A THESIS

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THE CHEMISTRY OF *ARISTOLOCHIA* SPECIES

THE STRUCTURE

OF

ARISTOLACTONE

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CONTENTS

Page

THE STRUCTURE OF ARISTOLACTONE

EXPERIMENTAL

III MISCELLANEOUS EXPERIMENTS

INTRODUCTION

While engaged on a general investigation of the chemical constituents of Aristolochia species, Stanlake and Williams[2] isolated a new crystalline lactone, designated aristolactone, from the light petroleum - soluble fractions of A. rotunda and A. serpentaria. Preliminary investigation of the structure by the same authors[1,2] established the empirical formula as $C_{15}H_{20}O_2$ and molecular weight determinations, ultraviolet and infrared absorption spectra provided further evidence that aristolactone was sesquiterpenoid. During hydrogenation experiments, the fully saturated hexahydroaristolactone $C_{15}H_{26}O_2$, was produced and comparison of its infrared spectrum with that of tetrahydroalantolactone showed a remarkable similarity. Differences did occur, however and the two compounds were certainly not identical but it gave rise to the idea that aristolactone might belong to the same class of sesquiterpene lactones as the alantolactones and santonin - i.e. the perhydronaphthalene group. Although this similarity was noted by Stanlake and Williams[1], they subsequently ignored any elaboration of this idea and concentrated their attention solely on possible structures containing one six-membered ring, in addition to the lactone ring. The evidence cited in support of this theory was as follows:-

i) bands appearing at 980 and 1020 $cm.^{-1}$ in the infrared absorption spectra were attributed to a six-membered ring[3].

ii) dehydrogenation experiments failed to yield any signs of naphthalenoid or aromatic products but did give rise, in one instance, to a product having benzenoid type absorption in the ultraviolet region.

iii) consideration of the number of double bonds in

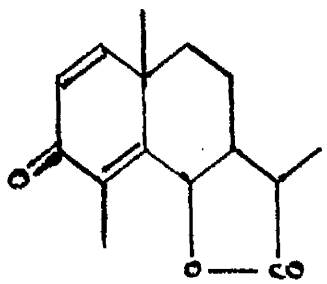
conjunction with the empirical formula restricted the choice of possible skeletons to those compounds having only one ring other than the lactone ring.

The spectral data can be criticised on the grounds that the bands quoted are not solely characteristic of six-membered rings but are also found in larger saturated ring systems[4,5], and, with the discovery in recent years of sesquiterpene lactones based on structures of the latter type [6,7,8,9,10,11], attention was turned to the possibility that a large ring structure might provide a more satisfactory explanation of the properties of aristolactone.

A survey has therefore been made of the various types of sesquiterpene lactones in an attempt to pin-point more precisely the class, if any, to which aristolactone belongs.

On the basis of their carbon skeletons, all known naturally occurring sesquiterpenoid lactones can be primarily sub-divided into three classes[6,1] these are the perhydronaphthalene group, the perhydroazulene group and the monocarbocyclic group.

The best known and most thoroughly investigated member of the perhydronaphthalene group is santonin (I), and other fully elucidated structures are alantolactone (IIa) and isoalantolactone (IIb)[13,14,15], ivasin (III)[16] and artemisin (IV)[17].



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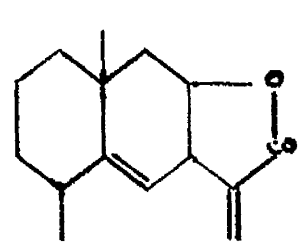

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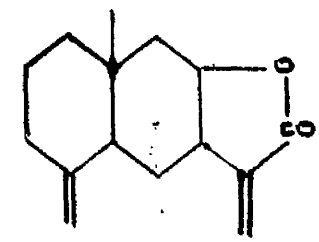

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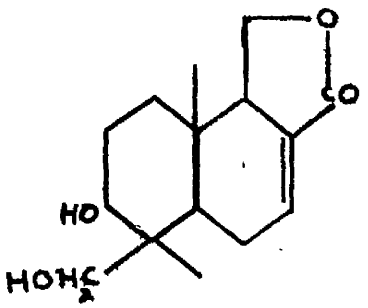

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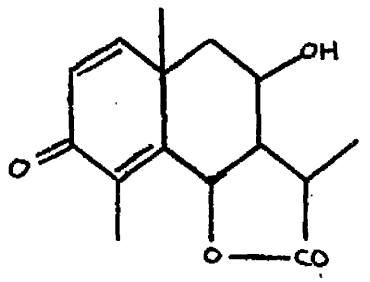
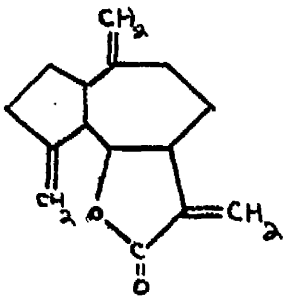


(IV)

Dehydrogenation of the above lactones yields mainly naphthalene derivatives, which are extremely useful as a guide to the original structures of the compounds.

Much attention has been focussed on the pseudoguaiane group in recent years and structures have been proposed for dehydrocostuslactone (V)[18], helenalin (VIa) and 11α,13-dihydrohelenalin (VIb)[19], artabsin (VII)[20], tomilin (VIII) and 11α,13-dihydrotomilin (IX)[21], matricin (X)[22], arbusculin (XI)[23] and geigerin (XII)[24].



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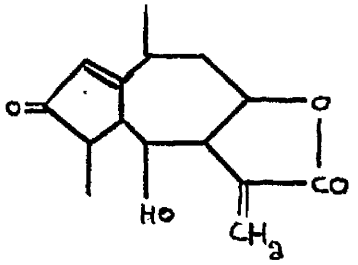
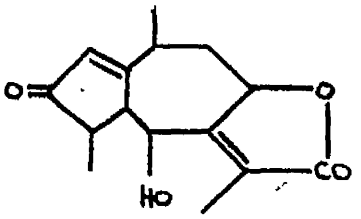


(VIa)



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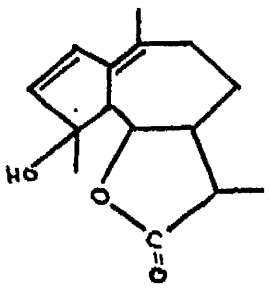

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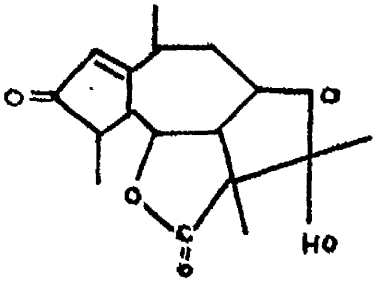

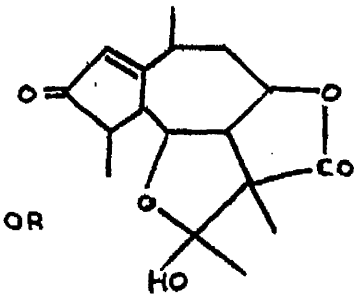

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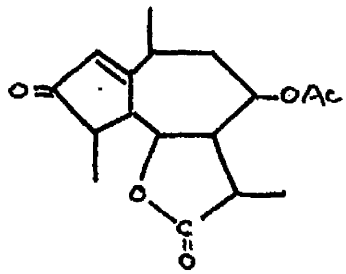

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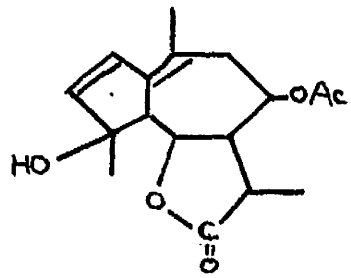

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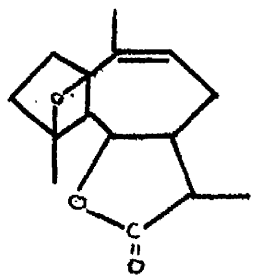

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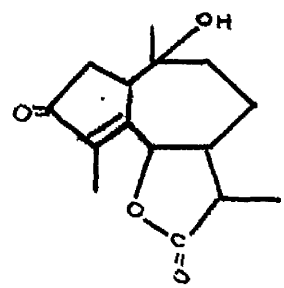
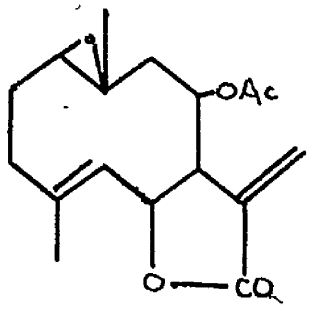


(XII)

On dehydrogenation, every member of this class yields an alkyl substituted azulene which is usually identified by the melting points of its adducts with trinitrobenzene and picric acid and by comparison of the absorption spectra with those of known azulenes.

