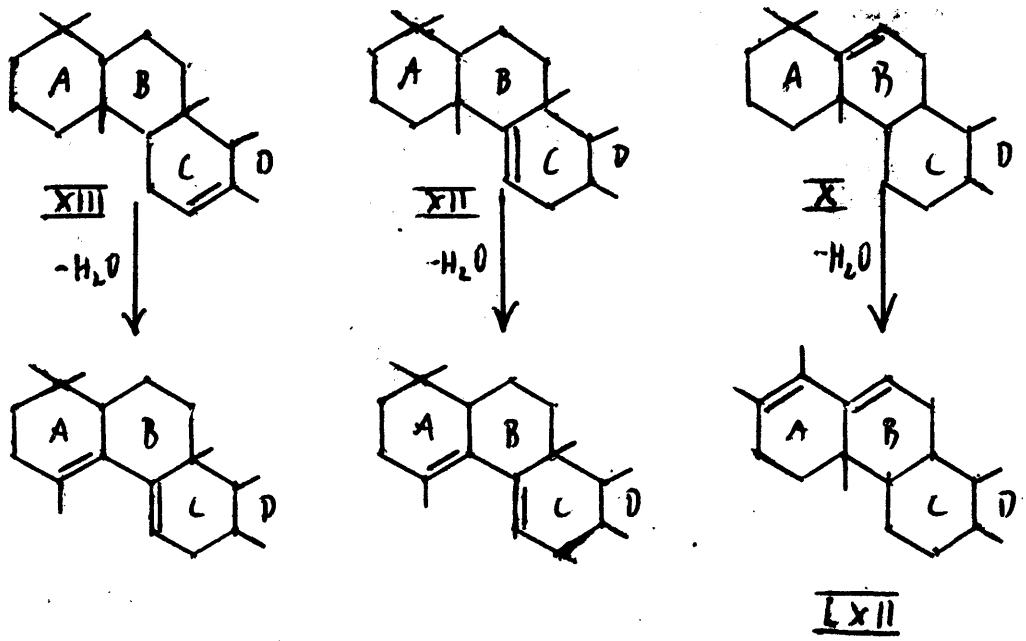
easily be isomerised to the trans- α-decalone structure. The double bond introduced by the partial dehydrogenation by which α-amyradienol is prepared, gives rise to a ketone which either contains a trans- α-decalone system, or a cis- α-decalone system in which isomerisation is impossible. The presence of a possible β-decalone system, which cannot under any circumstances be isomerised is ruled out by general structural considerations of α-amyrin. This evidence is best interpreted by the adoption of the structure (X)



for α-amyrin in which the need for stereochemical change is confined to the junction of rings A/B.

THE CHEMISTRY OF 1- α-AMYRADIENE

The nature of the changes which lead to the formation of 1- α-amyradiene has never really been elucidated. In the case of Ruzicka's formulation of α-amyrin (XIII) the reaction involves the migration both of the double bond originally present in α-amyrin and of the double bond introduced into ring A by the dehydration. Movement of the angular methyl group from C_5 to C_4 is also required. The alternative formulation (XII, p. 10) implies the same rearrangement in ring A without movement of the double bond of α-amyrin. Formula (X) supplies the most plausible mechanism - straightforward retro-pinacolin dehydration leading to the formation of 1- α-amyradiene.

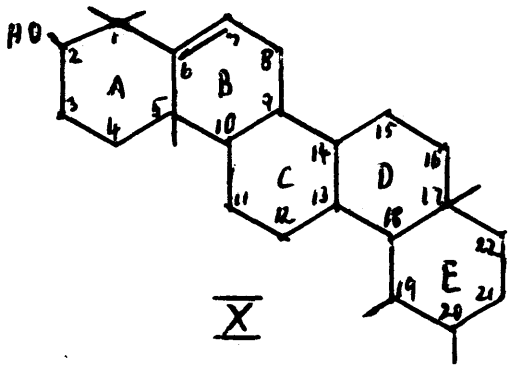


If any one of the double bonds of 1- α-amyradiene were amenable to the action of a reagent which would effect its rupture, considerable evidence as to the position of the bond would be available. Oxidation with potassium permanganate did not result in the formation of the expected di-oxy derivative. It was also found that osmium tetroxide , which usually attacks centres of unsaturation with the formation of α-glycols, had no effect on 1- α-amyradiene.

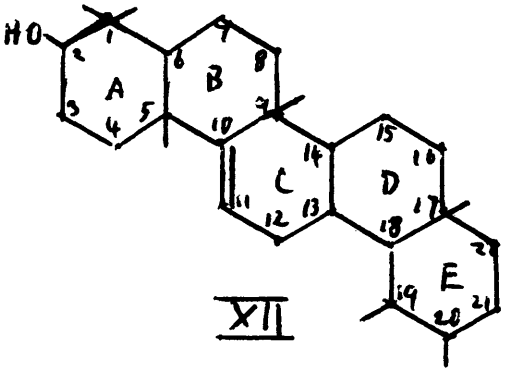
α-Amyrin derivatives are known to undergo the Ziegler reaction with N-bromosuccinimide with the formation of the corresponding α-amyradienyl derivatives (24). On the basis of any one of the three alternative structures of α-amyrin (X, XII, XIII - there is no positive evidence whatever in support of the fourth alternative structure of α-amyrin (XI, p. 10) and this is not being discussed in the present thesis) it should be possible to introduce further double bonds in 1- α-amyradiene in conjugation by means of the Ziegler reagent. One of these double bonds might be more amenable to treatment with an oxidising agent which would produce a tetracyclic derivative by scission of the unsaturated grouping. 1- α-Amyradiene was, indeed, found to react with

Ziegler's reagent, though most of the reaction product could not be obtained crystalline. Careful chromatography resulted in the isolation of a crystalline, halogen free material. This was, however, not one of the expected unsaturated derivatives, but a compound containing oxygen. All attempts to determine the nature of this material were, however, unsuccessful. The compound which contained two oxygen atoms in the molecule, could not be acetylated, nor would it react with any of the common ketonic reagents. The same product was also obtained when the reaction of 1-α-amyradiene with bromine was investigated, though the change of reagent did not lead to any improvement in the yield. Since the reactions were carried out under anhydrous conditions, the nature of the oxidation process leading to the formation of this compound is difficult to understand. It could be suggested that the primary product of the reaction was a bromine derivative which then underwent hydrolysis during the isolation of the compound, in particular during adsorption on alumina. In that case the two oxygen atoms would be present in the form of hydroxyl groups and the fact that they could not be acetylated would imply that they were located at sterically very hindered tertiary positions - a suggestion which would not be in agreement with any of the proposed formulations of α-amyrin.

Finally it must be pointed out that no comparable series of reactions is possible in the β-amyrin group of triterpenes. Though β-amyrin may be dehydrated by processes similar to those employed in the dehydration of α-amyrin, the products do not contain conjugated unsaturated systems, nor are they remarkable for a strong decrease in dextro-rotation. This is a difference in behaviour which cannot be readily explained by a difference in the position of a methyl group at the other end of the molecule. Far more does it indicate a difference in the position of the double bond, suggesting formulations (X) or (XII) for α-amyrin.



X

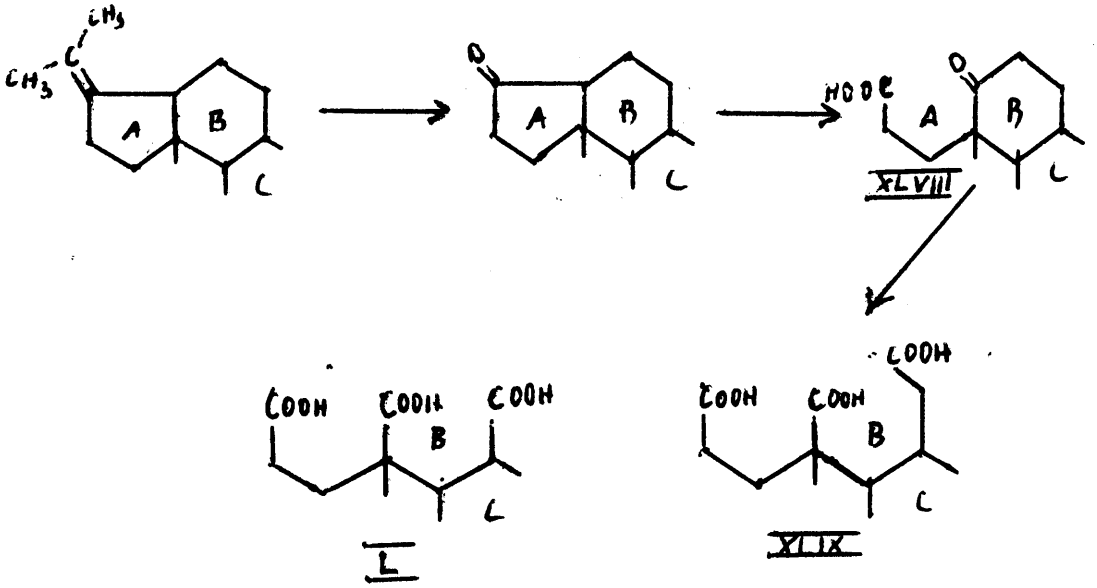


XII

Some of the experimental evidence favouring the adoption of formula (X), which has been obtained by the present author, has already been discussed in connexion with the oxidation of α-amyradienyl esters (p. 31), and there now follows a complete re-interpretation of the results obtained

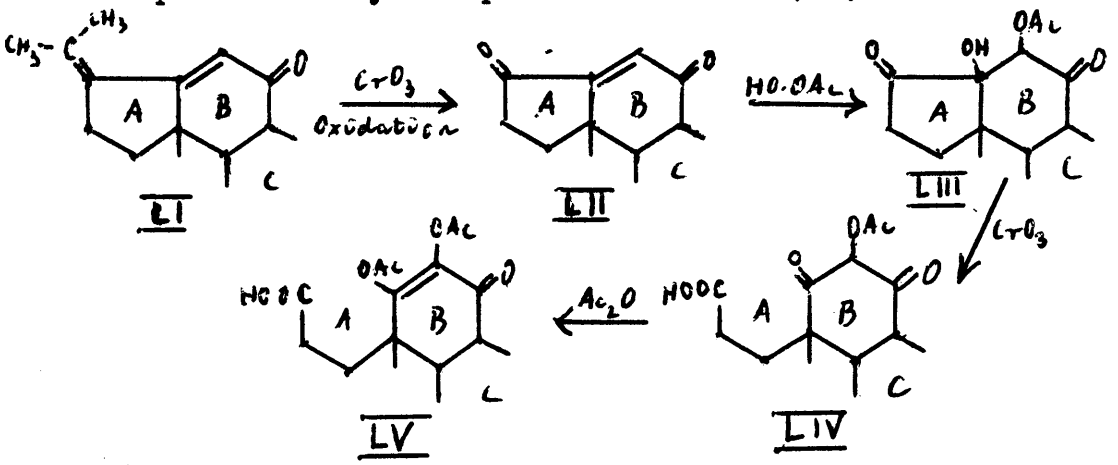
by Ruzicka (37, 38) in the degradative oxidation of α-amyradienone-I and α-amyradienone-II (p. 15).

Ruzicka's formulation for the final product of the oxidation of the α-amyradienones with chromic acid as (XIX, p. 16) is in itself difficult to justify. This type of oxidative degradation proceeds by the step-wise disruption of carbon to carbon bonds, where one or both of the carbon atoms involved carries a ketonic oxygen atom. Decarboxylation of the carboxylic acids which represent the final product of the reaction series does not occur under the conditions employed. In the case of Ruzicka's formulations for the α-amyradienones, the oxidation must necessarily proceed by the following intermediate stages:



The oxidation of the keto-acid (XLVIII) cannot proceed beyond stage (XLIX), whereas the formation of (XIX) requires further decarboxylation to (L). A similar type of degradation was carried out in the β-amyrin series (53), but in that case the intermediate acid corresponding to (XLIX) was isolated, and the conditions employed were far more vigorous. There is no indication given in Ruzicka's account (37) of the oxidation of α-amyradienone-I, of the evolution of carbon dioxide which must accompany decarboxylation.

Employing formula (X) for α-amyrin, however, it is possible to formulate the changes in a more plausible manner. In particular, it is possible to explain the prolonged alkaline treatment which is found necessary for the hydrolysis of (XIX, p. 16) (37). Thus on the basis of formula (X) for α-amyrin, α-amyradienone-I will be represented by the partial formula (LI)



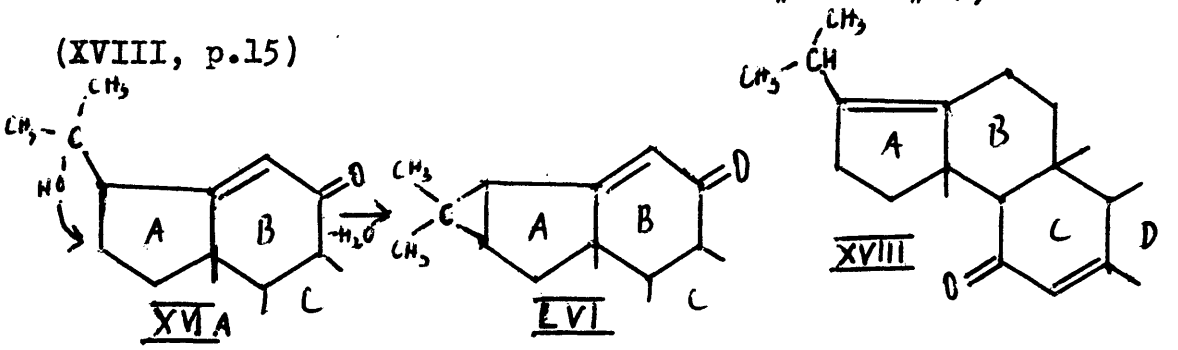
whose primary oxidation product will be the diketone (LII). Further oxidation in an acetic acid solution of chromic anhydride will then lead to the formation of the compound (LIII) by addition of peracetic acid to the double bond. This then undergoes further oxidation to (LIV). In the investigation under discussion (37) the immediate oxidation product was not isolated, but the crude acid fraction was subjected to vigorous acetylation - resulting in the formation of the enol-acetate (LV) in which there is only one possible position for the carbon - carbon double bond because of the presence of an angular methyl group at C_5, and which will therefore still contain the characteristic $\alpha\beta$-unsaturated keto-grouping. A comparison of the percentage contents of carbon and hydrogen of this compound and of the experimental values obtained by Ruzicka (37) shows that (LV) (or an isomer thereof) is a valid representation of the reaction product.

Found:-	C = 70.27%;	H = 8.14%
$C_{31}H_{46}O_7$ requs.:-	C = 70.20%;	H = 8.66%
$C_{31}H_{44}O_7$ requs.:-	C = 70.49%;	H = 8.33%

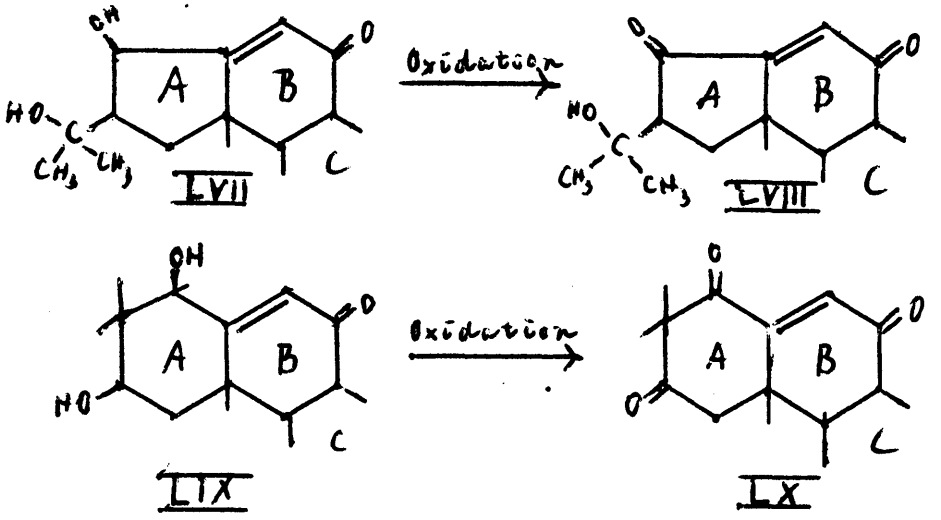
Neither the free tribasic acid, nor any of its esters were isolated.

It now becomes necessary to provide an alternative

formulation for α-amyradienone-II. With the location of a double bond between carbon atoms #6 and # 7, formula (XVIII, p.15)

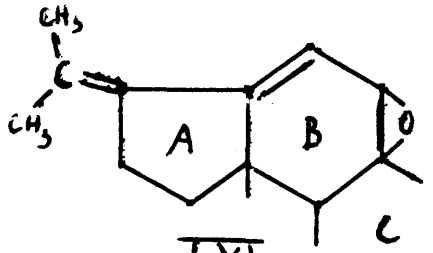


containing a double bond between carbon atoms #1 and #6 is clearly untenable. There is, however, no direct proof of the presence of an additional double bond in this compound, and it may therefore be formulated as (LVI). This is a structure often encountered in terpenoid substances (cf. thujane) and also found in the sterols (cf. i-cholesterol). The cyclopropane ring behaves as an ethylenic system and will react with osmium tetroxide with the formation of (LVII) or (LIX)



Treatment with lead tetraceta te will effect further oxidation to the diketone (LVIII) or the triketone (LX), respectively, both of which still contain thirty carbon atoms.