The third group, the nonocarbocyclic compounds, consists of lactones having only one ring other than the lactone ring itself. Only four examples in this class have so far been described and elaborated; these are pyrethrosin (XIII)[7], arctiopicrin (XIV)[8,9], xanthinin (XV) and xanthatin (XVI)[6,10,11]. Since, as already indicated, artabsinolactone belongs to this class of nonocarbocyclic lactones, the chemistry of the four compounds named above will be discussed in detail in the following section.



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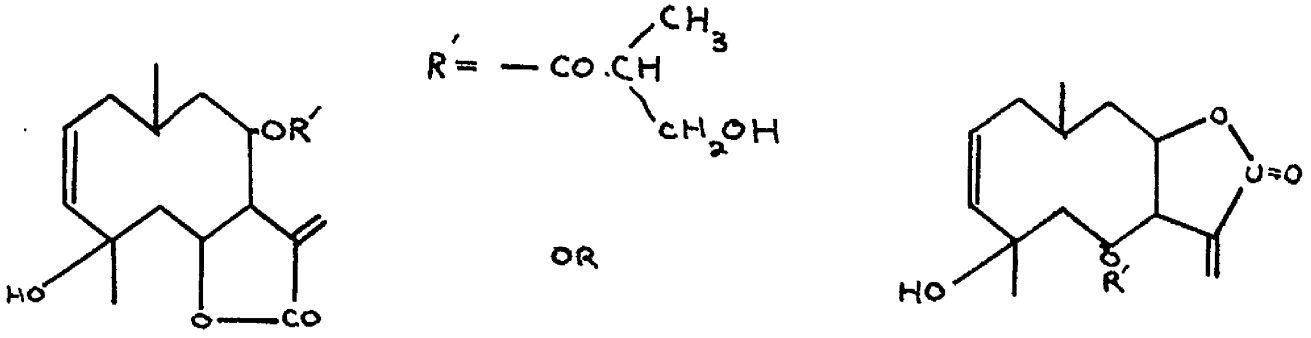

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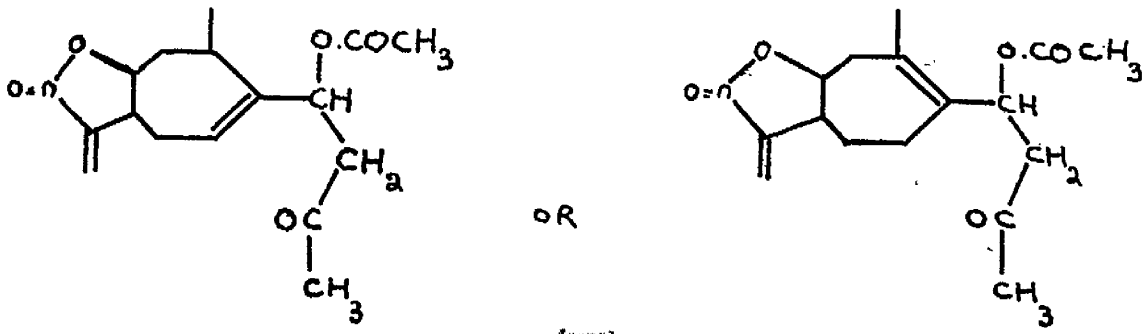

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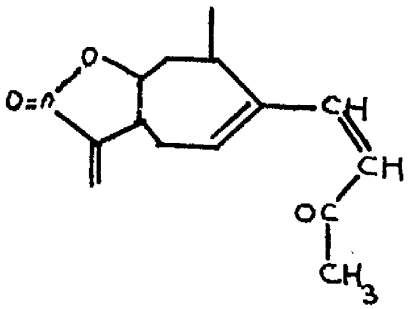


(XVI)

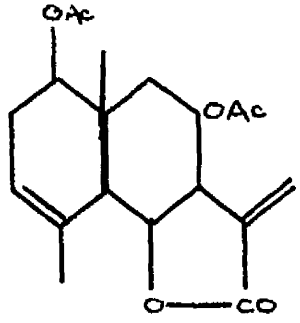
PYRETHROSIN

Although pyrethrosin was first isolated in 1891, the structure of this lactone, empirical formula $C_{17}H_{22}O_5$, was only recently determined by Barton and de Mayo[7]. Previous work by Haller et al. showed that pyrethrosin contained two double bonds and an acetoxy grouping, but, although oxidation with chromic acid afforded a dehydropyrethrosin, the fifth oxygen atom was not present in a hydroxyl group since pyrethrosin could not be acetylated. By means of zinc-dust distillation, the same workers produced an azulene (pyrethrazulene) which was tentatively identified as 2:4:8-trimethylazulene[25]. Barton's work supports the above findings but he states that formation of an azulene from pyrethrosin is a misleading indication of its carbon skeleton. While this is true in the sense that azulene formation does not imply the presence of a guaiane or other bicyclic skeleton, in the author's opinion, production of an azulene from a sesquiterpene lactone known to be monocarbocyclic may, in fact, be quite helpful.

Infrared absorption spectra provided further confirmation of a γ-lactone function with a band at 1760 cm.$^{-1}$; other bands appeared at 1735 and 1242 (acetate), and 1670 and 1650 cm.$^{-1}$ (two double bonds). The absence of bands associated with either hydroxyl or carbonyl function allowed only one possible combination for the last oxygen atom, as an ether linkage. Consideration of these facts in conjunction with the empirical formula shows that pyrethrosin can have only one ring other than the lactone ring, and hence it belongs to the monocarbocyclic series of lactones.

Ozonolysis of pyrethrosin yielded formaldehyde whereas similar treatment of the dihydro-derivative did not. Comparison of the ultra-violet absorption spectra of the two compounds indicated elimination of an $\alpha\beta$-unsaturated lactone function during hydrogenation. It follows therefore that one of the double bonds is present as an exocyclic methylene group, situated $\alpha\beta$ - with respect to the lactone carbonyl.

Evidence relating to the size of ring and to the relative position of the substituents came from the production of a compound designated cyclopyrethrosin acetate, during an attempt to acetylate under acid conditions. This compound, which could also be produced under the relatively mild conditions of oxidation with sodium dichromate in acetic acid at room temperature, was proved to have structure (XVII). The retention of the $\alpha\beta$-unsaturated lactone function was established exactly as before and hydrogenation indicated the presence of a second double bond. When treated with aqueous sodium hydrogen carbonate solution, the dihydro compound (XVIII; R=Ac) yielded the corresponding hydroxy derivative (XVIII; R=H), and this in turn could be oxidised to the keto-acetate (XIX; R=Ac) which showed infrared absorption at 1777 (γ-lactone), 1727 and 1254 (acetate), 1710 (cyclohexanone) and 1655 cm.$^{-1}$ (isolated double bond).



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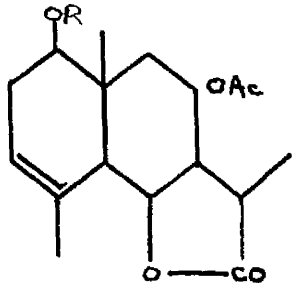

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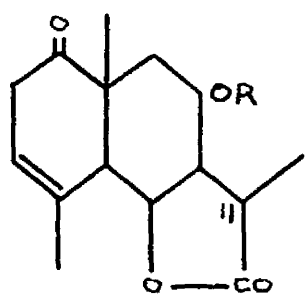
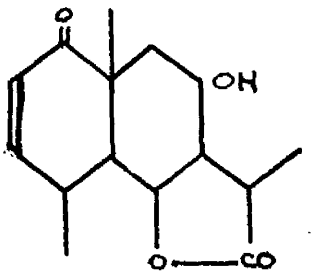


(XIX)

Proof that the double bond of the keto-acetate was placed $\beta\gamma$- with respect to the ketone group, was obtained by treatment with base when the $\alpha\beta$-unsaturated ketone (XX) was isolated.



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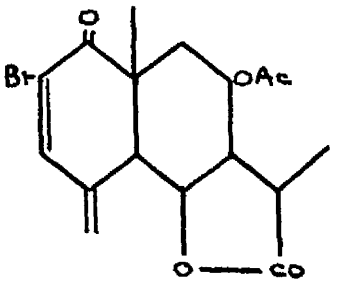
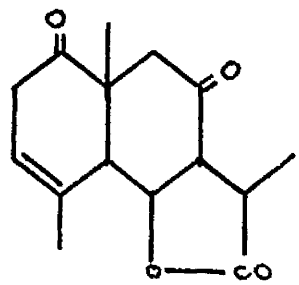


(XXI)

Further confirmation was provided by the characteristic ultraviolet absorption spectrum of the bromodienone (XXI), obtained by bromination of the keto-acetate (XIX; R=Ac) and subsequent dehydrobromination. The position of the maximum at 286 mμ precludes a homoannular conjugated system.

As well as producing the $\alpha\beta$-unsaturated ketone (XX), the action of base on (XIX; R=Ac) also yielded two stereoisomeric hydroxy ketones (XIX; R=H), differing in configuration at $C_{(11)}$. Chromic acid oxidation of both isomers gave rise to the corresponding diketones (XXII), which were not isolated but which were characterised by the appearance of a strong absorption maximum at 305 mμ, after mild treatment with base. When the more readily available hydroxy ketone (XIX; R=H) was oxidised with chromic acid, then treated with alkali, the product (XXII; R=H) responsible for the absorption at 305 mμ was characterised as the crystalline 2:4-dinitrophenylhydrazone and its methyl ester.



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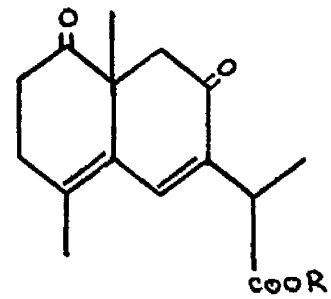
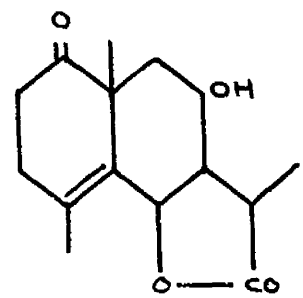


(XXIII)

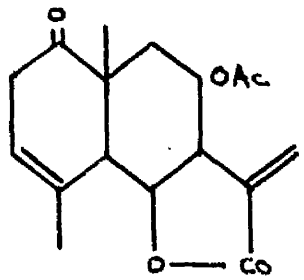
This proved to be the final link in the elucidation of cyclopyrethrosin acetate, since the same crystalline 2:4-dinitrophenylhydrazone and methyl ester 2:4-dinitrophenylhydrazone were obtained similarly from ψ-santonin (XXIV) by oxidation and treatment with base.



(XXIV)

Finally, whilst investigating the chromic acid oxidation of pyrethrosin, two crystalline products were isolated. Of these, one was probably identical with Rose and Haller's dehydropyrethrosin. This compound, $C_{17}H_{20}O_5$, showed infrared bands at 1777 (γ-lactone), 1723 and 1251 (acetate), 1703 (cyclohexanone) and 1667 and 1650 cm.$^{-1}$ (double bonds), and possessed an ultraviolet absorption spectrum typical of an $\alpha\beta$-unsaturated lactone. It is represented by structure (XXV), since selective hydrogenation produced the acetoxy-ketone (XIX; R=Ac). The

second oxidation product, $C_{17}H_{22}O_6$, showed infrared bands at 1760 (γ-lactone), 1747 and 1215 (acetate), 1705 (cyclohexanone) and 1677 cm.$^{-1}$ (double bond), whilst the ultraviolet spectrum similarly confirmed retention of the $\alpha\beta$-unsaturated lactone system. The compound was formulated accordingly as the hydroxy ketone (XXVI). The uptake of



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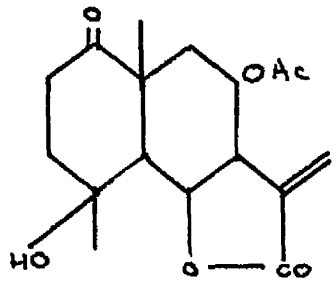
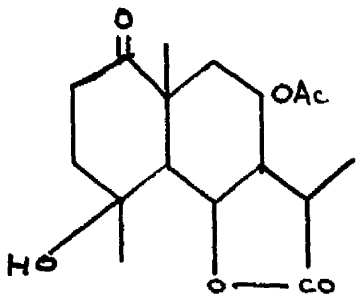


(XXVI)

one mol. of hydrogen results in the formation of the saturated product (XXVII) and this can be dehydrated with thionyl chloride and pyridine to the exocyclic methylene compound (XXVIII), which showed infrared bands at 1770 (γ-lactone), 1723 and 1254 (acetate), 1707 (cyclohexanone) and 1647 and 890 cm.$^{-1}$ (exocyclic methylene).



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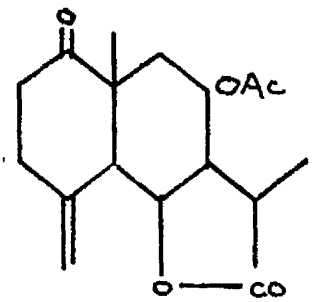
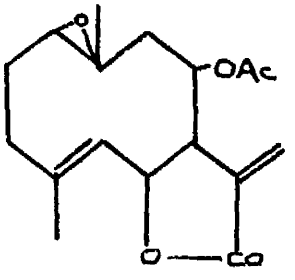


(XXVIII)

In his discussion of the structure of pyrethrosin itself, Barton interprets the cyclisation reaction as involving opening of the ethereal

oxygen ring by the acetylium ion, accompanied by formation of a new carbon - carbon link due to interaction with an ethylenic linkage. Consequently, the position of the newly formed acetoxy grouping must represent one end of the ethereal oxygen ring and the position of the new carbon - carbon bridge is the other. From the formation of the hydroxy compound (XXVI), the double bond involved in the cyclisation must be in position $C_{(3)} - C_{(4)}$ or $C_{(4)} - C_{(5)}$ and the ultimate conclusion is that pyrethrosin has structure (XXIX).



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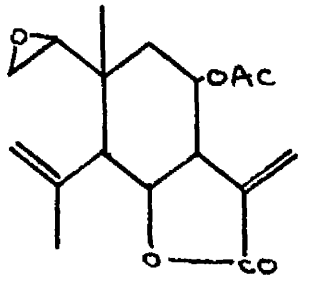


(XXX)

One other structure (XXX) is unacceptable since dihydropyrethrosin contains no methylene group and structure (XXIX) is thus preferred.

Dehydrogenation of dihydrocyclopyrethrosin acetate (XVIII; R=Ac) yielded chamazulene (1:4-dimethyl-7-ethylazulene), formed by shift of a methyl group, thus evading the relatively difficult elimination of the angular methyl.

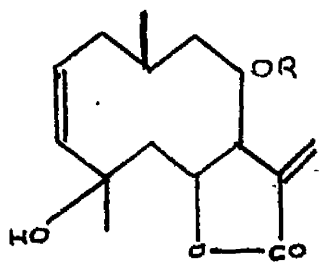
ARCTIOPICRIN

This sesquiterpenoid lactone is a much more recent discovery than pyrethrosin, being first reported in 1945 by Cavallito, Bailey and Kirchner [26]. Much of the early difficulty experienced in the elucidation of the structure can be attributed to the fact that it polymerises readily due to aerial oxidation. Consequently, determination of the correct empirical formula was almost impossible. Cavallito and his co-workers first assigned the formula $C_{15}H_{20}O_5$ to arctiopicrin, but later [27] they amended this to $C_{18}H_{24}O_6$. It was only after the Czechoslovakian workers under Sorm had obtained crystalline products from a hydrogenation experiment and had determined physical constants, molecular weights (cryoscopic) and elemental analyses of these products that the correct formula, $C_{19}H_{28}O_6$, was advanced [8]. This formula was borne out when a specially purified sample of arctiopicrin, stored under petrol, gave a satisfactory analysis for $C_{19}H_{28}O_6$.

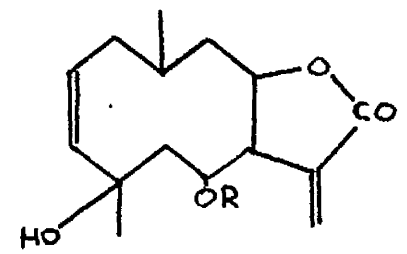
Despite the difficulty of formulation, however, Cavallito and his associates were able to establish correctly that arctiopicrin contained two double bonds, (one of which was methylenic and conjugated with the lactone carbonyl), an ester function and two hydroxyl groups, both esterifiable.