The absorption spectrum of α-amyradienone-I is not in agreement with the proposed formulation (LI, p. 54). This type of compound could be expected to show maximum absorption at λ = 2900A - 3000A, whereas the observed spectrum actually displays maximum absorption at λ_{max} = 2500A. There is, however, no direct chemical evidence to show that the compound is, in fact, a ketone. The tautomeric oxide (LXI)

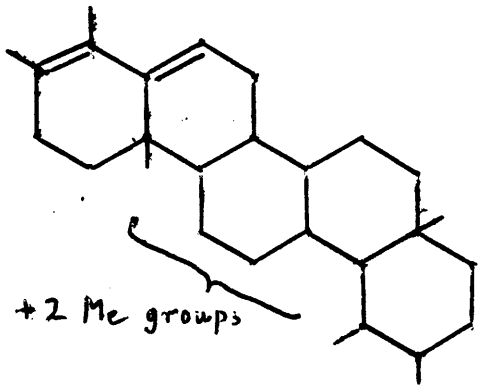


LXI

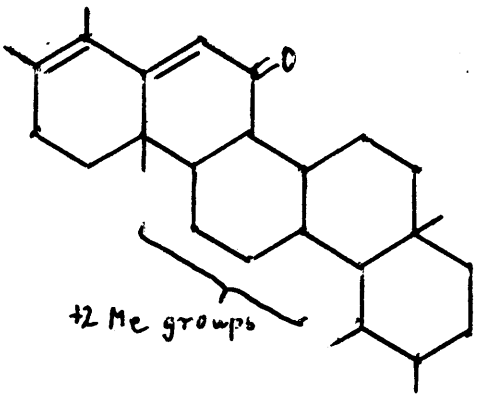
will show maximum absorption at λ = 2400A - 2500A, the chromophore being the conjugated system of carbon - carbon double bonds, in which three of the four carbon atoms involved are tertiary - a condition which favours displacement of the maximum towards the red end of the spectrum.

On the basis of formula (X, p. 10) for α-amyrin

the alternative formulae (LXII) and (LXIII) may be suggested for 1- α-amyradiene and α-amyradienone-III (p. 13) respectively, retro-pinacolin dehydration having occurred.



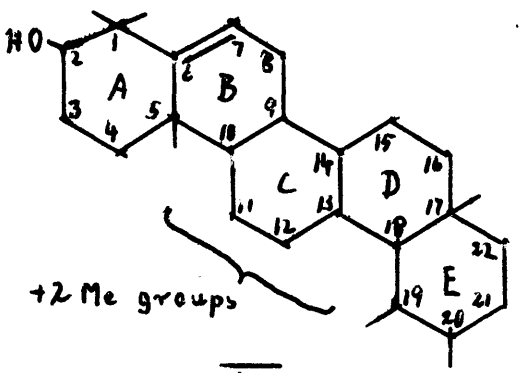
LXII



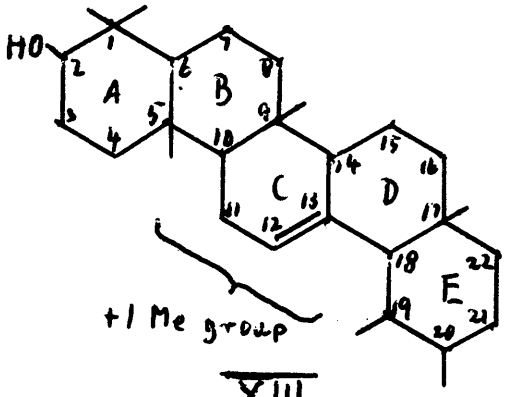
LXIII

OXIDATIVE DEGRADATION OF RING A

Before attempting oxidations in ring A it is necessary to protect the reactive methylene group adjacent to the double bond of α-amyrin. This may be achieved by its oxidation to carbonyl with the formation of α-amyrenonyl derivatives (p. 7), in which the positions adjacent to both the double bond and the carbonyl group are extremely unreactive, or by partial dehydrogenation to α-amyradienyl derivatives (p. 9), the conjugated system of double bonds again being notable for its unreactivity. Ring A contains the most reactive and sterically the most unhindered centre of the α-amyrin molecule, and this is therefore the most suitable position for initiating a step wise degradation of the compound. Successful degradation would also show at a very early stage, whether the unsaturated centre of α-amyrin was incorporated in a ring adjacent to ring A, as is postulated in formulation (X) for α-amyrin,



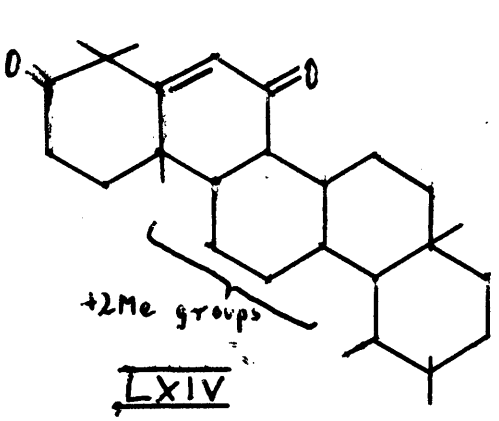
X



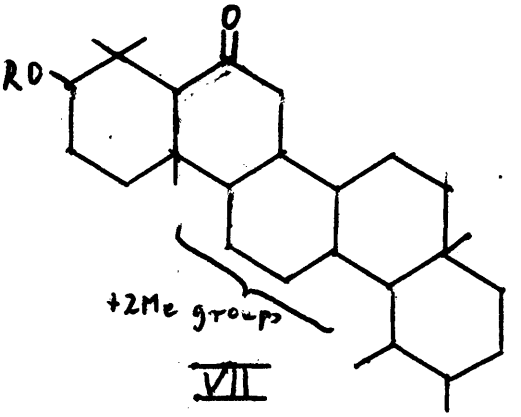
XIII

or whether the hydroxyl group and the double bond of α-amyrin were more widely separated, as is suggested by Ruzicka's formulation (XIII). Both methods of protection of the other reactive centre of the molecule, the methylene group adjacent to the double bond, were employed, but in neither case was it possible to carry out the projected reaction series because of the anomalous nature of the intermediate reactions.

Oxidation of the hydroxyl group in ring A and of the reactive methylene group adjacent to the double bond may be accomplished simultaneously by controlled oxidation with chromic acid with the formation of α-amyrenedione (LXIV).



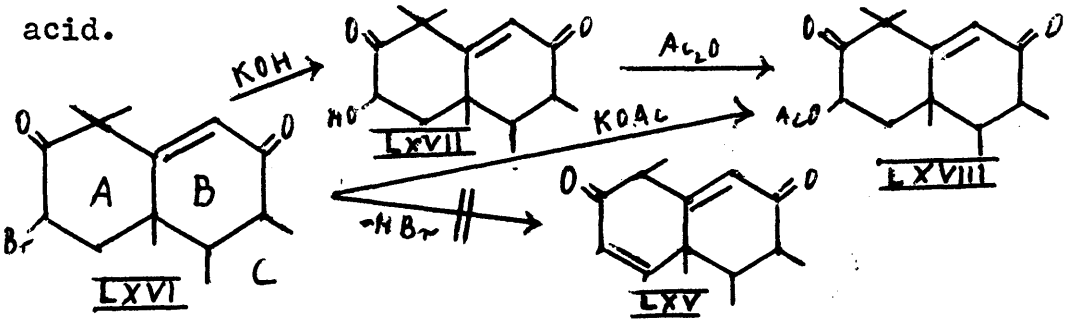
LXIV



VII

Further degra-dation of this was initiated by the introduction of bromine at the position α to the carbonyl group at C_2. (The position α to the carbonyl group adjacent to the double bond is unreactive; thus α-amyrenonyl acetate can be recovered unchanged in 80% yield

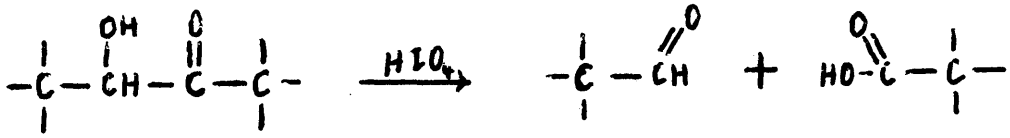
on vigorous treatment with bromine (54)). In analogy to the reactions of α-amyranonyl derivatives (VII, p. 9) it was expected that this bromo-ketone (bromo-α-amyrenedione (LXVI)) would readily undergo dehydrohalogenation with the formation of the αβ-unsaturated ketone (LXV) which would then afford a ready opportunity for the fission of the ring. Bromo-α-amyrenedione, however, proved to be completely stable to the action of hot glacial acetic acid.



Treatment of the bromo-ketone with organic bases did not yield crystalline products and the compound was finally refluxed with anhydrous potassium acetate in dry etha-nol. It was found that the expected dehydrohalogenation did not take place, but that, instead, the bromine atom was replaced by an acetoxy group. Bromo-α-amyrenedione was therefore treated with alcoholic potassium hydroxide which replaced the bromine atom by a hydroxyl group with the formation of the α-ketol α-amyrenedionol (LXVII). This compound is very sensitive to alkali and the reaction conditions must be rigorously controlled to ensure both

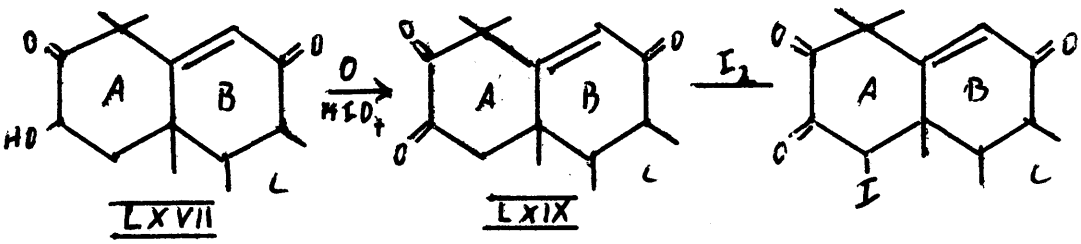
maximum hydrolysis of bromo- α-amyrenedione and minimum destruction of the reaction product, α-amyrenedionol. The α-ketol can be acetylated to the acetoxy derivative obtained by the action of anhydrous potassium acetate on bromo- -amyrenedione, and may therefore be formulated as (LXVIII, p. 61). The α-ketol (LXVII) is remarkable for an unusual red colour reaction with alcoholic ferric chloride.

In general α-ketols react quantitatively with the Malaprade reagent (periodic acid) with the production of an acidic and an aldehydic fragment:



The reagent is reduced to iodic acid and no further. When α-amyrenedionol was treated with periodic acid there was isolated in addition to the expected aldehydo-acid a yellow neutral product which contained one atom of iodine per molecule. To elucidate the mechanism of this reaction, the preparation of similar cyclic α-ketols was undertaken. Intensive study of the literature, in fact, showed that the action of the Malaprade reagent had only been studied in the case of aliphatic α-ketols. Investigations were concentrated on the preparation of a simple

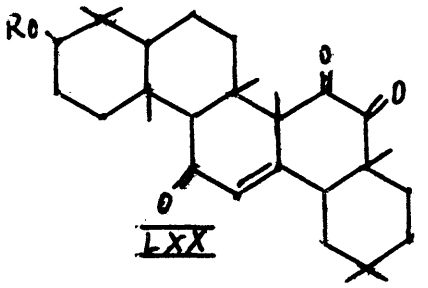
cyclic α-ketol with a carbon skeleton similar to that of the relevant ring of α-amyrin. It was finally possible to prepare 1:1-dimethyl-3-hydroxycyclohexanone-2 (v. Experimental section of this thesis). The compound reacted with the Malaprade reagent with the formation of a neutral and an acid product. The substances were oils and could not be purified sufficiently for identification, but the important point to note was, that, in the case of the cyclic α-ketols reduction of the Malaprade reagent does not stop at the iodic acid stage. There was a plentiful liberation of free iodine in the reaction at room temperature and this fact seems to indicate that cyclic α-ketols are stronger reducing agents than their aliphatic counterpa-rts. On this basis it was possible to formulate the changes in the reaction of α-amyrenedionol with periodic acid as follows:



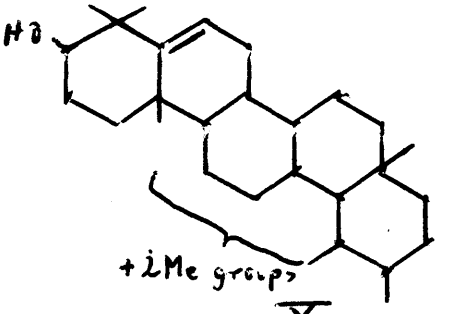
LXVII LXIX

It is unlikely that the colour of the iodine derivative formed in this reaction is due to the presence of one atom of halogen. It has, however, been noted that a derivative of a member of the β-amyrin group of triterpenes, the

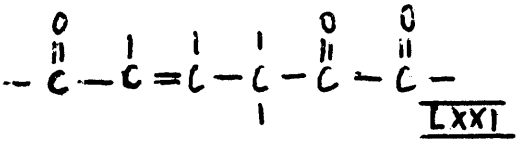
compound (LXX) (55)



LXX



X



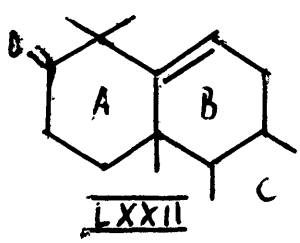
LXXI

is bright yellow in colour. Normal α-diketones are colourless and it would appear that the grouping (LXXI) is necessary for the development of colour. It is only on the basis of formula (X) for α-amyrin that a molecule containing this grouping can be derived from α-amyrenedione. This compound (LXIX, p. 63) will then react with the iodine liberated in the oxidation of the α-ketol (LXVII) with substitution of the halogen on the carbon atom α to the new carbonyl group at C_4. It is interesting to note that dehalogenation with zinc and glacial acetic acid also effects partial reduction of the diketone, the extent of this reduction depending on the degree of activity of the zinc employed. A zinc dust preparation which has been pretreated with dilute hydrochloric acid and thoroughly degreased with organic solvents will remove not only the iodine atom, but also one oxygen atom with

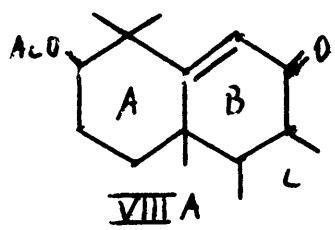
the formation of a compound of unusual ultra-violet absorption spectrum. There is an intense absorption band (ε_{max} = 18 000) at λ_{max} = 2630A; α-amyradienone-III (p. 13) shows a similar absorption maximum in addition to the characteristic spectrum pertaining to the grouping

$$-CH=CH-CH=CH-C=O$$

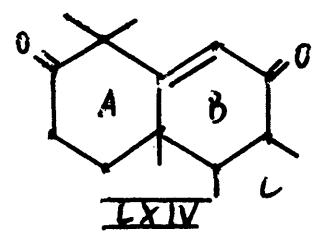
and the nature of the chromophore involved has never been clearly identified. Neither α-amyrenonyl acetate (VIIIA) α-amyrenone (LXXII) nor α-amyrenedione (LXIV)



LXXII



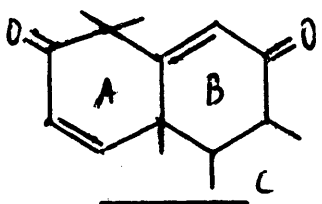
VIII A



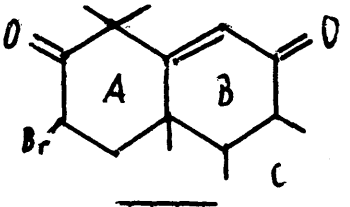
LXIV

all of which were specially prepared for comparison purposes, were found to react in any way with zinc and glacial acetic acid. It was, however, possible to effect reduction of α-amyrenedionol (LXVII, p. 61) by this means under certain circumstances, and after numerous attempts it was possible to isolate from the reaction product which generally contained a large proportion of starting material, a compound identical with the one formed in the dehalogenation of the iodo-derivative. It could also be shown that, when dehalogenation was carried out with zinc of low

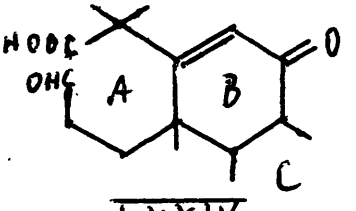
activity, the product isolated was α-amyrenedionol, identical with the compound prepared by hydrolysis of bromo- α-amyrenedione. It was not, however, possible to elucidate the structure of the deoxy- α-amyrenedionol. This was to a large measure due to the extreme experimental difficulties besetting the preparation of even α-amyrenedionol. Deoxy- α-amyrenedionol may be considered as a dehydration product (LXXIII) of α-amyrenedionol



LXXXIII



LXVI



LXXIV

though this suggestion does not appear very plausible in view of the reluctance shown by bromo- α-amyrenedione (LXVI, p. 61) to undergo dehydrohalogenation. The investigation of this problem requires very large scale facilities which were not available to the author.

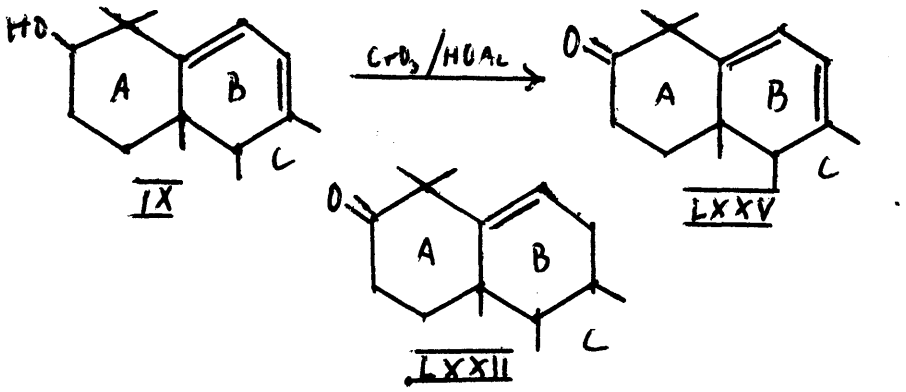
The acid product of the Malaprade oxidation of α-amyrenedionol was identified as a monobasic acid whose neutral methyl ester could be oxidised by treatment with potassium permanganate under mild conditions to an amorphous acid product. The acid therefore had the expected constitution (LXXIV).

In this case, too the preparative difficulties are such that the acid could not be considered a suitable material for further step-wise degradation of the α-amyrin molecule and no more detailed investigations in this direction were undertaken.

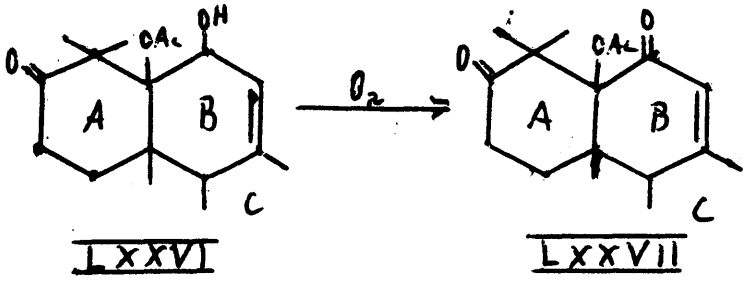
It is interesting, too, to note the anomalous reaction of α-amyrenedionol with lead tetracetate. The product no longer exhibits selective absorption in the ultraviolet region of the spectrum between 2200A and 3200A and contains one oxygen atom less than α-amyrenedionol. This is a further indication of the close relationship which exists between ring A and the α,β-unsaturated keto-group of which the double bond of α-amyrin forms a part. It is not possible to duplicate this reaction with α-amyrenonol which does not possess the α-ketol structure in ring A. The exact nature of the changes in this reaction was not elucidated.

The effect of alterations in the environment of the double bond on the course of the reactions in ring A was also studied, and the alternative method of removing the reactive methylene group adjacent to the double bond (the partial dehydrogenation of α-amyrin to α-amyradienol) was therefore investigated.

α-Amyradienone (LXXV) was first obtained by Jacobs (22) by somewhat vigorous oxidation of α-amyradienol (IX, p. 9) with chromic acid.

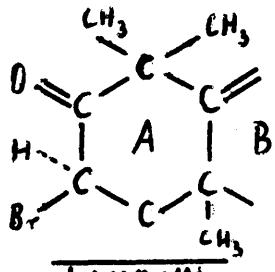


Since the reagent is known to attack the conjugated system of the compound under these conditions (41) the method was modified to that employed in the preparation of α-amyrenone (56) (LXXII, p. 65). In addition to α-amyradienone, which is formed in good yield (its physical properties differ somewhat from those described by Jacobs - loc. cit. - the melting point in particular being markedly higher), there is also obtained a high melting (320°) product whose analysis corresponds to the addition of peracetic acid to one of the double bonds of α-amyradienol with the formation of the compound (LXXVI) and its oxidation to (LXXVII).

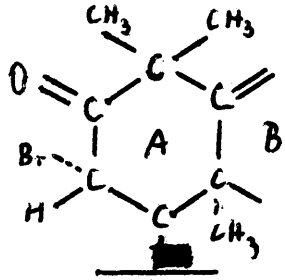


The ultra-violet absorption spectrum of the compound no longer shows the presence of a system of two carbon - carbon double bonds located in one ring, but exhibits instead an absorption band at λ_{max} = 2520A with a maximum extinction coefficient of ϵ_{max} = 8 000 due to the $\alpha\beta$-unsaturated keto-grouping present in (LXXVII). Similar compounds have been obtained by previous workers in the oxidation of α-amyradienyl derivatives (41, 57).

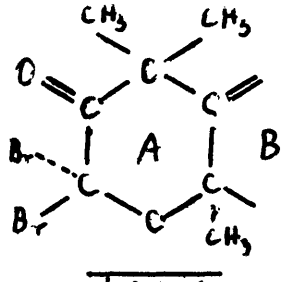
Though the overall yield of α-amyradienone in a two stage preparation is better than the yield of α-amyrenedione in a single stage oxidation, this advantage is entirely offset by the variety of bromination products derived from α-amyradienone as compared with the obvious homogeneity of bromo- α-amyrenedione. On treatment of α-amyradienone with bromine in glacial acetic acid at room temperature, two isomeric mono-bromides and a dibromide are obtained, which may be formulated as (LXXVIII), (LXXIX), and (LXXX),



LXXVIII

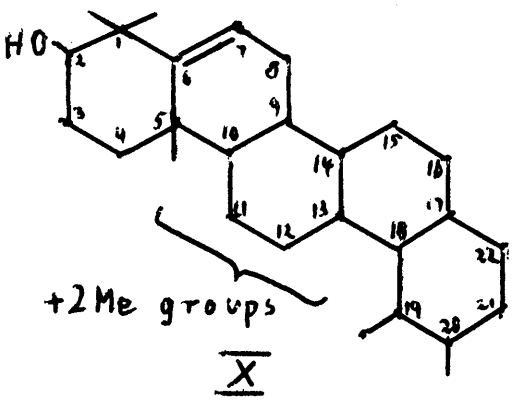


LXXIX

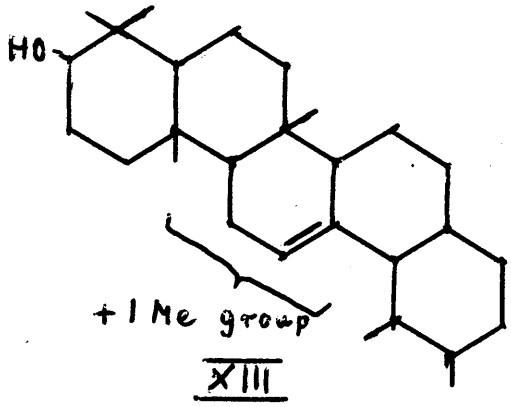


LXXX

the monobromides differing only in the steric arrangement of the substituent bromine atom. Dibromides of the type (LXXX) are readily obtained from sterols of similar structure. It is, however, not clear why it is possible to obtain these various derivatives from α-amyradienone and not from α-amyrenedione. The construction of Stuart models of the ring systems concerned shows that, irrespective of whether formula (X) or formula (XIII) is used to represent the α-amyrin molecule



X

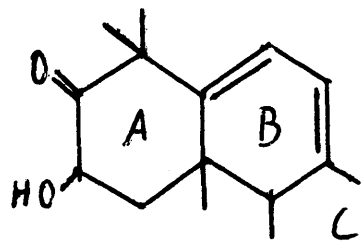


XIII

this remarkable difference in reactivity cannot principally be due to some form of steric interference between the additional carbonyl group in α-amyrenedione and substituents at C_3. It should be noted that the introduction of a double bond between carbon atoms #8 and #9 will have a distorting effect on the molecule tending to remove rings C, D and E from the proximity of ring A, whereas the introduction of a double bond between carbon

atoms #11 and #12 will have precisely the opposite effect. Here, too, then the evidence may be interpreted in favour of formula (X) rather than Ruzicka's formulation of α-amyrin (XIII). Until the configuration of the entire α-amyrin molecule is known it will be impossible to estimate the extent to which this type of molecular strain will affect the reactivities at various points of reaction.

Because of the variety of the bromination products which could be separated from each other only with great difficulty and because of the obvious possibility of Walden inversion during hydrolysis, sufficient α-amyradienonol (LXXXI)



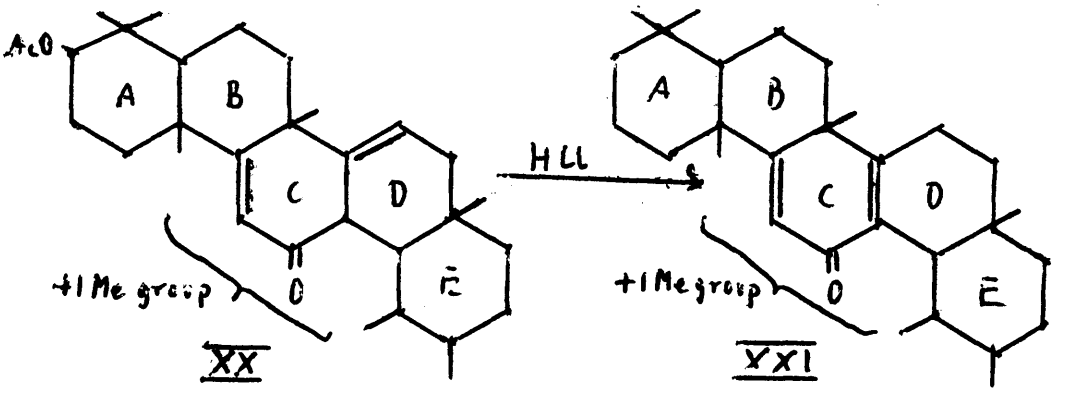
LXXXI

for further degradative investigations could not be prepared. A small amount of crystalline material was finally obtained after careful hydrolysis of a relatively pure specimen of one of the mono-bromo- α-amyradienones, and this was immediately treated with periodic acid. No crystalline product could be obtained from this reaction, though both

neutral and acidic amorphous compounds were isolated. The neutral material was colourless, the apparent chromophore (LXXI, p. 64) no longer being present.

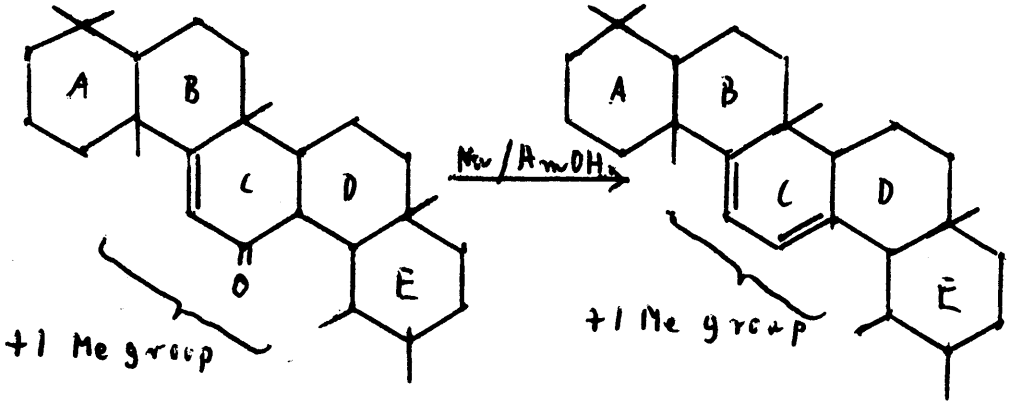
THE OXIDATION OF iso-∝-AMYRENONYL DERIVATIVES WITH SELENIUM DIOXIDE

The dubious interpretation of his experimental results which was published by Ruzicka (27, 28) (p. 17) made an investigation of the chemistry of the iso-∝-amyradienonyl derivatives necessary. iso-∝-Amyradienonol was formulated by Ruzicka as (XX, p. 18)



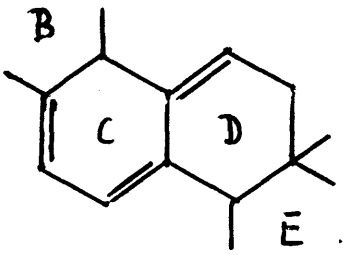
XX XXI

and iso-∝-amyradienonol-II as (XXI, p. 18). In analogy to iso-∝-amyrenonol (VIII, p. 9)

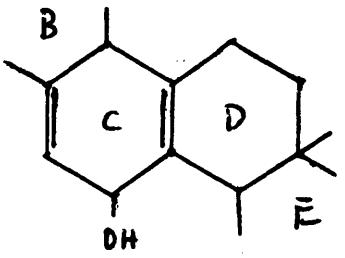


VIII IX

which can be reduced to α-amyradienol (IX, p. 9) with sodium and amyl alcohol, the compound (XX) should react with sodium and amyl alcohol with the formation of a conjugated triene derivative (LXXXII)



LXXXII

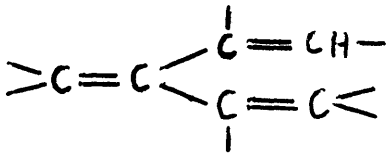


LXXXIII

whereas the compound (XXI) should under similar conditions yield the unsaturated alcohol (LXXXIII) or the amyl alcohol adduct thereof. Neither of the reactions took the expected course and the experimental results are discussed below.

That the product of the oxidation of iso- α-amyrenonyl acetate with selenium dioxide cannot have the structure suggested by Ruzicka (loc. cit.) is already clearly indicated by the considerable difference in the ultra-violet absorption spectra of starting material and product. iso- α-Amyradienonyl acetate shows an absorption maximum at λ_{max} = 2350A (ε_{max} = 16 000) which suggests the presence of a conjugated system of two carbon - carbon double bonds distributed between two rings. But

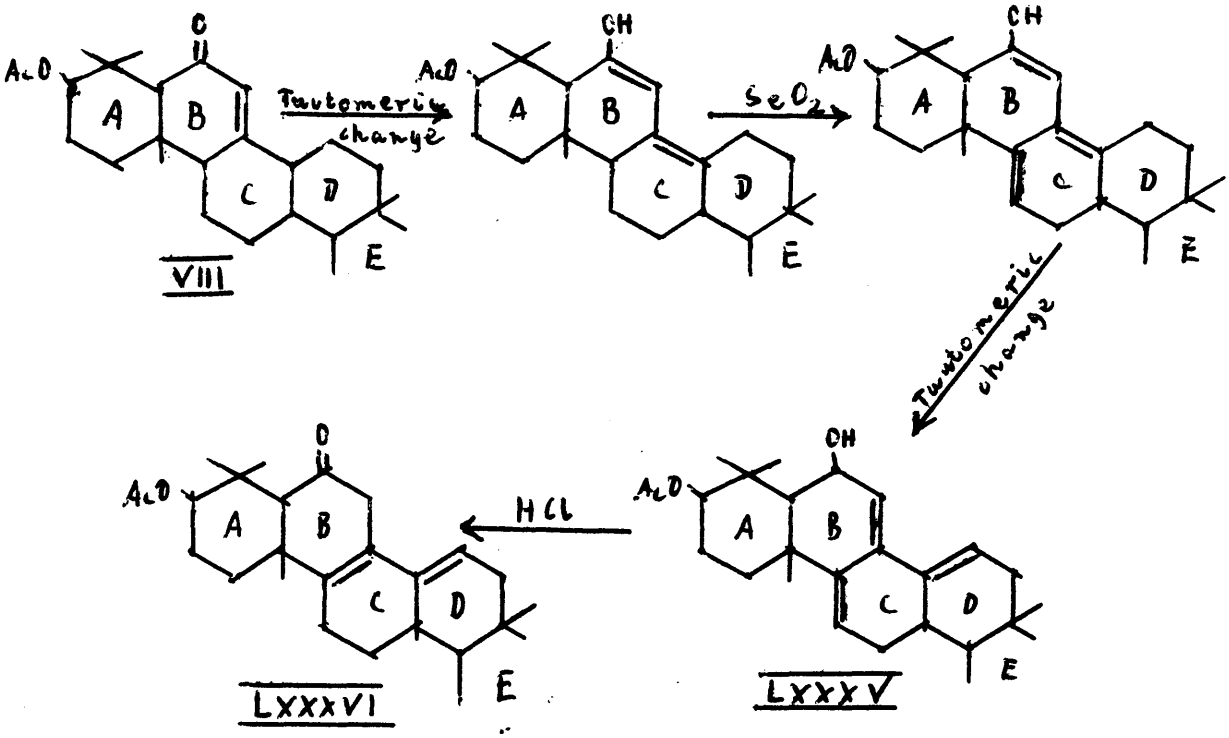
oxidation of one of the double bonds of iso- α-amyradienonyl acetate with osmium tetroxide yields an α-glycol which still shows a similar absorption spectrum (27). The presence of a further double bond is therefore necessary giving rise to the system (LXXXIV)



LXXXIV

Addition of one molecular proportion of hydrogen effects reduction to iso- α-amyrenonyl acetate (27), so that one of the double bonds of the triene system must have arisen from the keto-enol tautomerism of the carbonyl group. Addition of two molecular proportions of hydrogen effects reduction to α-amyradienyl acetate. This is further proof that the iso- α-amyrenonyl system is not present in iso- α-amyradienonyl acetate, since iso- α-amyrenonyl derivatives cannot be hydrogenated even under the most drastic conditions. The isolation of the acid $C_{11}H_{18}O_2$ (p. 22) at a later stage of the degradations carried out by Ruzicka (loc. cit.), makes the location of the double bond oxidised by osmium tetroxide between carbon atoms #14 and #15 imperative. Ascribing formula (X) to α-amyrin, one may formulate the reaction leading to the formation of iso- α-amyradienonyl acetate as a

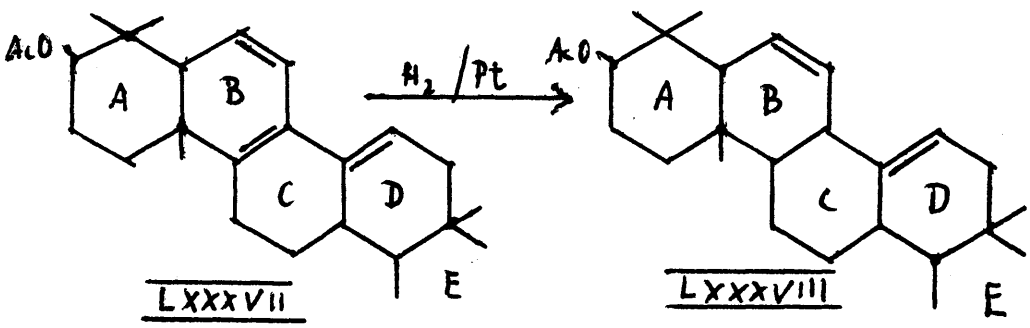
series of tautomeric changes as follows:



There is in any case no mechanism for the introduction of isolated double bonds by means of selenium dioxide. Generally the reaction proceeds by way of oxidation of a hydrogen atom attached to a reactive methylene group (α to a double bond) to hydroxyl and subsequent elimination of water with formation of a conjugated system of double bonds. Further evidence against Ruzicka's formulation of the compound was obtained by reduction with sodium and amyl alcohol.

iso- α-Amyradienonyl-II acetate is obtained from iso- α-amyradienonyl acetate by isomerisation with mineral

acid (27). It was found that it could be reduced with sodium and amyl alcohol to a highly unsaturated compound giving a brown colour reaction with tetranitromethane, and containing a conjugated system of two double bonds located in one ring - its ultra-violet absorption spectrum showed a strong absorption band (ε_{max} = 10 000) at λ_{max} = 2800A. The formation of this compound cannot be explained at all on the basis of Ruzicka's formulation of iso- α-amyradienonyl-II acetate (XXI, pp. 18, 73). But if the structure (LXXXVI) is assigned to iso-α-amyradienonyl-II acetate (v. scheme, p. 76), it is possible to formulate the reduction product as (LXXXVII).