It was at this stage that Sorm's school began work on arctiopicrin during a detailed investigation of sesquiterpene lactones found in Compositae. Four crystalline substances (labelled A, B, C and D) were isolated by chromatography of the hydrogenation product. Of these,

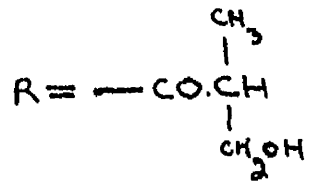
A and B have empirical formula $C_{19}H_{32}O_5$, and are stereoisomeric hydrogenolysis products. Both compounds contain only one active hydrogen atom and form mono-phenylurethane derivatives. Compounds C and D are also stereoisomers, but are normal hydrogenation products of formula $C_{19}H_{32}O_6$, forming diacetates and bis-phenylurethanes. All four compounds show infrared bands at 1775 cm.$^{-1}$, with splitting at 1730 cm.$^{-1}$ due to the ester function. Sorm has proposed structures (XXXI) and (XXXII) as possible alternatives for arctiopicrin[9]. Therefore, confining ourselves to the first of these, (simply to avoid duplication of all subsequently derived structures), compounds A and B can be represented by (XXXIII) and C and D by (XXXIV).

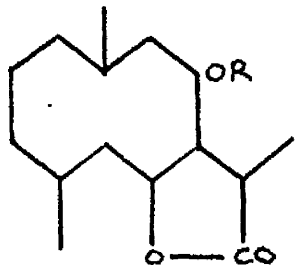


(XXXI)

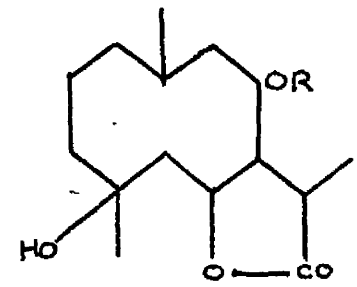


(XXXII)



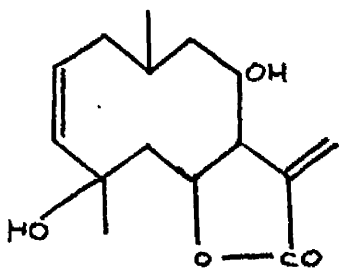


(XXXIII)

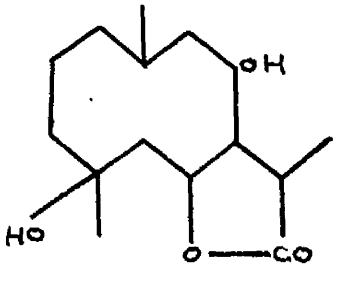


(XXXIV)

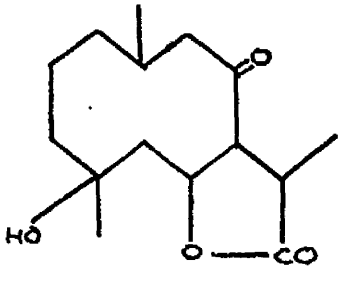
Hydrolysis of compound C_2 tetrahydroarctiopicrin, with methanolic potassium hydroxide, neutralisation and treatment with diazomethane yielded two compounds. One, $C_{15}H_{26}O_4$, had infrared bands corresponding to a γ-lactone ring and two hydroxyls and is therefore the parent saturated alcohol of arctiopicrin. Analysis of the acid component, isolated as the methyl ester, gave the molecular formula $C_5H_{10}O_3$ as expected. It was found that the acid could be readily dehydrated and the hydroxyl group is therefore placed β- with respect to the carboxyl. The only two possibilities are β-hydroxy-n-butyric acid and β-hydroxy-isobutyric acid and comparison of the infrared spectra of these acids with that of the hydrolysis product indicated the latter to be β-hydroxy-isobutyric acid. This was finally confirmed by lithium aluminium hydride reduction of both authentic and experimentally derived acid, with conversion of the diols to the same bis-phenylurethane derivative, giving identical melting points and mixed melting point. Hence, arctiopicrin is an ester of β-hydroxyisobutyric acid with a mono-carbocyclic sesquiterpene compound, $C_{15}H_{22}O_4$, designated arctiolide (XXXV).



(XXXV)



(XXXVI)

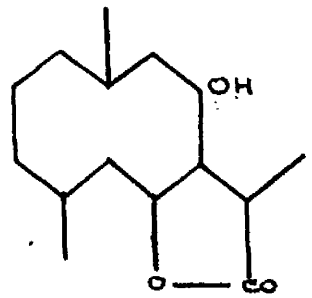


(XXXVII)

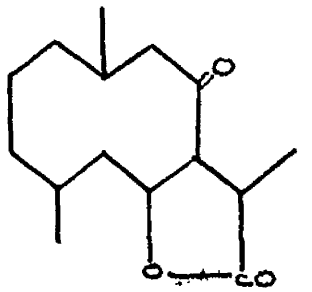
Tetrahydroarctiolide (XXXVI) showed an infrared band at 1767 $cm.^{-1}$

for the γ-lactone carbonyl compared with 1776 cm.$^{-1}$ in arabiopicrin. Chromic acid oxidation of (XXXVI) yielded the hydroxy-keto-lactone (XXXVII).

Compound B (XXXIII), from the hydrogenation of arabiopicrin, was hydrolysed to β-hydroxylaevulyric acid and the hydroxylactone $C_{15}H_{26}O_3$ (XXXVIII), which could be oxidised with chromic acid to the keto-lactone $C_{15}H_{24}O_3$ (XXXIX). This keto-lactone showed infrared bands at 1770 (γ-lactone) and 1705 cm.$^{-1}$ (ketone). Oxidation of a crude mixture of stereoisomers of (XXXVIII) gave a second keto-lactone $C_{15}H_{24}O_3$, stereoisomeric with (XXXIX).

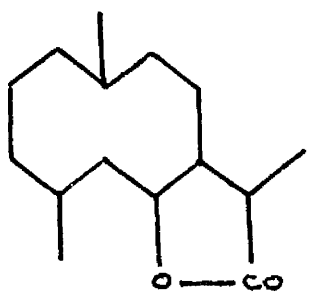


(XXXVIII)

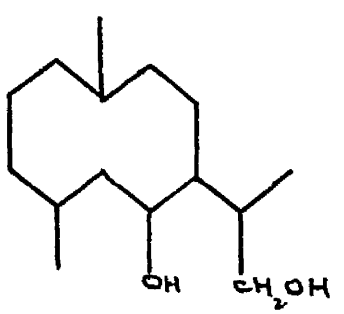


(XXXIX)

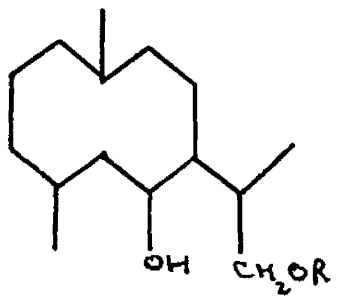
The second keto-lactone was converted to the liquid lactone (XL) $C_{15}H_{26}O_2$, via the thioketal. This saturated, unsubstituted lactone (XL) was reduced to the corresponding diol (XLI) with lithium aluminium hydride and converted to the monobenzoate (XLII; R=C_6H_5CO-).



(XL)



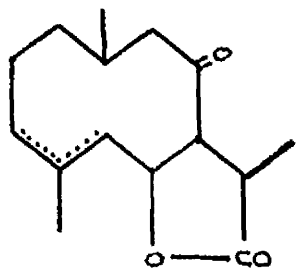
(XLI)



(XLII)

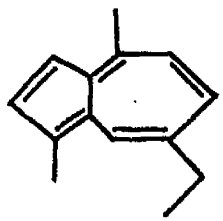
The keto compound obtained from chromic acid oxidation of the monobenzoate, showed absorption bands at 1700 (ketone) and 1723 cm.$^{-1}$ (ester carbonyl).