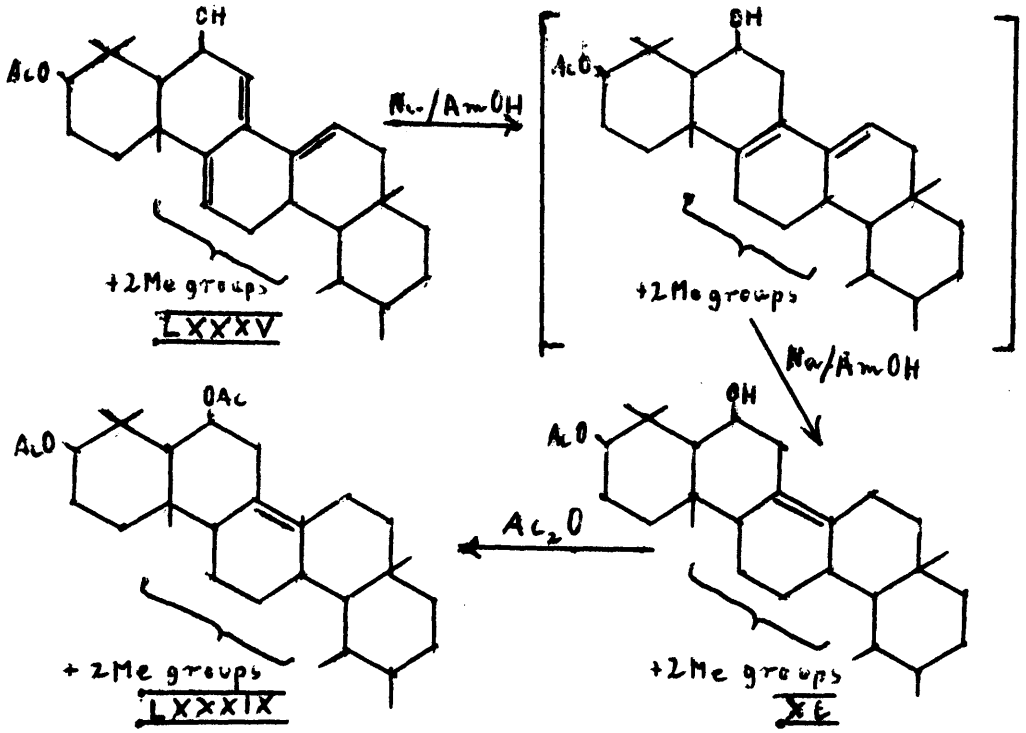
LXXXVII LXXXVIII

When this compound was treated with hydrogen in the presence of platinum catalyst, it absorbed one molecular proportion of hydrogen with the formation of a less unsaturated compound, which, however, still gave a deep yellow colour reaction with tetranitromethane and therefore probably contained more than one double bond. This product showed

no selective absorption in the ultra-violet region of the spectrum between 2200A and 3200A. It was thus the double bond between rings B and C which had been reduced with the formation of the compound (LXXXVIII).

<u>iso</u>- α-amyradienonyl acetate on the other hand was reduced by treatment with sodium and amyl alcohol to a compound which showed no selective absorption in the ultra-violet region of the spectrum, and to which the formula $C_{34}H_{54}O_4$ was assigned on the basis of its analysis. This compound developed a faint yellow colour with the tetra-nitromethane reagent, showing the presence of one double bond. In this reaction therefore, sodium and amyl alcohol has effected the reduction of at least one carbon - carbon double bond. Since this reagent, however, will only effect 1:4 reduction of conjugated carbon - carbon double bonds, Ruzicka's formulation of <u>iso</u>- α-amyradienonyl acetate as (XX, pp. 18, 73) is clearly impossible, since this does not contain a conjugated system of carbon - carbon double bonds. Here, again, the reaction may readily be explained by assigning the formula (LXXXV, scheme, p. 76) to <u>iso</u>- α-amyradienonyl acetate. Successive 1:4-reductions of the conjugated systems proceed as outlined below and in the final product the hydroxyl group can no longer

revert by a simple tautomeric change to a carbonyl group. Dehydration, too, is no longer favoured by the presence of a double bond in the same ring and the compound is therefore readily acetylated in the next stage of the reaction to yield the di-acetate (LXXXIX).

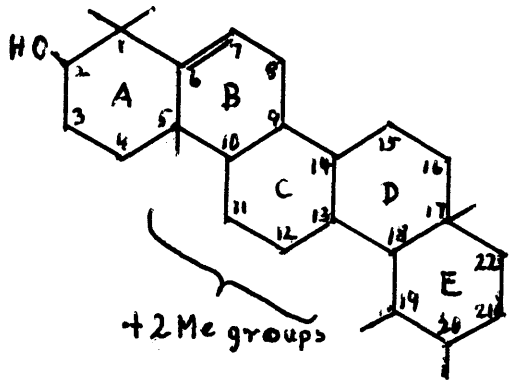


iso- α-Amyradienonyl acetate (LXXXV, i.e.: α-amyratrienediol mono-acetate) cannot be further acetylated with ketene, but apparently the presence of a double bond in the same ring prevents this. Thus α-amyrenonol-II and iso- α-amyrenonol (p. 31 ff) must exist in partially enolic form, since isomerisation with alkali is possible. Nevertheless, neither of the compounds can be induced to form an enol-acetate even under the most drastic conditions,

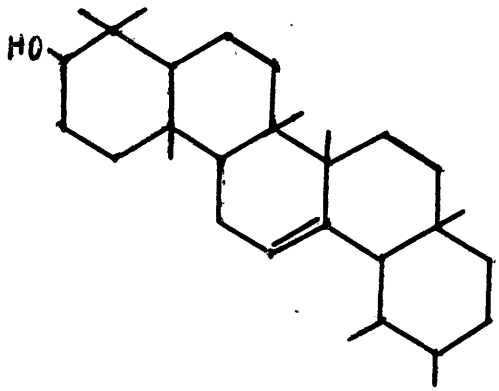
whereas α-amyranonyl acetate (VII, p. 9) which does not contain this double bond, readily forms an enol-acetate. The double bond present in (LXXXV) would appear to exert a similar inhibiting influence; in the reduction product (XC) this double bond in ring B is no longer present and acetylation proceeds in a normal manner.

CONCLUSION

The experimental results have led to the proposal of the structure

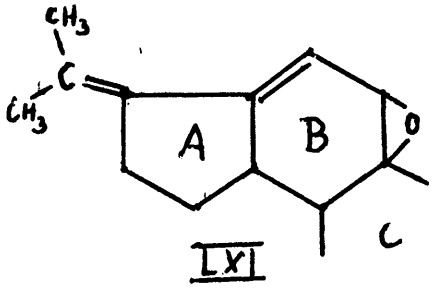


for α-amyrin in preference to the structure



proposed by L. Ruzicka (26, 27, 28). Particular attention is drawn in this connexion to the <u>cis</u>- α-decalone to <u>trans</u>- α-decalone type of isomerism which certain oxidation products of α-amyrin undergo (p. 31 ff) and to the results of the reduction processes applied to derivatives of α-amyrin obtained by the oxidation of <u>iso</u>-α-amyrenonyl acetate with selenium dioxide (p. 73 ff).

It must be pointed out that this new formulation (X) of α-amyrin, though accounting very well for all the experimental results obtained by the present author, is not much more satisfactory than Ruzicka's formulation (XIII) in accounting for the experimental results obtained by other investigators. It makes necessary the adoption of formula (LXI, p. 57)



LXI

for α-amyradienone-I, to account for the ultra-violet absorption spectrum of the compound, though there is no experimental evidence in its favour. Furthermore it leaves the position of two of the angular methyl groups undecided. The isolation of sapotalene on dehydrogenation of α-amyrin suggests the presence of an angular methyl group at C_{14}. Migration of this group to C_8 would, however, be required during the oxidation of iso-α-amyrenonyl acetate with selenium dioxide, if the postulated reaction mechanism (p. 76) is correct; but the same is true of Ruzicka's formulation of the changes (p. 23). In any case the latter accounts for neither the spectrographic evidence nor the products of reduction (p. 73 ff).

In view of this evidence the present author favours a structure of α-amyrin in which no angular methyl group is located at C_{14}.

The most recent degradative oxidations carried out by L. Ruzicka (p. 24) have not been discussed in detail. In their present formulation they are quite irreconcileable with the evidence presented in this dissertation. The reinvestigation of these reactions is of prime importance though it has not been possible to undertake this work.

It has also been shown that the Malaprade reagent (periodic acid) is not as quantitative in its action on cyclic α-ketols as it has been shown to be in the case of similar aliphatic compounds. This has led to some unusual results which have, in part, been elucidated.

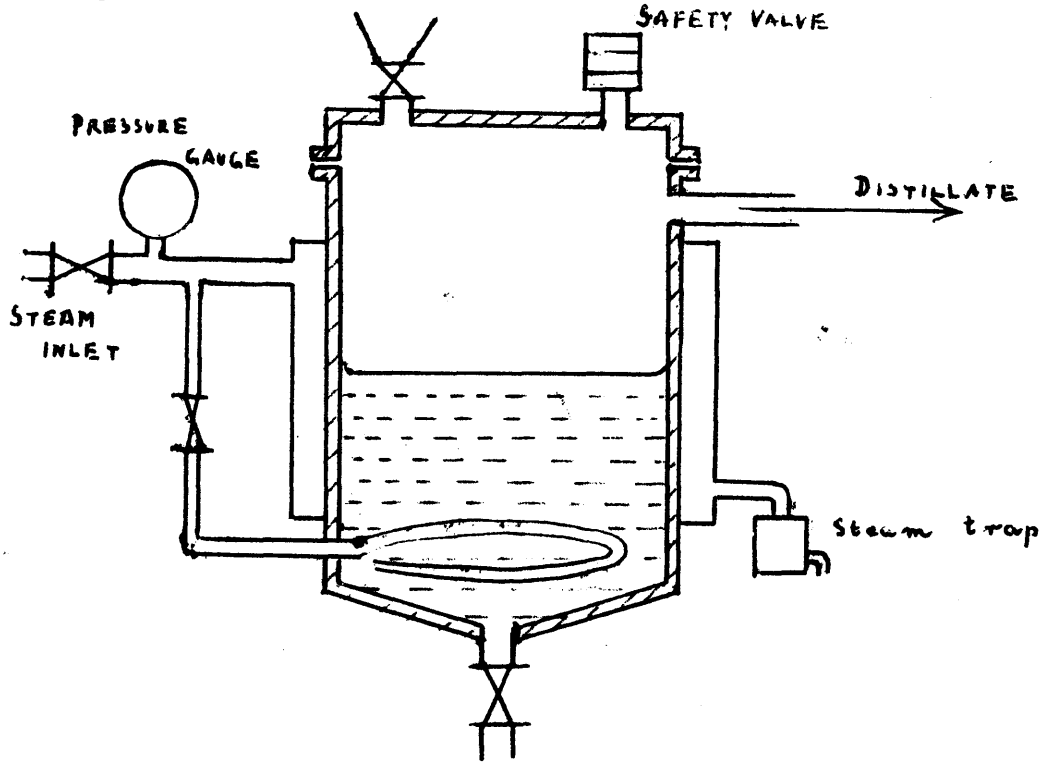
Finally it must be emphasised that research progress in this field must necessarily be slow until ample supplies of pure α-amyrin and its derivatives are available.

EXPERIMENTAL

N.B. All melting points are uncorrected for exposed stem. All optical rotations are determined in chloroform solution, all spectrographic data in ethanol solution. Micro-analyses were executed by Miss A.J. Henderson and Mr. W. McCorkindale, The Royal Technical College, Glasgow, and Messrs. Weiler & Strauss, Oxford. Spectrographic determinations were carried out by Miss J.N. Black.

The Isolation of Mixed Amyrins:

a) Removal of volatile oils:- Elemi resin, in 15 lb batches, was packed into a steam jacketed still of the design shown.



Water (4 l) was added and steam at a pressure of 15lbs/in^2 admitted into the jacket. When the contents of the still reached a temperature of approximately 80°C, a slow stream of live steam was passed through the mass. The distillate initially contained up to 10% of volatile oil, but this proportion rapidly decreased to 0.5% after four hours (10 l of distillate). Steam distillation was, however,

continued for a further 48 hours during which the proportion of oil in the distillate remained constant at 0.2% - 0.3%. The later fractions of the oil solidified on cooling. The solid volatile matter was identified as a mixture of elemol and elemicin (G. Silverstone, Priv. Com.). The contents of the still were then allowed to cool to room temperature and the solid residue chipped out. Any of the material which could not be removed by mechanical means was dissolved out with boiling chloroform.

Yield:- 5kg/batch

b) Removal of resin acids:- The crude mixed amyrins were finely powdered in a mortar and the powder (2kg) dissolved in methylated ether (5 l). The ethereal solution was thriced washed with 10% caustic soda solution (3 l) several times with water, once with 10% hydrochloric acid (3 l), twice more with water, and dried over anhydrous sodium sulphate. The ether was the removed by evaporation and the solid residue refluxed with methylated spirits (3 l for eight hours. At the end of this perios all the material had passed into solution and this was poured into a beaker. After two days at room temperature the contents of the vessel had completley solidified and the solid, crystalline mixed amyrins were collected on a filter with suction and thoroughly washed with cold

methylated spirits. The material was dried at 100°C, ground and redried.

Yield:- 600g; m.p. 150° - 160°C

α-Amyrin Benzoate:

Mixed amyrins (250 g.) were dissolved in dry, redistilled pyridine (150 c.c.) and the solution heated on the steam bath with stirring. Benzoyl chloride (175 c.c.) was added gradually and heating and stirring continued for six hours. The reaction mixture was then allowed to cool and dissolved in benzene (750 c.c.) and dilute (10%) hydrochloric acid (1 l.). The benzene solution was thoroughly washed with further quantities of dilute hydrochloric acid until free from pyridine. It was then vigorously shaken with an aqueous (10%) solution of caustic soda (2 x 500 c.c.), washed with water and superficially dried over anhydrous sodium sulphate. The solution was then concentrated to a thick oil and diluted with boiling ethanol until a permanent turbidity was reached. It was then allowed to crystallise and the solid product collected on a filter and washed with methylated spirits until relativley colourless. After drying at 100°, the material was ground and shaken with 250 c.c. portions of ether until the clearing point (i.e.: the point of complete fusion) of the material had reached 210°. The

ether washings were evaporated to dryness and the solid residue recrystallised by dissolving in the minimum of benzene at the boiling point and adding twice the volume of ethanol. For a satisfactory separation of the two isomers, α - and β-amyrin, this first crystallisation is of prime importance. Conditions must be so adjusted (by concentration and/or addition of benzene) that crystallisation commences at the surface, the prismatic crystals settling to the bottom of the vessel only after they have reached a certain size. If crystals appear in the form of needles, the material must be redissolved. If these conditions are observed one further crystallisation from the same solvent will generally give the pure α-amyrin benzoate. In all cases filtration must be carried out as soon a s the solution has cooled to room temperature and the supernatant liquor should first be decanted without disturbing the solid mass of crystals which should then be washed with a small amount of a solution of benzene (50 c.c.) in ethanol (200 c.c.) before filtration.

Yield:- 70 g. - 110 g. (varying from batch to batch)

m.p. 192° - 194°C

β-Amyrin Benzoate:

β-Amyrin benzoate is readily obtained by recrystallisation of the solid residue after the extraction of α-amyrin with ether from the mixed benzoylation product. Three recrystallisations from benzene - acetone give pure β-amyrin benzoate in scintillating plates.

Yield:- 30 g. - 45 g. (varying from batch to batch)

m.p. 235° - 236°C

α-Amyrin:

α-Amyrin benzoate was hydrolysed with alcoholic potassium hydroxide according to the method described by Newbold (M.Sc. Thesis, Manchester 1944, 146).

Yield:- 94% crude, m.p. 178° - 180°C

1- α-Amyradiene:

α-Amyrin (45 g.) was dissolved in dry benzene (250 c.c.) and the solution shaken with phosphorus pentoxide (85 g.). Agitation must be sufficiently vigorous to keep the pentoxide in fine suspension and a reciprocating shaker was found to be the only type satisfactory for the purpose. Shaking was continued for twenty-four hours and the reaction mixture then extracted with water and well washed. The benzene solution was then concentrated to ca. 60 c.c. and a little ether added.

When shaking had not been sufficiently vigorous a non-crystallisable syrup was obtained at this stage and could not be purified under any circumstances. At the end of a successful preparation 1- α-Amyradiene crystallised in prisms.

Yield:- 18 g.; m.p. 193°C

$[\alpha]_D^{Mb} = -112^\circ$ (c = 0.6)

Attempted oxidations of 1- α-Amyradiene:

a) Using osmium tetroxide:- 1- α-Amyradiene (2.4 g.) was dissolved in dry redistilled ether (200 c.c.) (minimum amount) and osmium tetroxide (3 g. = 2 Mol) added to the solution, which then darkened rapidly. The reaction mixture was kept at room temperature for six days and the ether then evapprated. The residue was dissolved in benzene (50 c.c.) and ethanol (25 c.c.), and shaken at 20° with a solution of mannitol (7.4 g.) and potassium hydroxide (4.6 g.) in water (11 c.c.) and ethanol (23 c.c.) for two hours. The reaction mixture was then well extracted with benzene (4 x 50 c.c.), the benzene solution washed with water, dried over anhydrous sodium sulphate and the benzene removed in vacuo. The solid residue crystallised from benzene - ether as prisms and was

recrystallised several times from the same solvent.

Yield:- 2.23 g.; m.p. 193°C undepressed on admixture of an authentic specimen of 1- α-amyradiene.

$[\alpha]_D^{16} = -114^\circ$ (c = 0.85)

b) Using potassium permanganate:- 1- α-Amyradiene (1 g.) was dissolved in carbon tetrachloride (70 c.c.) and the solution shaken with aqueous potassium permanganate (98 c.c. N/10, 2 Oxygen atoms/Mol) and 10% sulphuric acid (10 c.c.) for twenty-four hours at room temperature. At the end of this period the residual permanganate was found to be equivalent to 8 c.c. N/10. The precipitated manganese dioxide was removed by filtration, the aqueous solution further extracted with carbon tetrachloride and the combined carbon tetrachloride solutions washed with water and caustic soda solution (10%). The solvent was removed by evaporation and the solid residue recrystallised several times from benzene - alcohol.

Yield:- 730 mg.; m.p. 202° - 203°C

m.p. on admixture of a specimen of 1- α-amyradiene (m.p. 193°C) = 195° - 197°C

$[\alpha]_D^{16} = -92^\circ$ (c = 0.8)

Found:- C = 87.0%, 86.4%; H = 11.6%, 11.5%

$C_{30}H_{48}$ (60%), $C_{30}H_{48}O$ (40%) requs. C = 86.9%; H = 11.6%

The Action of Bromine on 1- α-Amyradiene:

1- α-Amyradiene (1 g.) was dissolved in carbon tetrachloride (70 c.c.) and a solution of bromine (0.8 g.) in glacial acetic acid (4 c.c.) and carbon tetrachloride (15 c.c.) added at room temperature. Rapid evolution of hydrogen bromide began after the addition of 5 c.c. of the reagent solution and the reaction mixture was finally kept at room temperature for sixteen hours. The solvent and the slight remaining excess of reagent were removed in vacuo. The resinous residue was dissolved in ether (100 c.c.) and the solution washed with water and alkali. It was dried over anhydrous sodium sulphate, the ether removed by evaporation and the resinous residue dissolved in dry petrol and chromatographed on alumina (Merck).

Fraction	Solvent	wt. mat. eluted	Remarks
1.	150 c.c. petroleum	55 mg.	resin
2.	" " "	300 "	"
3.	" " "	105 "	"
4.	" " "	45 "	crystals (from EtOH) m.p. 106° - 111°C
5.	" " "	130 "	crystals (from EtOH) m.p. 110° - 113°C
6.	" " "	30 "	crystals (from EtOH) m.p. 109° - 113°C
7.	" " "	negligible	
8. - 12.	benzene (700 c.c.) then acetone (100 c.c.)	325 mg.	resin
		1070 mg.	
wt. resin before chromatog.:-		960 "	

Fractions 4. - 6. were combined and recrystallised several times from methanol.

Yield:- 120 mg.; m.p. 116°C

$[\alpha]_D^{15} = +33.5°$ (c = 1.1)

Found:- C = 82.1%; H = 11.27%

$C_{30}H_{48}O_2$ requs.:- C = 81.8%; H = 10.91%

Attempted Acetylation of the compound m.p. 116°C

The compound (60 mg.) was dissolved in dry pyridine (2 c.c.) and acetic anhydride (1 c.c.). The reaction mixture was heated on the steam bath for twenty minutes, allowed to cool and poured into ice-water (50 c.c.). The precipitated solid was collected on a filter and recrystallised from methanol.

Yield:- 45 mg.; m.p. 116°C undepressed on admixture of a specimen of the starting material.

Reaction of the compound m.p. 116°C with ketonic reagents:

The compound did not react with 2:4-dinitrophenylhydrazine nor with semicarbazide hydrochloride.

The Action of N-bromosuccinimide on 1-α-Amyradiene:

1-α-Amyradiene (1 g.) was dissolved in dry carbon tetrachloride (50 c.c.) and the solution refluxed with freshly prepared N-bromosuccinimide (1.1 g.) for three hours. At the end of this time most of the reagent had

been debrominated and the succinimide was removed by filtration. The cold solution was then washed successively with 1% sodium hydroxide solution, water and N sulphuric acid, and dried over anhydrous sodium sulphate. The solvent was removed in vacuo and the resinous residue dissolved in dry light petroleum (b.p. 40° - 60°) and chromatographed. The crystalline material obtained from the column (230 mg.) was recrystallised several times from methanol.

Yield:- 165 mg.; m.p. 116°C undepressed on admixture of a specimen obtained by the action of bromine on 1- α -amyradiene.

$[\alpha]_D^{15} = +31.2^\circ$ (c = 1.2)

α-Amyradienyl Benzoate:

α-Amyrenonyl benzoate (15 g.) was dissolved in dry carbon tetrachloride (375 c.c.) and the solution refluxed with 80% N-bromosuccinimide (10 g.) for three hours. The succinimide produced was removed by filtration and the cold solution thoroughly washed with dilute alkali, sodium thiosulphate and water. It was dried over anhydrous sodium sulphate and the solvent removed in vacuo. The red residual syrup was refluxed with acetone (60 c.c.) for fifteen minutes and hot methanol added to the boiling solution

until there was a faint permanent turbidity. α-Amyradienyl benzoate crystallised from the dark brown solution in colourless plates and was twice recrystallised from ethanol.

Yield:- 11 g.; m.p. 175° - 176°C

α-Amyradienol:

α-Amyradienyl benzoate (2 g.) was refluxed with a solution of potassium hydroxide (5 g.) in water (3 c.c.) and ethanol (50 c.c.) for eight hours. The reaction mixture was then poured into water and the precipitated solid extracted with ether. The ethereal solution was washed free from alkali and alcohol and dried. The product was recrystallised twice from chloroform - petroleum.

Yield:- 1.7 g.; m.p. 166°C

N.B. This procedure was adopted for the hydrolysis of all esters of α-amyrin derivatives, except where otherwise stated.

α*Amyradienyl Acetate:

α-Amyradienol (1.5 g.) was dissolved in dry pyridine (10 c.c.) and acetic anhydride (5 c.c.). The reaction mixture was heated on the steam bath for fifteen minutes and then poured into ice-water (150 c.c.) with vigorous stirring. When all the ice had melted the precipitated

product was collected on a filter, washed free from pyridine and acetic acid and recrystallised several times from chloroform - methanol.

Yield:- 1.5 g.; m.p. 167° - 168°C

N.B. This procedure was adopted for the acetylation of all α-amyrin derivatives possessing a secondary hydroxyl group at C_2 in ring A, unless otherwise indicated.

α*Amyradienyl Acetate:

α-Amyrin acetate (10 g.) was treated with N-bromosuccinimide as described above. The product was recrystallised from ethanol.

Yield:- 6.2 g.; m.p. 167° - 168°C undepressed on admixture of a specimen prepared by acetylation of α-amyradienol.

Attempted Oxidations of α-Amyradienyl Acetate:

a) Using Mercuric Acetate:- α-Amyradienyl acetate (2 g.) was dissolved in ethanol (100 c.c.) and to the boiling solution a solution of mercuric acetate (4 g.) in ethanol (40 c.c.) and glacial acetic acid (10 c.c.) was rapidly added. The reaction mixture was refluxed for four hours and filtered hot. The material which crystallised from the solution on cooling was recrystallised

several times from ethyl aceate until free from unreacted mercuric acetate.

Yield:- 1.4 g.; m.p. 166° - 168°C undepressed on admixture of an authentic specimen of α-amyradienyl acetate.

b)Using osmium tetroxide:- α-Amyradienyl acetate (1.6 g.) was dissolved in dry absolute ether (50 c.c.) and osmium tetroxide (1 g. = 1 Mol) added to the cold solution. The reaction mixture was kept at room temperature in absence of light for 14 days and the ether then evaporated. The residue was dissolved in benzene (40 c.c.) and ethanol (20 c.c.), and shaken at 20° with a solution of mannitol (5g.) and potassium hydroxide (3.2 g.) in water (8 c.c.) and ethanol (16 c.c.) for two hours. The reaction mixture was then well extracted with benzene (5 x 30 c.c.), the benzene solution washed with water, dried over anhydrous sodium sulphate and the benzene removed in vacuo and the residue recrystallised from chloroform - methanol.

Yield:- 1 g.; m.p. 164° - 166°C undepressed on admixture of an authentic specimen of α-amyradienol.

Perbenzoic Acid:

Perbenzoic acid was prepared according to the method

described in "Organic Syntheses" (XVI, p. 8).

Yield:- 72% theor.

iso- α-Amyrenonyl Acetate and α-Amyrenonyl Acetate:

α-Amyradienyl acetate (1.6 g.) was dissolved in a solution of perbenzoic acid (o.6 N) in dry chloroform (50 c.c.) and the reaction mixture maintained at a temperature of 2° for 14 days. The solution was then washed with sodium carbonate and water and dried over anhydrous sodium sulphate. The resinous residue obtained on removal of the solvent could not be crystallised and was chromatographed on alumina (Brockmann, 40 g.)

Fraction	Solvent	wt. mat. eluted	Remarks
1. - 7.	2.000 l. petroleum	negligible	
8.	0.250 " benzene	230 mg.	crystals (MeOH) m.p. 274° - 278°C
9.	" " "	250 "	Crystals (MeOH) m.p. 276° - 278°C
10.	" " "	40 "	crystals (MeOH) m.p. 272° - 277°C
11. - 12,	0.500 " "	50 "	resin
13.	0.250 " "	110 "	crystals (MeOH) m.p. 270° - 274°C
14.	0.250 " "	30 "	crystals (MeOH) m.p. 268° - 272°C
15. - 19.	1.250 " "	400 "	resin (non-cryst.)
20. - 22.	0.300 " acetone	230 "	resin
		1340 mg.	

wt, resin before chromatography: 1420 mg.

Fractions 8. - 10. were combined and recrystallised several times from chloroform - methanol.

Yield:- 300 mg.; m.p. 282° - 283°C undepressed on admixture of an authentic specimen of iso- α-amyrenonyl acetate.

$[\alpha]_D^{16}$ = +89° (c = 1.3)

Fractions 13. and 14. were combined and recrystallised several times from chloroform - methanol.

Yield:- 90 mg.; m.p. 275° - 276°C undepressed on admixture of an authentic specimen of α-amyrenonyl acetate. $[\alpha]_D^{16}$ = +96° (c = 0.7)

iso- α-Amyrenonyl Acetate:

iso- α-Amyrenonyl acetate was prepared according to the method of Seymour, Sharples and Spring (J.C.S., 1939, 1075)

Yield:- 45% theor.; m.p. 283° - 284°C

$[\alpha]_D^{16}$ = +87° (c = 2.1)

α-Amyrenonyl Acetate:

α-Amyrin acetate (1 g.) was oxidised with chromic acid according to the method of Spring and Vickerstaff (J.C.S. 1937, 249). The product was recrystallised from chloroform - methanol.

Yield:- 600 mg.; m.p. 276°C

$[\alpha]_D^{15}$ = +98° (c = 1.7)

α-Amyrenonyl-II Benzoate and α-Amyrenonyl Benzoate:

α-Amyradienyl benzoate (5 g.) was dissolved in glacial acetic acid (225 c.c.). The solution was heated on the steam bath and 50 c.c. of a mixture of hydrogen peroxide (30%, 40 c.c.) and glacial acetic acid (40 c.c.) added with vigorous stirring during twenty minutes. Stirring and heating was continued until all the material precipitated by the oxidising solution had redissolved (2 hours) and the remainder of the hydrogen peroxide solution then added. The hot solution was stirred for a further hour and then diluted with hot water to faint permanent turbidity. It was then allowed to cool, the crystalline product collected, washed with a little methanol to facilitate wetting and well with water and then dried at 100° in air. The mother liquors were largely diluted with water and the precipitated solid collected, washed with water and dried in vacuo.

The crystalline material was dissolved in chloroform (3 c.c.) and ethanol (200 c.c.) and the solution concentrated at atmospheric pressure until crystallisation set in at the boiling point. The crystalline material was removed from the boiling solution by filtration and the filtrate further concentrated. The first three fractions of crystals thus obtained were combined and

recrystallised from chloroform- methanol. α-Amyrenonyl-II benzoate crystallised as large needles.

Yield:- 2.1 g.; m.p. 242°C

$[\alpha]_D^{18} = +21°$ (c = 3.0)

Ultra-violet absorption spectrum:-

Rising absorption from λ = 2650A to λ = 2300A

Extinction coefficient:- ε_{max} = 18 000

Found:- C = 81.14%, 81.5%; H = 9.8%, 9.8%

$C_{37}H_{52}O_3$ requires:- C = 81.6%; H = 9.6%

The material precipitated from the acetic acid oxidation mother liquors was dissolved in the ethanolic filtrate from the fractional crystallisation. Concentration and crystallisation at the boiling point gave two fractions which were combined and recrystallised from chloroform - methanol. α-Amyrenonyl benzoate crystallised in well-formed plates.

Yield:- 800 mg.; m.p. 275° - 276°C undepressed on admixture of an authentic specimen of -amyrenonyl benzoate.

$[\alpha]_D^{18} = +108°C$ (c = 4.4)

Ultra-violet absorption spectrum:-

Rising absorption from λ = 2650A to λ = 2300A

Extinction coefficient:- ε_{max} = 18 000

Found:- C = 81.32%; H = 9.55%

$C_{37}H_{52}O_3$ requires:- C = 81.6%; H = 9.6%

α-Amyrenonyl Benzoate:

α-Amyrin benzoate (1.5 g.) was oxidised with chromic acid according to the method of Spring and Vickerstaff (J.C.S. 1937, 249). The reaction product was isolated and crystallised from chloroform - methanol in the form of plates.

Yield:- 900 mg.; m.p. 274° - 275°C

$[\alpha]_D^{18} = +107.2°$ (c = 3.3)

α-Amyrenonyl Acetate and α-Amyrenonyl-II Acetate:

α-Amyradienyl acetate (5 g.) was oxidised with hydrogen peroxide by the method employed for the oxidation of α-amyradienyl benzoate (p. 99). The first four fractions obtained by fractional crystallisation at the boiling point of ethanol were combined and recrystallised from chloroform - methanol. α-Amyrenonyl acetate crystallised in the form of large plates.

Yield:- 3.6 g.; m.p. 276°C undepressed on admixture of an authentic specimen of α-amyrenonyl acetate.

$[\alpha]_D^{18} = +96°$ (c = 1.7)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2520A

Extinction coefficient:- ϵ_{max} = 12 000

Found:- C = 79.9%; H = 10.2%

$C_{32}H_{50}O_3$ requires:- C = 79.6%; H = 10.45%

The amorphous material obtained from the acetic acid reaction mother liquors was dissolved in the ethanolic mother liquors from the fractional crystallisation and the solution concentrated to very small bulk. A crystalline product separated after several days at room temperature, and was recrystallised from chloroform - methanol.

Yield:- 700 mg.; m.p. 193°C

This represents a constant composition mixture of α-amyrenonyl acetate and α-amyrenonyl-II acetate. It was dissolved in boiling methanol and the solution concentrated until crystallisation set in. The first crystalline fraction isolated at the boiling point of methanol melted indistinctly above 220° and was discarded (110 mg.). The next two fractions were isolated and recrystallised from chloroform - methanol. α-Amyrenonyl-II acetate crystallised as needles.

Yield:- 350 mg.; m.p. 200° - 202°C undepressed on admixture of a specimen obtained by acetylation of α-amyrenonol-II (v. inf.)