The positions of the ketonic carbonyl bands in the infrared spectra of various derivatives of aretiopicrin would indicate a medium sized ring in the skeleton [28,29,30]. The secondary hydroxyl group is esterified and the relative positions of the lactone ring and the ester function may be deduced by analogy with matricin [31]. In this case, wavelength of the lactone carbonyl absorption dropped from 1775-77 to 1765 cm.$^{-1}$, after removal of the esterifying radicle; this drop is attributed to the presence of keto or ester grouping neighbouring to the lactone ring. Since a similar drop (from 1778 to 1767 cm.$^{-1}$) is observed in the aretiopicrin and aretiolide spectra, it can be assumed that the esterified secondary hydroxyl group is attached to $C_{(8)}$. The other (tertiary) hydroxyl group is allylic, as shown by its susceptibility to hydrogenolysis, and its location at $C_{(4)}$ was deduced from the infrared absorption spectra of the unsaturated keto-lactone (XLIII), produced by dehydration of (XXXVII). Bands at 815 and 1664 cm.$^{-1}$ indicate a trisubstituted double bond and the ketone absorption lies at 1705 cm.$^{-1}$, which proves that no conjugation exists. If the tertiary hydroxyl group

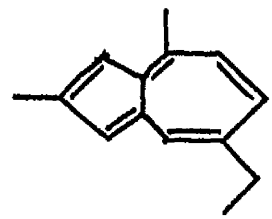


(XLIII)

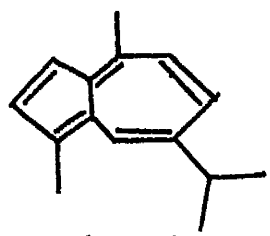
was located at the only other possible position $C_{(10)}$, it is almost certain that dehydration would produce an $\alpha\beta$-unsaturated ketone. The position of this hydroxyl at $C_{(4)}$ establishes the position of one double bond; the second double bond is known to be exocyclic and conjugated with the lactone carbonyl. No conclusive evidence was obtained by various dehydrogenation experiments, the products being a mixture of naphthalenic material with 3- and 3e-chamazulene (XLIV and XLV)[32], and 3- and 3e-guaiazulene (XLVI and XLVII)[33,34].



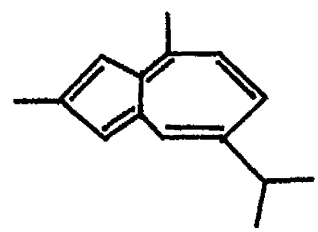
(XLIV)



(XLV)



(XLVI)



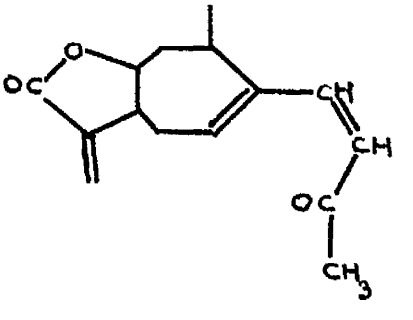
(XLVII)

The only structures which explain all the above evidence are (XXXI) and (XXXII) and so far, no further results have been published as to which is more likely.

XANTHININ AND XANTHATIN

Xanthinin is a naturally occurring sesquiterpene lactone, isolated[11] from the dried leaves of Xanthium pennsylvanicum, Compositae. It is an acetoxy-keto-lactone, $C_{17}H_{22}O_5$, and removal of the elements of acetic acid yields a second keto-lactone, $C_{15}H_{18}O_3$, named xanthatin. The first workers to extract this species of Xanthium chromatographed their product on alumina and obtained xanthatin, which they thought to be an actual plant constituent[35]. Two schools of research have published work on this topic - one American[6,10,11], the other Czechoslovakian[36] - and most of their work is in total agreement. Therefore, although minor differences of opinion do occur, it is convenient, for the greater part, to consider both sets of results at the same time.

Both schools have proposed the same structure (XLVIII) for xanthatin. This compound has an ultraviolet absorption maximum at 275 mμ, characteristic of a 2:4-dienone, and the infrared bands are in good agreement with those expected for a γ-lactone, conjugated dienone and trisubstituted double bond.

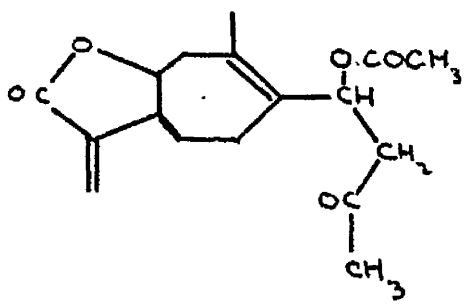


(XLVIII)

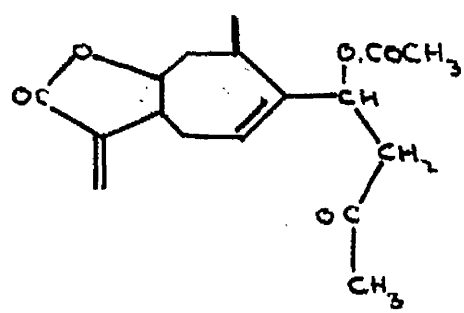
The formation of xanthatin from xanthinin can be illustrated by the partial formulae (XLIX).

(XLIX)

Sorm's structure for xanthinin (L)[36] differs from that proposed by Goldsman and Deuel (LI)[6] in the position of the cyclic double bond.



(L)

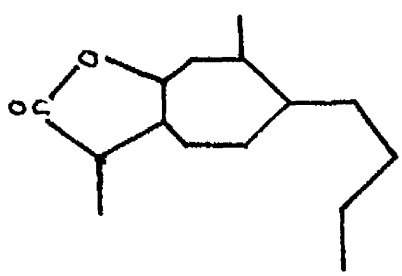


(LI)

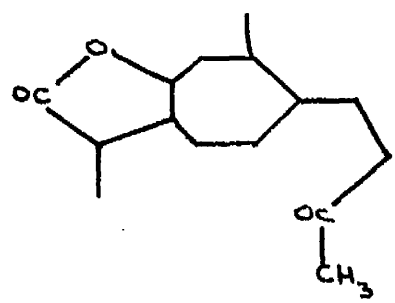
There is a direct contradiction in the two infrared spectra. Sorm and his associates were unable to repeat the findings of Goldsman and Deuel, who reported a band at 814 cm.$^{-1}$ in xanthinin and at 812 cm.$^{-1}$ in dihydroxanthinin; a band in this position at approximately 810 cm.$^{-1}$ is typical of a trisubstituted double bond. Sorm suggested that the trisubstituted double bond only arises after elimination of acetic acid from xanthinin has occurred and is not present in xanthinin or its dihydro-derivative. In support of this theory, he quoted infrared bands at 814 and 812 cm.$^{-1}$ in xanthatin and dihydroxanthatin respectively but

reported that he could find no trace of such bands in xanthinin or dihydroxanthinin.

Hydrogenation experiments revealed the presence of three double bonds in xanthatin, the hexahydro-derivative being obtained as a liquid which analysed satisfactorily for $C_{15}H_{24}O_3$. Xanthinin, on the other hand, consumed nearly four mols. of hydrogen (according to Horn), and chromic acid oxidation of the product yielded two compounds which were separated by chromatography. The first product, $C_{15}H_{26}O_2$, corresponded to the fully saturated parent lactone (LII), showing infrared bands at 1765 (γ-lactone) and 1388 cm.$^{-1}$ (C-methyl). The other product was a saturated keto-lactone (LIII), $C_{15}H_{24}O_3$, with bands at 1767 (γ-lactone), 1715 (ketone) and 1365 cm.$^{-1}$ (methyl group of CH_3CO-). From this, it was deduced that xanthinin contained two double bonds; a third molecule of hydrogen is taken up during elimination of acetic acid (hydrogenolysis) and part of a fourth molecule reduces the ketone group originally present.



(LII)



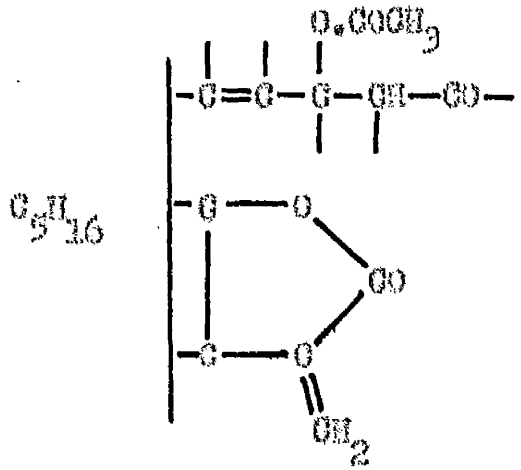
(LIII)

Goldman claims that xanthinin takes up only three mols. of hydrogen. This discrepancy is unimportant and is probably due to a number of factors such as activity of catalyst, solvent and duration of

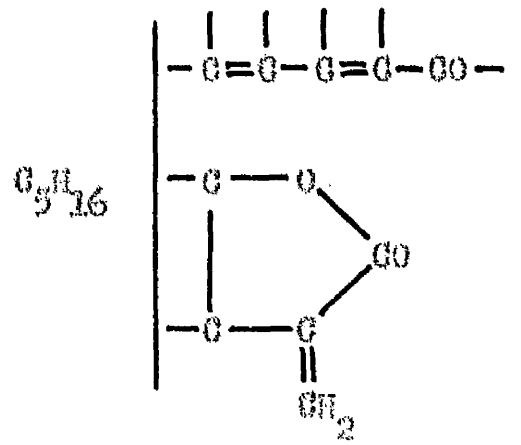
hydrogenation. Both schools agree that xanthinin contains two double bonds and an acetoxy group which can be readily removed by hydrogenolysis and is therefore probably allylic; also, xanthatin contains three double bonds, two of which form a conjugated dienone system with the ketone group already present. By deduction from the appropriate molecular formulae, it is evident that both lactones are monocarbocyclic.