$[\alpha]_D^{16} = +14^\circ$ (c = 0.9)

Found:- C = 79.3%; H = 10.8%

$C_{32}H_{50}O_3$ requires:- C = 79.6%; H = 10.45%

α-Amyrenonol-II

a) From α-Amyrenonyl-II Acetate:- α-amyrenonyl-II acetate (250 mg.) was dissolved in benzene (5 c.c.) and a solution of potassium hydroxide (250 mg.) in water (2 c.c.) and ethanol (20 c.c.) added. The reaction mixture was refluxed for 100 minutes and poured into ice-water (150 c.c.) containing concentrated sulphuric acid (0.2 c.c.). The organic material was extracted with ether, the ethereal solution purified and dried, and the ether removed by evappration. The residue was recrystallised from methanol and from chloroform - petroleum (b.p. 60° - 80°). α-Amyrenonol-II crystallised in the form of long feathery needles.

Yield:- 150 mg.; m.p. 211° - 213°C

$[\alpha]_D^{lb} = -16°$ (c = 1.8)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2500A

Extinction coefficient ε_{max} = 12 000

b) From α-Amyrenonyl-II Benzoate:- α-Amyrenonyl-II benzoate was hydrolysed in the manner described under a). The product was recrystallised from chloroform -petroleum (b.p. 60° - 80°). The product did not differ from the α-amyrenonol-II prepared by hydrolysis of α-amyrenonyl-II

acetate.

m.p. 211° - 212°C undepressed on admixture of an authentic specimen of α-amyrenonol-II obtained by hydrolysis of α-amyrenonyl-II acetate.

$[\alpha]_D^{16}$ = -15.5° (c = 3.4)

Found:- C = 81.9%; H = 10.7%

$C_{30}H_{48}O_2$ requires:- C = 81.8%; H = 10.7%

α-Amyrenonyl-II Acetate:-

α-Amyrenonol-II (1 g.) obtained by hydrolysis of α-amyrenonyl-II benzoate, was acetylated (Note, p.95). The product crystallised as needles from chloroform - methanol.

Yield:- 950mg.; m.p. 200° - 201°C

$[\alpha]_D^{16}$ = +11° (c = 1.8)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2500A

Extinction coefficient ε_{max} = 11 000

α-Amyradienyl Acetate:

a) α-Amyrenonyl-II benzoate (3 g.) was dissolved in dry technical amyl alcohol (120 c.c.) at the boiling point and sodium (7.5 g.) added during twenty minutes. When all the sodium had dissolved, more amyl alcohol (20 c.c.) was added and refluxing continued for 40 minutes.

The alcoholic solution was washed once with water (200 c.c.) and the amyl alcohol then removed by steam distillation. The precipitated solid was extracted with ether from its aqueous suspension, the ethereal solution purified and dried and the ether removed by evaporation. The residue was refluxed with acetic anhydride (20 c.c.) for one hour. The reaction mixture was poured onto crushed ice with vigorous stirring and allowed to stand over night. The solid precipitate was collected on the filter and recrystallised from chloroform - methanol several times.

Yield:- 1.6 g.; m.p. 168°C undepressed on admixture of an authentic specimen of α-amyradienyl acetate.

$[\alpha]_D^{19} = +313^\circ$ (c = 1.6)

Found:- C = 81.9%; H = 10.5%

$C_{32}H_{50}O_2$ requires:- C = 82.3%; H = 10.7%

b) α-Amyrenonyl-II acetate (300 mg.) was reduced with sodium and amyl alcohol in a similar manner and the product after acetylation recrystallised from chloroform - methanol.

Yield:- 200 mg.; m.p. 166°C undepressed on admixture of an authentic specimen of α-amyradienyl acetate.

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2830A

Extinction coefficient ϵ_{max} = 9 000

c) α-Amyrenonyl benzoate (1 g., prepared by oxidation of α-amyradienyl benzoate with hydrogen peroxide) was reduced with sodium and amyl alcohol in a similar manner and the product acetylated.

Yield:- 0.5 g.; m.p. 168° - 169°C undepressed on admixture of an authentic specimen of α-amyradienyl acetate.

$[\alpha]_D^{28}$ = +318° (c = 1.2)

Attempted Isomerisation of α-Amyrenonyl-II Acetate:

The acetate (120 mg.) was dissolved in chloroform (0.5 c.c.) and glacial acetic acid (2 c.c.) and the solution treated for one hour at 40° with concentrated hydrochloric acid (0.1 c.c.). The reaction mixture was then poured into water and the product extracted with ether. The ethereal solution was freed from acid and dried and the ether removed by distillation. The residue was recrystallised several times from chloroform - methanol.

Yield:- 88 mg.; m.p. 200° - 201°C undepressed on admixture of a specimen of α-amyrenonyl-II acetate.

Attempted Isomerisation of α-Amyrenonyl Acetate:

α-Amyrenonyl acetate (120 mg.) was treated with hydrochloric acid in the manner described above. The product was recrystallised several times from chloroform -

Attempted Isomerisation of iso- α-Amyrenonol:

iso- α-Amyrenonol (200 mg.) was dissolved in ethanol (40 c.c.) and the solution refluxed with concentrated hydrochloric acid (10 c.c.) for five hours. The product crystallised from the reaction mixture on cooling.

Yield:- 170 mg.; m.p. 240°C undepressed on admixture of an authentic specimen of iso- α-amyrenonol.

Attempted formation of the enol-acetate of α-amyrenonol-II:

α*Amyrenonyl-II acetate (400 mg.) was dissolved in acetic anhydride (12 c.c.) and the solution refluxed in the presence of anhydrous sodium acetate (400 mg., freshly fused) for twenty-four hours. The deep brown reaction mixture was poured into water and t he product extracted with ether. The ethereal solution was purified and the ether evaporated. The residue was recrystallised several times from chloroform - methanol.

Yield:- 150 mg.; m.p. 201°C undepressed on admixture of a specimen of α-amyrenonyl-II acetate.

Attempted formation of the enol-acetate of α-amyrenonol:

a) α-Amyrenonyl acetate (250 mg.) was treated with acetic anhydride in the presence of anhydrous sodium acetate as described in the previous experiment. The product was isolated by extraction with ether and recrystallised

from chloroform - methanol.

Yield:- 150 mg.; m.p. 276°C undepressed on admixture of a specimen of α-amyrenonyl acetate.

b) α-Amyrenonyl acetate (600 mg.) was dissolved in pyridine (6 c.c.) and acetic anhydride (4 c.c.) and the solution was refluxed for twenty-four hours. The reaction mixture was cooled and poured onto ice with vigorous stirring. The solid precipitate was removed by filtration, dried and recrystallised from chloroform - methanol.

Yield:- 320 mg.; m.p. 276°C undepressed on admixture of a specimen of α-amyrenonyl acetate.

Attempted hydrogenation of iso- α-Amyrenonyl Acetate:

iso- α-Amyrenonyl acetate (2 g.) was dissolved in glacial acetic acid at 80° and the solution shaken in the presence of platinum oxide (100 mg.) in an atmosphere of hydrogen for several days, the temperature being maintained by the external application of steam. The volume of gas in the cold apparatus was measured before and after shaking.

Volume of hydrogen absorbed at N.T.P. = 20.32 c.c.

equivalent to the reduction of 103 mg. platinum oxide. The catalyst was removed by filtration and the glacial acetic acid removed by evaporation in vacuo at 40°.

The crystalline residue was recrystallised several times from chloroform - methanol.

Yield:- 1.72 g.; m.p. 283° - 285°C undepressed on admixture of an authentic specimen of iso- α-amyrenonyl acetate.

$[\alpha]_D^{16} = +85°$ (c = 0.9)

iso- α-Amyrenonyl Benzoate:

iso- α-Amyrenonyl benzoate was prepared according to the method of Seymour, Sharples and Spring (J.C.S. 1939, 1075).

Yield:- 4.7 g. from 10 g. α-amyrin benzoate; m.p. 211°C.

Attempted Oxidation of iso- α-Amyrenonyl Benzoate:

iso- α-Amyrenonyl benzoate (4 g.) was treated with hydrogen peroxide in the manner used for the oxidation of α-amyradienyl esters. Only unchanged iso- α-Amyrenonyl benzoate was recovered from the reaction mixture.

Yield:- 3.6 g.; m.p. 211° - 213°C undepressed on admixture of a specimen of iso- α-amyrenonyl benzoate.

α-Amyranonol-II:

α-Amyrenonol-II (600 mg.) was dissolved in glacial acetic acid (150 c.c.) and the solution shaken in the presence of platinum oxide (200 mg.) in an atmosphere of

hydrogen for six hours at room temperature. Absorption of hydrogen was complete after one hour.

Volume of hydrogen absorbed at N.T.P. = 73.2 c.c.

" " " required for

reduction of PtO_2 (200 mg.) " = 39.6 c.c.

Hydrogen used for reduction = 33.6

α-Amyrenonol-II absorbs 1.11 Mol of hydrogen on hydrogenation.

The catalyst was removed by filtration and the glacial acetic acid removed by evaporation in vacuo at 40°. The crystalline residue was recrystallised from chloroform - methanol. α-Amyranonol-II crystallised in the form of large hexagonal plates.

Yield:- 530 mg.; sintering at 244°C, m.p. 258° - 260°C.

$[\alpha]_D^{18} = +58.5^\circ$ (c = 2.0).

Found:- C = 81.9%; H = 11.58%

$C_{30}H_{50}O_2$ requires:- C = 81.5%; H = 11.32%

<u>α-Amyranonyl-II Acetate:</u>

<u>a) From α-Amyranonol-II:-</u> α-Amyranonol-II was acetylated (400 mg.), (Note, p. 95). α-Amyranonyl-II acetate crystallised from ethanol in the form of needles.

Yield:- 380 mg.; sintering at 206°C, m.p. 217°C

$[\alpha]_D^{16} = +59.3^\circ$ (c = 2.2)

b) From α-Amyrenonyl-II Acetate:- α-Amyrenonyl-II acetate (300 mg.) was dissolved in glacial acetic acid (250 c.c.) at 20°C and the solution shaken in the presence of platinum oxide (200 mg.) in an atmosphere of hydrogen for sixteen hours. α-Amyrenonyl-II acetate absorbed 1.20 Mol of hydrogen. The catalyst was removed by filtration and the acetic acid removed by evaporation in vacuo at 40°. α-Amyranonyl-II acetate was recrystallised from chloroform - ethanol in the form of needles.

Yield:- 280 mg.; m.p. 216°C undepressed on admixture of a specimen of α-amyranonyl-II acetate.

$[\alpha]_D^{18} = +59.5°$ (c = 1.8)

Found:- C = 79.5%; H = 11.04%

$C_{32}H_{52}O_3$ requires:- C = 79.3%; H = 10.74%

iso-α-Amyrenonol:

α-Amyrenonyl-II benzoate (3 g.) was dissolved in ethanol (140 c.c.) at the boiling point and a solution of potassium hydroxide (11 g.) in water (10 c.c.) and ethanol (20 c.c.) added. The reaction mixture was refluxed for six hours. A solution of potassium hydroxide (7 g.) in water (5 c.c.) and ethanol (20 c.c.) was then added and refluxing continued for eight hours. The reaction mixture was poured into water (1 l.) and the product

extracted with ether (6 x 60 c.c.). The ethereal solution was washed free from alkali and dried. The residue after removal of the ether by evaporation, was recrystallised from methanol and from chloroform - petroleum (b.p. 100° - 120°).

Yield:- 2.1 g.; m.p. 239°C undepressed on admixture of an authentic specimen of iso- α-amyrenonol.

$[\alpha]_D^{18}$ = +75.2° (c = 2.1)

iso- α-Amyrenonyl Acetate:-

iso- α-Amyrenonol (obtained from α-amyrenonyl-II benzoate) (500 mg.) was acetylated (Note, p. 95). The product was recrystallised from chloroform - methanol.

Yield:- 480 mg.; m.p. 285°C undepressed on admixture of a specimen of iso- α-amyrenonyl acetate (p. 98).

$[\alpha]_D^{18}$ = +83.6° (c = 3.0)

Irradiation of α-Amyradienol with visible light:

α-Amyradienol (500 mg.) and erythrosin (500 mg.) were dissolved in air-free methanol (100 c.c.) and the solution illuminated with two 150 W. incandescent tungsten filament lamps at a distance of 4 ins. for 4 days. The solution was then evaporated to dryness in vacuo and the residue extracted with benzene.. The solution was washed with alkali and water, the benzene dried and evaporated and the solid residue recrystallised from

chloroform - petroleum (b.p. 60° - 80°).

Yield:- 450 mg.; m.p. 164°C undepressed on admixture of an authentic specimen of α-amyradienol.

iso- α-Amyradienonyl-I acetate:

iso- α-Amyrenonyl acetate (1.5 g.) was dissolved in glacial acetic acid (stabilised, 170 c.c.) and the solution refluxed with selenium dioxide (4.5 g.) for 24 hours. The precipitated selenium was removed by filtration, the acetic acid solution concentrated at 35° in vacuo to 30 c.c., and poured into water. The product was extracted with ether and the ethereal solution washed with 10% caustic soda solution, water, potassium cyanide solution to remove colloidal selenium, and water, and dried. The residue after removal of the ether was recrystallised from chloroform- methanol. iso- α-Amyradienonyl acetate crystallised in the form of needles.

Yield:- 700 mg.; m.p. 222° - 223°C

$[\alpha]_D^{20} = +24°$ (c = 3.6)

Attempted acetylation of iso- α-Amyradienonyl-I Acetate:

iso- α-Amyradienonyl-I acetate (7 mg.) was dissolved in dry redistilled ether (3 c.c.). The solution was cooled to 0° and a slow stream of ketene passes through the solution at that temperature for ten minutes. The

reaction mixture was then diluted with chloroform (6 c.c.) and concentrated at atmospheric pressure to 4 c.c. It was then diluted with redistilled methanol (8 c.c.) and again concentrated at atmospheric pressure to 4 c.c. iso- α-Amyradienonyl-I acetate crystallised on cooling.

Yield:- 4 mg.; m.p. 223°C undepressed on admixture of an authentic specimen of iso- α-amyradienonyl-I acetate.

iso- α-Amyradienonyl-II Acetate:

iso- α-Amyradienonyl-II acetate (1.25 g.) was dissolved in glacial acetic acid (300 c.c.) and the solution saturated with dry hydrogen chloride. The reaction mixture was maintained at room temperature for 20 hours and the acetic acid then evaporated in vacuo at 42°. The crystalline residue was recrystallised from chloroform - methanol. iso-α-Amyradienonyl-II acetate crystallised in the form of needles.

Yield:- 1.1 g.; m.p. 254° - 255°C

$[\alpha]_D^{18} = +163°$ (c = 2.6)

Deoxy-iso- α-Amyradienonyl-II acetate:

iso- α-Amyradienonyl-II acetate (1 g.) was dissolved in dry redistilled amyl alcohol (40 c.c.). The solution was refluxed and sodium (2.5 g.) added in portions.

When the addition of metal was complete, the reaction mixture was refluxed for thirty minutes. Amyl alcohol (7 c.c.) was then added and refluxing continued for a further 40 minutes. The amyl alcohol solution was immediately poured into water (200 c.c.) and the mixture shaken vigorously. The aqueous layer was separated and discarded and the amyl alcohol removed from the product by steam distillation. The resinous product was extracted from the resulting aqueous suspension with ether and the ethereal solution washed, and dried over anhydrous sodium sulphate. The residue after removal of the ether was refluxed with acetic anhydride (20 c.c.) for two hours and the reaction mixture poured into ice-water with vigorous stirring. Stirring was continued for four hours and the suspended resin extracted with ether and washed with 10% sodium carbonate solution. The residue after removal of the solvent could not be crystallised. It was therefore dissolved in dry petroleum (b.p. 40° - 60°) and chromatographed on alumina (Spence, standardised after Brockmann, activity III - IV, 18 g.).

Fraction	Solvent	wt. mat. eluted	Remarks
1.	200 c.c. Petroleum	70 mg.	resin cryst. from methanol
2.	100 " "	120 "	crystals
3.	100 " "	40 "	resin, non-cryst.
4. - 8.	500 " "	50 "	" " "
9. - 11.	300 " Pet:Benz.; 4:1	negligible	
12. - 14.	200 " Pet:Chloroform; 4:1	90 mg.	resin cryst. from methanol
15. - 19.	500 " Pet:Chloroform; 4:1	30 "	resin, non-cryst.
20. - 21.	200 " Ethan:Chloroform; 1:4	80 "	" " "
22. - 26.	500 " Ethanol:Chloroform; 1:4	110 "	" " "
27.	150 " Acetone	60 "	" " "
		620 mg.	
wt. resin before chromatography:-		700 "	

Fractions 1. and 2. were combined and recrystallised from methanol.

Yield:- 130 mg.; m.p. 178° - 179°C

$[\alpha]_D^{16} = -693.0^\circ$ (c = 0.7)

Ultra-violet absorption spectrum:-

Absorption band at $\lambda_{max} = 2800A$

Extinction coefficient $\epsilon_{max} = 10\,000$

Found:- C = 82.1%; H = 10.1%

$C_{32}H_{48}O_2$ requires:- C = 82.7%; H = 10.33%

Fractions 12. - 14. were combined and recrystallised from chloroform - methanol.

Yield:- 40 mg.; m.p. 253°C undepressed on admixture of a specimen of iso- α-amyradienonyl-II acetate.