Crystalline dihydroxanthinin was isolated by interrupting the hydrogenation of xanthinin after the uptake of one mol. Removal of acetic acid from this compound resulted in the production of dihydroxanthatin which possessed the characteristic ultraviolet absorption of xanthatin itself. This proves that the double bond which reduces first is not the one which forms part of the dienone system of xanthatin. Unlike xanthatin, xanthinin has no characteristic ultraviolet spectrum but it does possess fairly intense end-absorption in the 210-220 mμ region, which is generally typical of α-methylonic γ-lactones[37,38,39]. Dihydroxanthinin shows a greatly decreased end-absorption which suggested that one of xanthinin's two double bonds might be conjugated with the lactone carbonyl and exocyclic to the lactone ring. Confirmation of this was provided by ozonolysis experiments in which formaldehyde was obtained from xanthinin but not from dihydroxanthinin. Further, dihydroxanthinin was shown to contain an extra C-methyl group and it would not react with diazomethane to form a pyrazoline derivative, although xanthinin readily yielded such a compound. By similar methods, xanthatin was also shown to contain a conjugated methylene group, as would be expected. The preceding evidence can be summarised in the following partial structures (LIV) and (LV) for xanthinin and xanthatin

respectively.



(LIV)

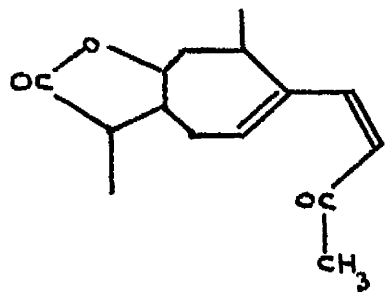


(LV)

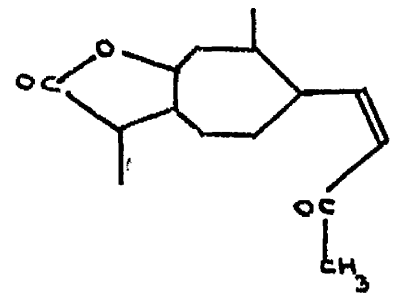
The methods used by the two schools to elucidate the rest of the structure are quite different and are best considered separately. Sorm's interpretations are more firmly based on experimental evidence than the Americans' and are probably more satisfactory for that reason.

Sorm established the presence of the methyl ketone in xanthatin by three methods. The liquid hexahydro-derivative (LIII) showed an infra-red band at 1365 cm.$^{-1}$, mentioned previously, which is typical of a methyl group adjacent to a carbonyl. The iodoform test was positive and, lastly, acetic acid was produced by Beckmann rearrangement of the oxime of (LIII).

Ozonolysis of xanthatin, dihydroxanthatin (LVI) and tetrahydro-xanthatin (LVII) yielded methylglyoxal in every case, identified as its bis.-2:4-dinitrophenylhydrazone, and as methyl glyoxime. Tetrahydro-xanthatin was not entirely homogeneous and it had a low intensity maximum at 278 mμ, corresponding to about 6% unchanged dihydroxanthatin, from which it was prepared.

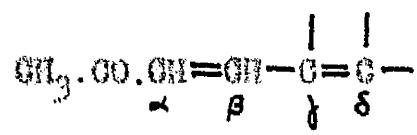


(LVI)



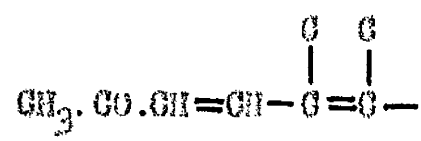
(LVII)

The principal ultraviolet absorption occurs at 226 mμ , which agrees very well with Woodward's rule for the wavelength of absorption of a monosubstituted αβ -unsaturated ketone[40]. On this basis, the conjugated dienone system of xanthatin was formulated (LVIII).



(LVIII)

Oxidation of dihydroxanthatin (LVI) with potassium permanganate in acetic acid yielded a lactone dicarboxylic acid, $C_{11}H_{16}O_6$, isolated as its p-bromophenacyl ester. The acid showed an infrared band at 1770 cm.$^{-1}$ (γ-lactone). An acid of this nature could only be formed if the γδ -double bond of the dienone system is in the carboxylic ring. The partial structure (LVIII) can therefore be extended to (LIX).

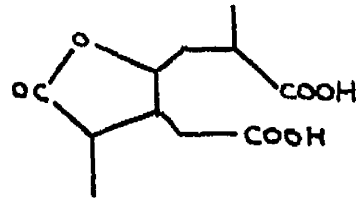


(LIX)

A dienone system, substituted as shown would have a maximum at 230 mμ ;

calculated according to Woodward's rule[40], and this agrees very well with the wavelength found (275 mμ). This structure (LIX) also explains the infrared band at 814 cm.$^{-1}$ in dihydroxanthatin (and xanthatin).

Kuhn-Roth oxidation indicates the presence of two C-methyl groups in the dicarboxylic acid, and, since one is known to be attached to the lactone ring, the other must be a ring substituent in dihydroxanthatin. Chromic acid oxidation of xanthatin yields methylsuccinic acid[6], therefore the dicarboxylic acid contains all the carbon atoms of the methylsuccinic acid and the carbon atoms of the carboxyl groups are the γ- and δ -carbons of the conjugated dienone system. Hence the lactone dicarboxylic acid can be formulated as (IX) and xanthatin by (XLVIII).

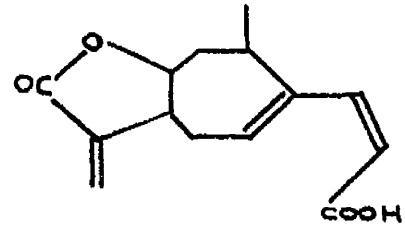


(IX)

In support of their structure for xanthinin, (I), Sorm and his colleagues were able to show that dihydroxanthinin yielded formaldehyde when treated with osmium tetroxide and then lead tetra-acetate. Since ozonolysis did not afford any formaldehyde and infrared spectra showed no band characteristic of a methylene group, the formation of formaldehyde can only be explained by shift of a double bond, resulting in a semi-cyclic vinylidene compound. Sorm also points out that formation of methylsuccinic acid from xanthinin after non-specific oxidation is not necessarily proof

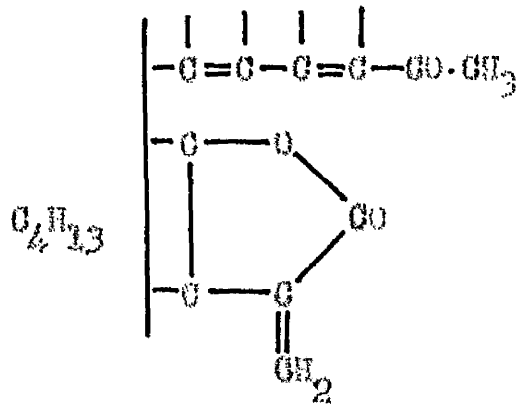
of the location of the double bonds in the molecule, since xanthatin may be a primary product of the oxidation. When considered in conjunction with the spectral evidence, the structure proposed by the Czechoslovakian would appear to be more probable.

Geissman and his colleagues established the methyl ketone group by hypoiodite oxidation of xanthinin and xanthatin. They obtained iodoform and a new crystalline compound, xanthatic acid, (LXI) in both cases. Confirmation of the $\alpha\beta,\gamma\delta$-unsaturated acid grouping was obtained from the absorption spectra. Xanthatic acid, which bears two alkyl substituents on the unsaturated system, shows an intense absorption at 252 mμ. The reference compound quoted, sorbic acid (hexa-2,4-dienoic acid) bears only one alkyl substituent and absorbs at 254 mμ. Consequently, the author would have expected xanthatic acid to absorb at approximately 265 mμ. If the $\gamma\delta$-double bond of the dienoic acid had been in the adjacent tetrasubstituted position, however, the theoretical wavelength of absorption would have been higher still, and in this respect, the wavelength quoted (252 mμ) supports structure (LXI).