$[\alpha]_D^{18} = +170^\circ$ (c = 0.4)

iso- α-Amyradienyl Acetate:

Deoxy-iso- α-amyradienonyl-II acetate (53 mg.) was added at room temperature to a suspension of freshly reduced Adam's platinum catalyst (60 mg.) in glacial acetic acid (6 c.c.). The reaction mixture was shaken for three hours in an atmosphere of hydrogen, though absorption of hydrogen was complete after 40 minutes. Deoxy-iso- α-amyradienonyl-II acetate absorbed 1.15 Mol of hydrogen on hydrogenation. During part of the hydrogenation period the reaction mixture was maintained at a temperature of 80°, since the hydrogenation product was insoluble in glacial acetic acid and tended to crystallise. The solution was diluted with chloroform (10 c.c.) and the platinum removed by filtration. The chloroform was removed from the solution by fractional distillation at atmospheric pressure and the product crystallised from the residual acetic acid solution. iso- α-amyradienyl acetate crystallised in the form of

prisms. It was twice recrystallised from chloroform - methanol.

Yield:- 30 mg.; m.p. 241°C

$[\alpha]_D^{18} = +89°$ (c = 0.48)

Found:- C = 82.0%; H = 10.62%

$C_{32}H_{50}O_2$ requires:- C = 82.4%; H = 10.73%

iso- α-Amyrenediol Diacetate:

iso- α-Amyradienonyl-I acetate (750 mg.) was dissolved in dry, redistilled amyl alcohol (30 c.c.) and sodium (1.8 g.) added portion wise to the boiling solution. Amyl alcohol (5 c.c.) was added after thirty minutes and refluxing continued (with exclusion of moisture) for a further forty minutes. The solution was then immediately poured into water (150 c.c.) and the mixture shaken vigorously. The aqueous layer was separated and discarded and the amyl alcohol removed from the product by steam distillation. The resinous product was extracted from the resulting aqueous suspension with ether, and the ethereal solution washed, and dried over anhydrous sodium sulphate. The residue after removal of the ether was refluxed with acetic anhydride (15 c.c.) for two hours and the reaction mixture poured into ice-water with vigorous stirring. The mixture was allowed to stand over

night and the precipitated product collected on a filter. It was redissolved in ether (50 c.c.) and the solution washed with 10% sodium carbonate solution (80 c.c.) and water. The residue after removal of the ether could not be crystallised from any of the common organic solvents. It was therefore dissolved in dry petroleum (b.p. 40° - 60°) and chromatographed on alumina (Spence, standardised after Brockmann, activity III - IV, 12 g.).

Fraction	Solvent	wt. mat. eluted	Remarks
1.	100 c.c. petroleum	80 mg.	cryst., m.p. 180°
2.	" " "	90 "	" " "
3.	" " "	92 "	" " 182°
4.	" " "	60 "	" " 180°
5.	" " "	20 "	resin
6.	" " "	negligible	
7. - 10.	400 " "	25 mg.	resin, non-cryst.
11. - 14.	" " benzene	50 "	" " "
15.	150 " acetone	140 "	" " "
		557 mg.	

wt. resin before chromatography:- 615 mg.

Fractions 1. - 5. were combined and recrystallised

three times from chloroform - methanol.

Yield:- 230 mg.; m.p. 186° - 187°C

$[\alpha]_D^{18} = +58°$ (c = 1.4)

Found:- C = 77.4%, 77.7%; H = 10.40%, 10.47%

$C_{34}H_{54}O_4$ requires:- C = 77.6%; H = 10.27%

Acetoxy- α-Amyrenedione-II:

α-Amyradienol (1.08 g.) was dissolved in glacial acetic acid (19 c.c.) at 30°. To the warm solution, a solution of chromic anhydride (240 mg.) in slightly aqueous acetic acid (3 c.c.) was added with stirring. The temperature of the reaction mixture was maintained at 45° for 50 minutes and at 25° - 30° for eighteen hours. It was then poured into water and the precipitated product extracted with ether. The ethereal solution was freed from acid (there were no organic acids present), dried and evaporated. The residue was recrystallised from acetone - methanol and chloroform - ethanol. Acetoxy-α-amyrenedione-II crystallised as plates.

Yield:- 540 mg.; m.p. 319° - 321°C

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2520A

Extinction coefficient ε_{max} = 7 000

Found:- C = 77.4%; H = 10.10%

$C_{32}H_{48}O_4$ requires:- C = 77.4%; H = 9.67%

α-Amyradienone-V:

α*Amyradienol (20 g.) was dissolved in glacial acetic acid (250 c.c.) and the solution kept at 35°C during the addition of a solution of chromic anhydride (3.3 g.) in water (5 c.c.) and glacial acetic acid (45 c.c.). When all the oxidising solution had been added (30 minutes), the reaction mixture was maintained at 30° for one hour with stirring and then at room temperature for a further fourteen hours. It was then poured into water (2 l.) and the product extracted with ether. The ethereal solution was freed from acid (there were no organic acids present) and dried over anhydrous sodium sulphate. The solvent was removed by evaporation and the resinous residue redissolved in acetone (40 c.c.). α-Amyradienone-V crystallised from this solution after four days at room temperature and was recrystallised from chloroform - methanol.

Yield:- 15.6 g.; m.p. 160°C

$[\alpha]_D^{18} = +397.5^\circ$ (c = 1.2)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2840A

Extinction coefficient ϵ_{max} = 13 000

Found:- C = 85.4%; H = 10.8%

$C_{30}H_{46}O$ requires:- C = 85.3%; H = 10.9%

Bromo- α-amyradienone-V:

α-Amyradienone-V (5 g.) was dissolved in glacial acetic acid (90 c.c.) and treated at room temperature with a solution of bromine (1.42 N) in glacial acetic acid (17 c.c. in the presence of a trace of hydrobromic acid. The solution was immediately poured into water, the precipitated solid collected on a filter, washed, dried and recrystallised from chloroform - methanol.

Yield:- 2 g.; m.p. 166° - 170° (decomp.)

$[\alpha]_D^{18}$ = +268° (c = 2.3)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2820A

Extinction coefficient ε_{max} = 11 000

Found:- C = 72.3%; H = 9.04%; Br = 16.7%

$C_{30}H_{45}OBr$ requires- C = 71.9%; H = 8.98%; Br = 16.0%

epi-Bromo- α-amyradienone-V:

epi-Bromo- α-amyradienone-V crystallised from the crystallisation mother liquors of the preparation of bromo- α-amyradienone-V in the form of needles and was recrystallised from chloroform - methanol.

Yield:- 200 mg.; m.p. 158° - 160°C (decomp.)

on admixture of a specimen of bromo- α-amyradienone-V, m.p. = 140° - 154°C.

$[\alpha]_D^{18}$ = +246° (c = 2.0)

Found:- Br = 16.2%

$C_{30}H_{45}OBr$ requires:- Br = 16.0%

Dibromo- α-amyradienone-V:

α-Amyradienone-V (4 g.) was dissolved in glacial acetic acid (80 c.c.) and titrated with a solution (1.5 N) of bromine in glacial acetic acid at room temperature in the presence of a trace of hydrobromic acid. The end point was reached when free bromine could be detected in the reaction mixture after it had been allowed to stand for two minutes at room temperature.

Volume of bromine solution used = 25 c.c. = 2 Mol Some of the product crystallised during the titration the remainder was precipitated by pouring the reaction mixture into water. The precipitate was collected by filtration and recrystallised from chloroform - ethanol until free from mono-bromo derivatives.

Yield:- 1.4 g.; m.p. 196°C (decomp.) $[\alpha]_D^{18}$ = +185° (c = 4.0)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2840A

Extinction coefficient ε_{max} = 8 000

Found:- C = 62.1%; H = 7.60%; Br = 28.2%

$C_{30}H_{44}OBr_2$ requs:- C = 62.1%; H = 7.59%; Br = 27.6%

α-Amyradienonol:

Bromo- α-amyradienone-V (600 mg.) was dissolved in ethanol (20 c.c.). The solution was added to a boiling solution of potassium hydroxide (2 g.) in ethanol (40 c.c.) and the reaction mixture boiled for two minutes. It was then poured into ice-water (200 c.c.) containing concentrated sulphuric acid (2 c.c.). The ethanol was removed by fractional distillation in vacuo at 24° and the product extracted from the aqeous suspension with ether. The ethereal solution was washed and dried and the solvent evaporated. The residue was dissolved in methanol (10 c.c.). The solution deposited a small quantity of crystals after evaporating slowly in the atmosphere for four weeks. The product was recrystallised from methanol.

Yield:- 20 mg.; m.p. 137°C.

α-Amyrenedione:

α-Amyrin (30g) was oxidised with hot chromic acid according to the method of Spring and Vickerstaff (J.C.S. 1934, 650). The product was recrystallised several times from ethanol.

Yield:- 11.5 g.; m.p. 192°C

Bromo- α-amyrenedione:

α-Amyrenedione(3 g.) was dissolved in glacial

acetic acid (20 c.c.) containing a trace of hydrobromic acid and treated at room temperature with a solution (1.42 N) of bromine in glacial acetic acid. The addition of bromine was stopped when the reaction mixture showed a positive reaction for free bromine after standing at room temperature for two minutes.

Volume of bromine solution used = 10.1 c.c. ≡ 1 Mol
The yellow reaction mixture was immediatley poured into ice-water (200 c.c.). The precipitated solid was collected by filtration, washed, dried in vacuo at room temperature and recrystallised from chloroform- methanol. Bromo- α-amyrenedione crystallised in the form of needles.
Yield:- 3.1 g.; m.p. 237°C (decomp.)
$[\alpha]_D^{28} = +104^\circ$ (c = 3.4)

Found:- C = 69.7%; H = 9.10%; Br = 16.3%
$C_{30}H_{45}O_2Br$ requs:- C = 69.6%; H = 8.71%; Br = 15.5%

Attempted dehydrohalogenation of Bromo- α-amyrenedione:

The compound (500 mg.) was refluxed with glacial acetic acid (30 c.c.) containing a trace of hydrobromic acid, for one hour. The reaction mixture was poured into water and the solid precipitate collected and washed. It was recrystallised from chloroform - methanol.
Yield:- 450 mg.; m.p. 236°C (decomp.) undepressed on admixture of a specimen of bromo- α-amyrenedione.

Acetoxy- α-amyrenedione:

Bromo- α-amyrenedione (2 g.) was dissolved in dry ethanol (40 c.c.) and the solution refluxed with freshly fused potassium acetate (5 g.) for 48 hours, with exclusion of moisture. The reaction mixture was poured into water (200 c.c.) and the precipitate extracted with ether. The ethereal solution was washed, dried and the solvent removed by evaporation. The resinous residue was dissolved in dry petroleum and chromatographed on alumina (Merck, 50 g.)

Fraction	Solvent	wt. mat. eluted	Remarks
1. - 5.	2 l. petroleum	negligible	
6. - 9.	2 l. benzene	"	
10.	250 c.c. ether	20 mg.	resin
11. - 14.	1000 " "	90 "	crystals, m.p. 258°C
15. - 20.	2000 " "	300 "	resin
21.	200 " "	15 "	"
22.	200 " "	negligible	
23.	50 " chloroform	20 mg.	resin, non-crystallisable
24. - 26.	150 " "	380 "	" " "
27.	250 " acetone	600 "	" " "
		1410 mg.	

wt. resin before chrom.:- 1700 "

Fractions 10. - 20. were combined and recrystallised several times from chloroform - methanol.

Yield:- 210 mg.; m.p. 263°C

$[\alpha]_D^{18} = +157°$ (c = 0.5)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2530A .

Extinction coefficient ε_{max} = 12 000

Found:- C = 77.1%; H = 9.60%

$C_{32}H_{48}O_4$ requires:- C = 77.4%; H = 9.67%

<u>α-Amyrenedionol:</u>

<u>a) From Bromo-α-amyrenedione:-</u> Bromo-α-amyrenedione (5 g.) dissolved in hot dry ethanol (100 c.c.) was rapidly added to a solution of potassium hydroxide (10 g.) in boiling dry ethanol (200 c.c.). The reaction mixture was boiled for three minutes and quickly poured into a solution of concentrated sulphuric acid (10 c.c.) in water (900 c.c.) containing crushed ice (100 g.) with vigorous stirring. The aqueous suspension was then concentrated in vacuo at 30° to 700 c.c. and the product extracted with ether (5 x 70 c.c.). The ethereal extract was thoroughly washed, dried over anhydrous sodium sulphate and the ether removed by evaporation in vacuo. The resinous residue was recrystallised several times from methanol.

α-Amyrenedionol crystallised in the form of prisms.

Yield:- 800 mg.; m.p. 229° - 231°C

$[\alpha]_D^{16}$ = +182° (c = 1.8)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2540A

Extinction coefficient ϵ_{max} = 10 000

Found:- C = 79.5%; H = 10.50%

$C_{30}H_{46}O_3$ requires:- C = 79.3%; H = 10.14%

b) From Acetoxy- α-amyrenedione:- Acetoxy- α-amyrenedione (50 mg.) was refluxed with a solution of potassium carbonate (1 g.) in water (1.5 c.c.) and ethanol (9 c.c.) for three hours. The cooled reaction mixture was poured into water (50 c.c.) and the product extracted with ether. The ethereal solution was washed and dried and the residue after evaporation of the ether, was recrystallised from methanol. α-Amyrenedionol crystallised in the form of prisms.

Yield:- 38 mg.; m.p. 229°C undepressed on admixture of a specimen of α-amyrenedionol prepared by hydrolysis of bromo- α-amyrenedione.

$[\alpha]_D^{18}$ = +180° (c = 0.4)

Iodo-oxy- α-amyrenedione and α-Amyrenonecarboxylic acid:

α-Amyrenedionol (2 g.) was dissolved in ethanol (500 c.c.) and the solution warmed to 40°. A solution of sodium metaperiodate (4 g.) in N sulphuric acid (40 c.c.) was added to the warm solution with stirring and the reaction mixture maintained at 40° to 50° for four hours. A yellow colouration developed after fifteen minutes and gradually intensified. No free iodine could be detected in the reaction mixture. The solution was kept at room temperature for sixteen hours longer, diluted with water (200 c.c.) and concentrated in vacuo at 30° to 200 c.c. The solid, bright yellow precipitate was extracted with ether and the ethereal solution washed with dilute sodium thiosulphate and water. Acid products were removed by washing with dilute (10%) sodium carbonate and water and the ethereal solution finally dried over anhydrous sodium sulphate. The ether was removed in vacuo and the resinous residue recrystallised from ethanol (yellow needles).

Yield:- 300 mg.; m.p. 234° - 236°C (decomp.)

$[\alpha]_D^{20} = -55.6°$ (c = 3.2)

Ultra-violet absorption spectrum:-

Absorption band at $\lambda_{max} = 2520A$

Extinction coefficient $\epsilon_{max} = 12\,000$

Found:- C = 62.0%; H = 7.65%; I = 21.3%

$C_{30}H_{43}O_3I$ requires:- C = 62.3%; H = 7.44%; I = 22.0%

The alkaline extracts were twice washed with ether (50 c.c.), the ether washings being discarded, and then neutralised with concentrated hydrochloric acid with vigorous stirring. The precipitated acid was extracted with ether, the ethereal solution washed and dried and the ether removed in vacuo. The crystalline residue was recrystallised several times from aqueous methanol.

Yield:- 450 mg.; m.p. 242° - 247°C (decomp.)

$[\alpha]_D^{16} = +192.5°$ (c = 1.5)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2540A

Extinction coefficient ϵ_{max} = 8 000

Found:- C = 74.3%; H = 9.14%

$C_{30}H_{46}O_4.1\ H_2O$ req:- C = 73.8%; H = 9.83%

Equiv. Wt. (by titration with N/100 NaOH):*

Found:- 410

$C_{29}H_{45}O_2.COOH.1\ H_2O$ requs.:- 488

Methyl-α-Amyrenonecarboxylate:-

The acid (500 mg.) was dissolved in absolute ether (10 c.c.) and a freshly prepared solution of diazomethane (10 c.c.) added at -5°. The reaction mixture was kept at -2° for sixteen hours and was then allowed to stand at room temperature for a further four hours. It was then cooled below 0° and a solution of diazomethane (5 c.c.)

previously kept in the refrigerator for 24 hours, added with stirring. The reaction mixture was kept at -2° for another 18 hours. It was then washed with dilute cold hydrochloric acid to destroy excess diazomethane, with water and with dilute (10%) sodium carbonate. The alkaline extracts were found to contain no organic material. The ester was isolated from its purified ethereal solution and recrystallised four times from aqueous methanol.

Yield:- 240 mg.; m.p. 160° - 163°C

$[\alpha]_D^{19}$ = +113.5° (c = 3.6)

Found:- C = 74.4%; H = 9.70%; MeO = 6.45%

$C_{31}H_{48}O_4.1\ H_2O$ requires:- C = 74.1%; H = 9.96%; MeO = 6.2%

Monomethyl- α-Amyrenonedicarboxylate:

Methyl- α-amyrenonecarboxylate (150 mg.) was dissolved in chloroform (8 c.c.) and glacial acetic acid (30 c.c.) A solution (N/10) of potassium permanganate (8 c.c.) was added and the solution kept at room temperature for five minutes. The reaction mixture was then poured into water, excess permangante removed with a little sulphurous acid and the organic matter extracted with ether, (6 x 40 c.c.). The ethereal solution was well washed with water and the acid fraction extracted with dilute (5%) caustic soda solution (3 x 15 c.c.). The alkaline solution was washed with ether and the acid precipitated with dilute hydrochloric

acid and extracted with ether. The ether was washed free from inorganic matter and dried. The resinous residue after evaporation of the solvent was precipitated as an amorphous solid from its concentrated chloroform solution with petroleum (b.p. 60° - 80°) at 50°.

Yield:- 40 mg.; m.p. 170° - 178°C

From the ethereal solution freed from acid, unchanged methyl-α-amyrenonecarboxylate was isolated.

Yield:- 80 mg.; m.p. 161° - 163°C undepressed on admixture of an authentic specimen of methyl α-amyrenonecarboxylate.

$[\alpha]_D^{18} = +115°$ (c = 0.8)

α-Amyrenedionol:

Iodo-oxy- α-amyrenedione (1 g.) was dissolved in hot glacial acetic acid and the solution treated with zinc dust (1.5 g.). The reaction mixture was maintained at 100°C for 30 minutes. The excess zinc dust was removed by filtration and the filtrate poured into water (50 c.c.) The product was extracted with ether, the ethereal solution washed with water, dilute (10%) sodium carbonate (10 c.c.) and water, and dried. The residue on evaporation of the

solvent was recrysta llised several times from chloroform - methanol.

Yield:- 700 mg.; m.p. 228° - 230°C undepressed on admixture of an authentic specimen of α-amyrenedionol.

$[\alpha]_D^{18} = +176°$ (c = 2.4)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2540A

Extinction coefficient ϵ_{max} = 12 000

Acetoxy- α-amyrenedione:

α-Amyrenedionol (500 mg.) obtained by reduction of iodo-oxy- α-amyrenedione, was acetylated (Note, p. 95). Acetoxy- α-amyrenedione crystallised from chloroform - methanol in the form of needles.

Yield:- 420 mg.; m.p. 266° - 268°C undepressed on admixture of an authentic specimen of acetoxy- -amyrenedione.

$[\alpha]_D^{19} = +159°$ (c = 1.0)

Found:- C = 77.2%; H = 9.95%

$C_{32}H_{48}O_4$ requires:- C = 77.4%; H = 9.67%

Deoxy- α-amyrenedionol-I

a) From iodo-oxy- α-amyrenedione:- Iodo-oxy- α-amyrenedione (200 mg.) was dissolved in hot glacial acetic acid (20 c.c.) and zinc dust (300 mg.), activated by

treatment with 2% hydrochloric acid and thorough washing with, successively, water, ethanol, acetone and ether, added to the hot (100°) solution with stirring. The reaction mixture was maintained at 100° for 45 minutes with frequent agitation and the product isolated by pouring the solution after removal of excess zinc dust, into water and extracting the organic matter with ether. The ether was removed by distillation and the residue recrystallised twelve times from chloroform - methanol.

Yield:- 40 mg.; m.p. 243° - 245°C

$[\alpha]_D^{18} = +190°$ (c = 1.8)

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2640A

Extinction coefficient ϵ_{max} = 16 000

Found:- C = 81.9%; H = 11.10%

$C_{30}H_{46}O_2$ requires:- C = 82.2%; H = 10.51%

b) From -α-Amyrenedionol:- α-Amyrenedionol (500 mg.) was treated with activated zinc dust in a similar manner. Deoxy-α-amyrenedionol was recrystallised many times from chloroform - methanol.

Yield:- 120 mg.; m.p. 241°C undepressed on admixture of a specimen of deoxy-α-amyrenedionol-I obtained by reduction of iodo-oxy-α-amyrenedione with activated zinc dust.

Ultra-violet absorption spectrum:-

Absorption band at λ_{max} = 2640A

Extinction coefficient ϵ_{max} = 16 000

Found:- C = 82.8%; H = 10.63%

$C_{30}H_{46}O_2$ requires:- C = 82.2%; H = 10.51%

The Action of Zinc dust on α-Amyrenonyl Acetate:

α-Amyrenonyl acetate (100 mg.) was dissolved in hot glacial acetic acid (10 c.c.). Activated zinc dust (p. 133 (150 mg.) was added with stirring and the reaction mixture maintained at 100° for 30 minutes. Unreacted zinc dust was removed by filtration, the solution diluted with benzene and thoroughly washed with water. The solvent was removed and the residue recrystallised from chloroform - methanol.

Yield:- 70 mg.; m.p. 176°C undepressed on admixture of an authentic specimen of α-amyrenonyl acetate.

The Action of Zinc dust on α-Amyrenone:

α-Amyrenone (100 mg.; prepared according to the method of Ruzicka, Mueller and Schellenberg, Helv. Chim. Acta, XXII, 758) was treated with activated (p. 133) zinc dust (150 mg.) in hot glacial acetic acid solution and the product isolated as described in the previous experiment.

Yield:- 65 mg.; m.p. 124° undepressed on admixture of an authentic specimen of α-amyrenone.

The Action of Zinc Dust on α-Amyrenedione:

α-Amyrenedione (100 mg.) was treated with activated (p. 133) zinc dust (150 mg.) in hot glacial acetic acid (10 c.c.) and the product isolated as previously described. (p. 135).

Yield:- 55 mg.; m.p. 193° undepressed on admixture of an authentic specimen of α-amyrenedione.

Deoxy- α-amyrenedionol-II:

α-Amyrenedionol (1g.) was dissolved in dry benzene (200 c.c.) and the solution warmed to 45°. Freshly prepared lead tetracetate (1 g.) was added and the solution kept at 45° for 30 minutes. The temperature was then raised to 65° for 45 minutes and the solution was finally refluxed for 15 minutes after standing at room temperature over night. Throughout these operations there was a gradual accumulation of a brown precipitate. This was filtered off with the aid of "Hyflo" filter-aid and the residual solution washed with dilute (10%) sodium carbonate solution. No organic material was precipitated from the alkaline solution on acidification. The neutral product was isolated from the benzene solution and recrystallised from chloroform - methanol. Deoxy- α-amyrenedionol-II crystallised in the form of needles.

Yield:- 850 mg.; m.p. 196°C

$[\alpha]_D^{18} = +91.4^\circ$ (c = 7.2)

Found:- C = 83.1%; H = 10.13%

$C_{30}H_{44}O_2$ requires:- C = 82.6%; H = 10.09%

α-Methylcyclohexanol:

o-Cresol (500 g.) was treated with hydrogen (387 l. at N.T.P.) in the presence of Raney Nickel catalyst (12 g.) at 120° and 15 - 100 atmospheres pressure until hydrogen absorption was very slow (four days). The liquid product was diluted with ether (300 c.c.) and the solution washed 10% sodium hydroxide solution (6 x 250 c.c.). It was then thoroughly washed with water till free from alkali and dried over anhydrous sodium sulphate. The ether was evaporated and the residual α-methylcyclohexanol distilled through a short (Vigreaux) fractionating column at atmospheric pressure.

Yield:- 265 g.; b.p. 167° - 168°C/763 mm.

The alkaline extract was washed several times with small amounts of chloroform and acidified with dilute (10%) sulphuric acid. The precipitated cresol was extracted with chloroform (3 x 200 c.c.), the chloroform solution washed with water and dried. The residue after removal of the solvent was distilled at atmospheric pressure and solidified on cooling.

Yield:- 190 g.; m.p. 36°C

α-Methylcyclohexanone:

α-Methylcyclohexanol (265 g.) was gradually added to a solution of potassium dichromate (480 g.) in water (2.4 l.) and concentrated sulphuric acid (220 c.c.) with vigorous stirring, the temperature being maintained below 65°. When the reaction was complete (i.e.: when no further evolution of heat was observed), the misture was allowed to cool and the product extracted with ether (3 x 200 c.c.). The ethereal solution was washed with dilute (10%) sodium hydroxide until the alkaline washings were colourless and then thoroughly with water, and dried. The ether was evaporated and α-methylcyclohexanone distilled at atmospheric pressure.

Yield:- 172 g.; b.p. 163° - 164°C/755 mm.

α-gem-Dimethylcyclohexanone:

a) Mixed dimethylcyclohexanones:- α-Methylcyclohexanone (170 g.) was gradually added to a suspension of sodamide (60 g.) in dry ether (900 c.c.) with vigorous stirring. The reaction mixture was then refluxed with continuous stirring for two hours to remove the ammonia formed in the course of the reaction, and cooled. A solution of dimethyl sulphate (215 g.) in dry ether (180 c.c.)

was added in small portions, care being taken to avoid excessive violence of reaction, and stirring and refluxing continued for a further two hours. The suspension was then carefully diluted with water (400 c.c.), the water washed with ether (150 c.c.) and the combined ethereal extracts (approx. 1 l.) vigorously shaken with aqueous ammonia (sp. gr. 0.88) to remove excess dimethyl sulphate. The ether solution was then washed with dilute (10%) hydrochloric acid, sodium carbonate and water, and dried over anhydrous sodium sulphate. The solvent was evaporated and the residue immediately shaken with a saturated solution of sodium bisulphite. The bisulphite compound of α-methylcyclohexanone was removed by filtration and washed on the filter with petroleum (b.p. 40° - 60°). The solvent was evaporated from the filtrate and the residual mixed dimethylcyclohexanones distilled at atmospheric pressure.

Yield:- 50 g.; b.p. 168° - 170°/761 mm.

b) Mixed semicarbazones:- The methylation product (94 g., two preparations) was added to a solution of semicarbazide hydrochloride (85 g.) and crystalline (hydrated) sodium acetate (130 g.) in ethanol (600 c.c.) after removal of the precipitated sodium chloride from the reagent solution.

The reaction mixture was cooled and kept at 2° for twelve hours. The crystalline semicarbazones were collected by filtration.

Yield:- 124 g.; m.p. 168° - 185°C

c) Pure α-gem-dimethylcyclohexanone:- The impure semicarbazones were wll washed with water and, after drying in vacuo, suspended in ether (350 c.c.). The ether insoluble residue (102 g.) was then fractionally recrystallised from ethanol the least soluble portion being isolated.

Yield:- 22 g.; m.p. 191° - 192°C

The pure semicarbazone (22 g.) of α-gem-dimethylcyclohexanone was refluxed with a solution of oxalic acid (75 g.) in water (250 c.c.) for thirty minutes. The liberated ketone was extracted with ether and the ether solution washed with dilute (10%) sodium carbonate solution and water, and dried over anhydrous sodium sulphate. The ether was evaporated and the residual α-gem-dimethylcyclohexanone distilled at atmospheric pressure.

Yield:- 10 g.; b.p. 169° - 169.5°C/753 mm.

Monobromo- α-gem-dimethylcyclohexanone:

α-gem-Dimethylcyclohexanone (6.5 g.) was dissolved in glacial acetic acid (130 c.c.) containing a trace of

hydrobromic acid, and treated at room temperature with a solution of bromine (1.63 N) in glacial acetic acid (70 c.c.) (64 c.c. ≡ 1 Mol). The reaction mixture was immediately poured into water (2 l.) and the precipitated heavy oil extracted with chloroform (4 x 120 c.c.). The solution was washed with water (8 x 400 c.c.) and dried and the solvent removed in vacuo at 25°. Bromo- α-gem-dimethyl-cyclohexanone crystallised from the residue after 14 hours at room temperature and was collected on a filter and washed with a little light petroleum (b.p. 40° - 60°). The material was very soluble in all organic solvents (least in petroleum) and could not be recrystallised. It rapidly decomposed above 60° and could not be distilled at 2 x 10^{-1} mm. pressure.

Yield:- 6 g.; m.p. 51° - 52°C.

1:1-dimethyl-3-hydroxycyclohexan-2-one:

Monobromo- α-gem-dimethylcyclohexanone (5 g.) was dissolved in ethanol (70 c.c.) and added to a cold solution of potassium hydroxide (10 g.) in water (30 c.c.) and ethanol (200 c.c.). The reaction mixture was maintained at room temperature for two hours and the diluted with water (100 c.c.) and kept at room temperature for a further 18 hours. The ethanol was removed by distillation in vacuo at 25° and the aqueous solution of the

product extracted with ether (7 x 80 c.c.) after acidification with dilute sulphuric acid. The ethereal solution was washed with water (3 x 100 c.c.) and dried and the ether evaporated. The residual product was distilled in vacuo.

Yield:- 1.2 g.; b.p. $52^{\circ}C/2 \times 10^{-2}$mm. Hg.

Osazone:- Recrystallised as dark red plates from ethylene glycol monomethyl ether.

m.p.; 224° - $228^{\circ}C$.

Found:- C = 48.4%; H = 4.16%; N = 21.7%

$C_{20}H_{20}O_8N_8$ requires:- C = 48.0%; H = 4.00%; N = 22.4%

The Action of Periodic Acid on 1:1-Dimethyl-3-hydroxycyclohexan-2-one:

The material (1 g.) was dissolved in ethanol (130 c.c.) and the solution treated with a solution of periodic acid ($NaIO_4$ = 2.1 g., N H_2SO_4 = 10 c.c.) at room temperature. Free iodine was gradually liberated until the solution was dark brown in colour. After 20 hours at room temperature, the solution was diluted with water (100 c.c.) and the iodine removed by addition of dilute sodium thiosulphate. The ethanol was removed by distillation in vacuo, the product extracted with ether and separated into a neutral and an acid fraction. Both were oils which could not be identified.

BIBLIOGRAPHY

List of Abbreviations:

J.C.S. . . . . Journal of the Chemical Society.

Helv. . . . . Helvetica Chimica Acta

J. Biol. C. . . . . Journal of Biological Chemistry

Ann. . . . . Liebig's Annalen der Chemie

****

1. Rose, Ann. 13, 192 (1835)
2. Ruzicka & Wirz, Helv. 23, 132 (1940)
3. Ruzicka & Schellenberg, Helv. 20, 1553 (1937)
4. Ruzicka & Marxer, Helv. 22, 195 (1939)
5. Zimmermann, Helv. 19, 247 (1936)
6. Beynon, Heilbron & Spring, J.C.S. 1937, 989
7. Ruzicka & Giacomello, Helv. 20, 299 (1937)
8. Ruzicka & Marxer, Helv. 23, 144 (1940)
9. Goodson, J.C.S. 1938, 999
10. Ruzicka & Wirz, Helv. 22, 948 (1939)
11. Vesterberg, Berichte der Deutschen Chemischen Gesellschaft, 20, 1242 (1887)
12. Elsevier's Encyclopaedia of Organic Chemistry, 14, 526 ff. (1940).
13. G.T. Newbold, MSc. Thesis, Manchester, 1944
14. Vesterberg, Berichte der Deutschen Chemischen Gesellschaft, 24, 3834 (1891)

15. Rollett, Monatshefte fuer Chemie, 43, 413 (1922)

16. Ruzicka, Huyser, Pfeiffer & Seidel, Ann. 471, 21 (1929)

17. Vesterberg, Berichte der Deutschen Chemischen Gesellschaft, 23, 3186, (1890)

18. Ruzicka et al., Helv. 15, 431 (1932)

19. Spring & Vickerstaff, J.C.S. 1937, 249

20. Ruzicka, Hoesli & Ehmann, Helv. 17, 442 (1934)

21. Spring, Journal of the Society of Chemical Industry, 1936, 1050

22. Jacobs & Fleck, J.Biol.C. 88, 137 (1930)

23. Ewen, Spring & Vickerstaff, J.C.S. 1939, 1303

24. Ruzicka, Jeger & Redel, Helv. 26, 1238 (1943)

25. Ruzicka, Jeger & Volli, Helv. 28, 768, 1628 (1945)

26. Ruzicka et al., Helv. 30, 140 (1947)

27. Ruzicka et al., Helv. 30, 1293 (1947)

28. Ruzicka et al., Helv. 32, 1075 (1949)

29. Ruzicka et al., Helv. 20, 1271 (1937)

30. Seymour, Sharples & Spring, J.C.S. 1939, 1075

31. Seymour & Spring, J.C.S. 1941, 319

32. Seymour, Sharples & Spring, J.C.S. 1939, 1078

33. G.T. Newbold, M.Sc. Thesis, Manchester, 1944, 75 ff.

34. Vesterberg & Westerlind, Ann. 428, 247 (1922)

35. Ruzicka, Silbermann & Furter, Helv. 15, 247 (1932)

36. Winterstein & Stein, Ann. 502, 223 (1932)

37. Ruzicka, Jeger & Volli, Helv. 28, 1628 (1945)

38. Ruzicka, Jeger & Volli, ibid., 768

39. Ewen, Gillam & Spring, J.C.S. 1944, 28

40. Simpson & Williams, J.C.S. 1938, 1712

41. Ruzicka et al., Helv. 28, 199 (1945)

42. Ruzicka et al., ibid., 209

43. Ruzicka et al., 31, 1746 (1948)

44. cf. Reference (41).

45. G. Silverstone (Priv. Communication)

46. Redel, Ph.D. Thesis 1947, 50, Eidgenoessische Technische Hochschule, Zuerich.

47. Clutterbuck & Reuter, J.C.S. 1935, 1467

48. Adams, Organic Reactions, 2, 368 (Wiley & Sons, N.Y.1944)

49. Ruzicka, Leuenberger, Schellenberg, Helv. 20, 1271 (1937)

50. Hueckel, Ann. 441, 1 (1925)

51. Spring & Vickerstaff, J.C.S. 1937, 251

52. Ewen & Spring, J.C.S. 1940, 1196

53. Ruzicka, Norymberski & Jeger, Helv. 28, 380 (1945)

54. G. Silverstone (Priv. Communication)

55. Ruzicka et al., Helv. 31, 140 (1948)

56. Ruzicka, Mueller & Schellenberg, Helv. 22, 758 (1939)

57. Beynon, Sharples & Spring, J.C.S. 1938, 1236