(LXI)

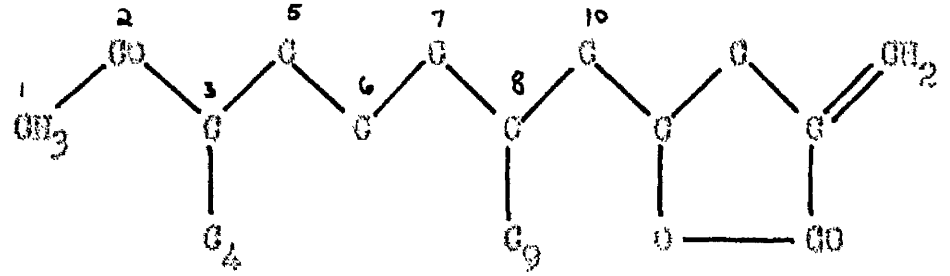
From the evidence advanced at this stage by Geissman and Dowel, xanthatin could be formulated as (LXII).



(LXII)

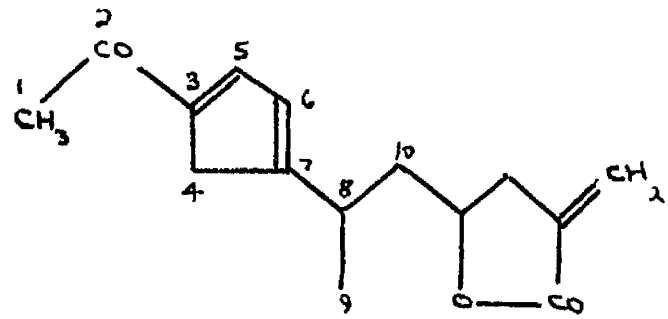
Xanthatin was shown to contain two C-methyl groups, one in the methyl ketone and the other probably in a secondary saturated location, since it yielded only half an equivalent of acetic acid.

From this point, the Americans fall back onto theoretical grounds for the final steps in the elucidation of the structures. They point out that the presence of an α-substituted γ-lactone and an additional C-methyl group suggests that xanthatin is sesquiterpenoid. If this is true, and the skeleton is composed of regularly arranged isoprene units, as in (LXIII), then the number of C-methyl groups would require that either $C_{(4)}$ or $C_{(9)}$ be used in ring formation.



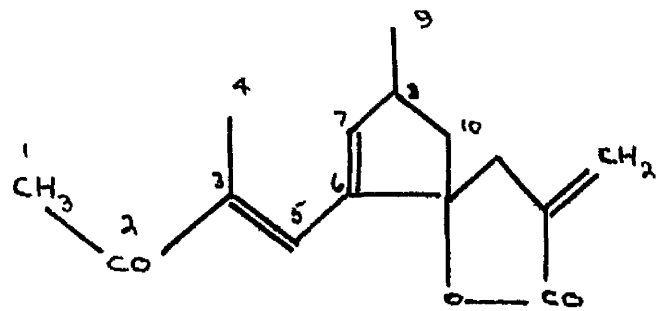
(LXIII)

Utilisation of $C_{(4)}$ in this way would lead to structure (LXIV), which, however, is rejected on the following spectroscopic evidence.



(LXIV)

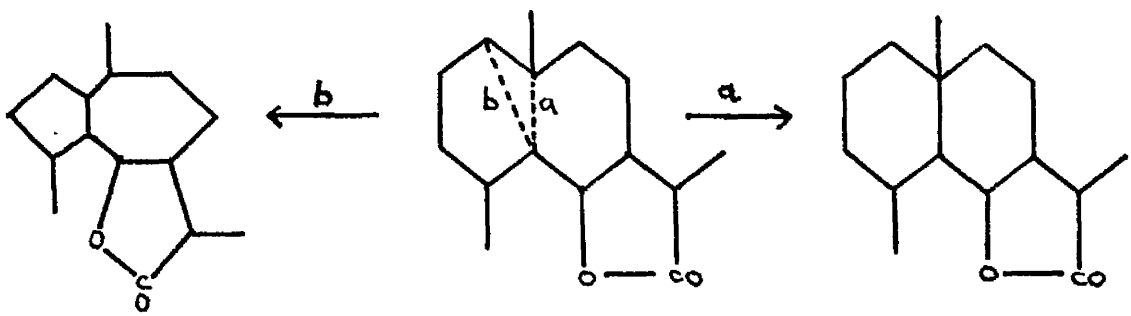
The ultraviolet absorption maximum of xanthatin at 276 mμ (275 mμ according to Soon) agrees closely with that expected for a linear dienone system carrying two alkyl substituents or for a system in which the γδ-double bond of the dienone is situated in a ring of at least six members[41]. On the same basis, structure (LXV) is also rejected, although the origin of this compound seems rather obscure, since it does not involve either $C_{(4)}$ or $C_{(9)}$ in ring closure. Further, structure (LXV) contains three C-methyl groups, when Geissman and Doucl themselves have shown that xanthatin contains only two.



(LXV)

Attention is drawn to the fact that xanthinin is obtained from a genus of Compositae, a family from which many sesquiterpene lactones have

been isolated. The lactones so far elucidated possess either the perhydronaphthalene[14,15,17] or perhydroazulene skeleton[18,19,20,21,22,23]. The relationship between the two classes may be illustrated as in scheme (LXVI).

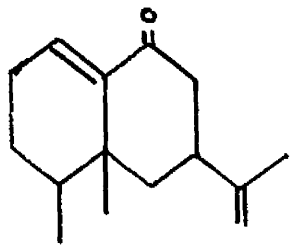


perhydroazulene perhydronaphthalene

(LXVI)

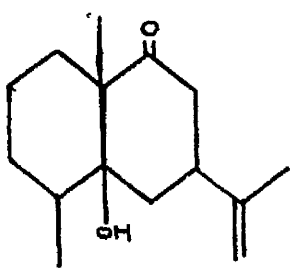
Xanthinin, containing seventeen carbons is probably related to the basic fifteen carbon xanthatin in the same way that proshanazulene, tomulin and pyrethrosin are related to similar parent lactones. While xanthatin cannot have a guaiane skeleton, it is probably a monocarbocyclic analogue of such a compound.

In proposing structure (XLVIII) for xanthatin, Geissman and Dowel are obviously prepared to consider compounds which are not composed of isoprene residues. However, Barton and de Mayo[12] define a terpenoid as "a compound whose carbon skeleton is either (a) theoretically composed from isoprenoid units or (b) has at some stage in its biogenesis, had a carbon skeleton so constructed." Sesquiterpenoid compounds which diverge widely from the isoprene rule are known. The outstanding example is eremophilone (LXVII)[12,42].

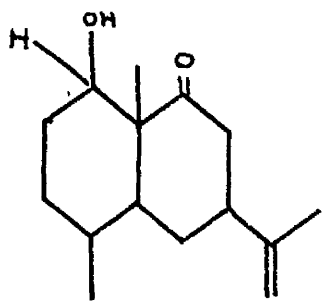


(LXVII)

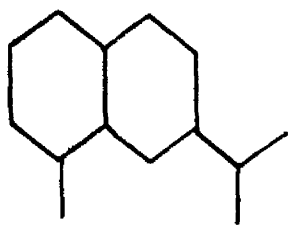
The explanation offered for the non-isoprenoid skeleton of this compound is that migration of a methyl group occurs during biogenesis. Sir Robert Robinson[42] suggested that eremophilone is formed in nature by dehydration and molecular rearrangement of compounds like (LXVIII) or (LXIX), both of which obey the isoprene rule. Therefore, on this basis, the structure of eremophilone can be reduced to the normal eudalenoid pattern (LXX).



(LXVIII)



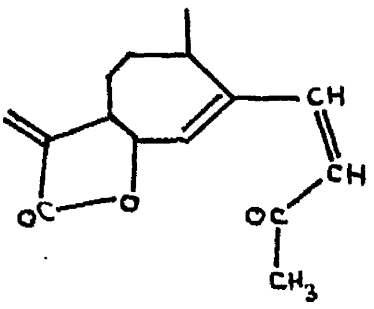
(LXIX)



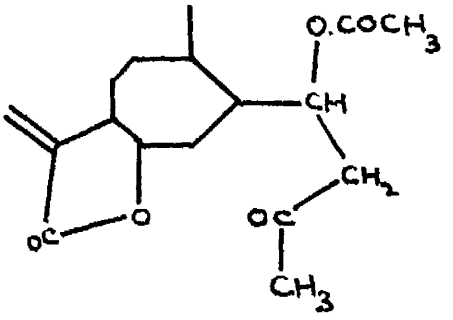
(LXX)

Both Goleman and Sorm point out that xanthatin (or its naturally occurring derivative xanthinin) is probably formed from isoprenoid precursors through rupture of a bond, or by failure to form a bond.

As a final word, Goldsman suggests that structures (LXXI) and (LXXII) are also possible for xanthatin and xanthinin respectively. Evidence against these structures is that after hydrogenation, no acidic hydrogenolysis products (other than acetic acid) were isolated, as would almost certainly be the case if the lactone oxygen was allylic.



(LXXI)



(LXXII)

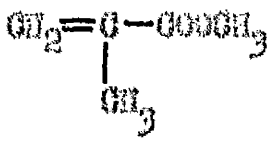
The largest fragment obtained after chromic acid oxidation was methylsuccinic and not methylglutaric acid. Therefore Goldsman and Deuel prefer structures (XLVIII) and (LI), although, as already shown, there is some doubt as to whether their structure for xanthinin is correct, in respect to the position of the double bond in the ring.

The completely negative results obtained from dehydrogenation experiments is support for the abnormal skeleton of xanthatin since otherwise some traces of azulenic, naphthalenic or benzenoid material would almost certainly have been found.

ARISTOLACTONE

The findings of Stenlake and Williams[1,2] formed the foundation on which the present work was built, and, although a number of their original experiments have been extended or repeated, many of their results are incorporated in the conclusions drawn by the author. A summary of their work is therefore presented.

Elementary analysis and molecular weight determination of aristolactone gave the molecular formula as $C_{15}H_{20}O_2$, and hydrogenation experiments established the presence of three double bonds. It follows, therefore, that aristolactone contains only one ring other than the lactone ring, i.e. it is monocarbocyclic. The lactone function was demonstrated experimentally by saponification, when an acidic fragment was obtained in quantitative yield, while no trace of a separate alcohol fraction was found. Aristolactone exhibited an ultraviolet absorption maximum at 211 mμ (ϵ, 11,500), which was attributed to an $\alpha\beta$-unsaturated lactone or ester grouping. Determination of the number of double bonds by the quantitative measurement of halogen absorption (iodine value) invariably gave fractional values (2.3 - 2.7) and it was thought this could be due to the $\alpha\beta$-unsaturated lactone or ester system, which is known to be unreactive. On the other hand, similar determinations on model compounds which were readily available, santonin (I; $\alpha\beta$-unsaturated carbonyl) and methyl methacrylate (LXXIII ; $\alpha\beta$-unsaturated ester), showed no uptake of halogen at all. It was concluded therefore, that some substitution of the aristolactone molecule was occurring, as well as addition of the bromine to the two reactive double bonds.



(LXXIII)

In carbon tetrachloride, aristolactone showed infrared absorption peaks at 1770 cm.$^{-1}$ (1736 cm.$^{-1}$ in paraffin mull, in confirmation of the γ-lactone function) and 890 and 1650 cm.$^{-1}$ (vinylidene group). The presence of the vinylidene was firmly established by isolation of formaldehyde as the dimedone derivative, after ozonolysis. Peaks at 782, 800 and 840 cm.$^{-1}$ were thought to indicate the presence of a tetrasubstituted double bond, although this was only a tentative suggestion.

Selective hydrogenation in presence of a palladium-charcoal catalyst, gave a crystalline dihydro-derivative, $[\alpha]_D$ -77°, $\lambda_{max.}$ 209 mμ (ε, 7,300), ozonolysis of which did not yield formaldehyde, indicating that the vinylidene grouping had been eliminated during the hydrogenation. A re-examination by the present author of the infrared spectrum of this compound (in solution and in potassium bromide disc) has shown that the band usually associated with the vinylidene grouping at 890-900 cm.$^{-1}$, is still present, although reduced to approximately half of its intensity compared with the spectrum of aristolactone. When platinum oxide was used as a catalyst in the hydrogenation of aristolactone, two mols. of hydrogen were rapidly absorbed but if isolated at this stage, the product was an oil. When allowed to run to completion, hydrogenation afforded the crystalline saturated

hexahydroaristolactone, $[\alpha] + 3^{\circ}$. Unlike aristolactone, this neutral compound, $C_{15}H_{26}O_2$, was stable to cold alkali but the infrared spectrum confirmed the continued presence of the lactone ring, with a peak at 1780 cm.$^{-1}$ (γ-lactone).

Tetrahydroalantolactone (II) was obtained in order to compare its infrared spectrum with that of hexahydroaristolactone. It was found that the two curves were strikingly similar, although differences did occur, showing that the two saturated lactones are not identical. The bands at 950 and 1013 cm.$^{-1}$ in the spectrum of tetrahydroalantolactone and at 951 and 1006 cm.$^{-1}$ in hexahydroaristolactone were attributed to the presence of a cyclohexane ring in the molecule[3]. (The infrared spectrum obtained from tetrahydroalantolactone was a replica of a literature curve which was published at a later date[43]).

The bands quoted are not specific indication of a cyclohexane ring, since published spectra of cyclononane derivatives[4] and cyclodecane and some of its derivatives[5], contain similar bands which could also be interpreted as indicative of a six-membered ring. In addition, during a discussion of bands exhibited by cyclopropane rings, Colo[44] states that oxygenated substituents have a greater absorption at approximately 1020 cm.$^{-1}$ than cyclopropane rings appear to have. Hence, identification of a cyclopropane ring in a compound containing oxygenated substituents is impossible on the basis of absorption in this region. A logical corollary therefore, is that a band at 1020 cm.$^{-1}$ is not absolutely reliable for identification of a cyclohexane ring, even when coupled with another band between 950 and 1000 cm.$^{-1}$.

Bands at 890 and 1650 cm.$^{-1}$ (vinylidene) in aristolactone are absent from the spectrum of hexahydroaristolactone, as would be expected, and two peaks at 1034 and 1064 cm.$^{-1}$ (ester group absorption) are replaced by a single peak at 1167 cm.$^{-1}$ in the saturated compound.

The action of alkali upon aristolactone

When aristolactone was dissolved in cold ethanolic potassium hydroxide, the optical rotation of the solution rose rapidly to a maximum positive value, followed by a very slow drop to a small negative value. Titration of aliquot portions withdrawn at various intervals showed that the increase in optical rotation did not involve utilisation of alkali, but the succeeding fall was accompanied by neutralisation of one equivalent. Acidification of the reaction mixture at the point of maximum rotation precipitated a neutral crystalline compound, ethyl oxoaristate, $C_{17}H_{26}O_3$, $[\alpha]_D$ + 317°, apparently formed from aristolactone by addition of the elements of ethanol.

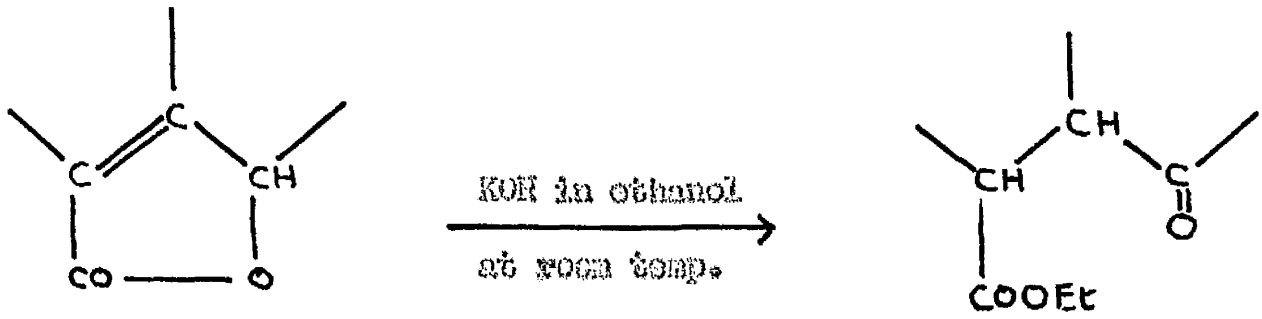
Ethyl oxoaristate was also obtained by the action of ethanolic ammonia on aristolactone, during an attempt to form an ammonia adduct. Some $\alpha\beta$-unsaturated lactones, such as alantolactone, are known to react in this way but the only product obtained from aristolactone was ethyl oxoaristate. This failure to form an adduct provides further evidence for the alternative $\beta\gamma$-lactone formulation which will be discussed in more detail in a later section.

Ethyl oxoaristate was shown to be a keto-ester, having bands at 1726 and 1186 cm.$^{-1}$ (ester carbonyl) and a shoulder at 1704 cm.$^{-1}$ (ketone carbonyl); the ultraviolet absorption spectrum confirmed the

presence of the ketone group, with a low-intensity maximum at 291 mμ, (ε, 250).

Using solution of potassium hydroxide in methanol (instead of ethanol), the corresponding methyl ester was obtained and saponification of this compound confirmed the presence of an ester, of equivalent weight 265 (theoretical, 264).

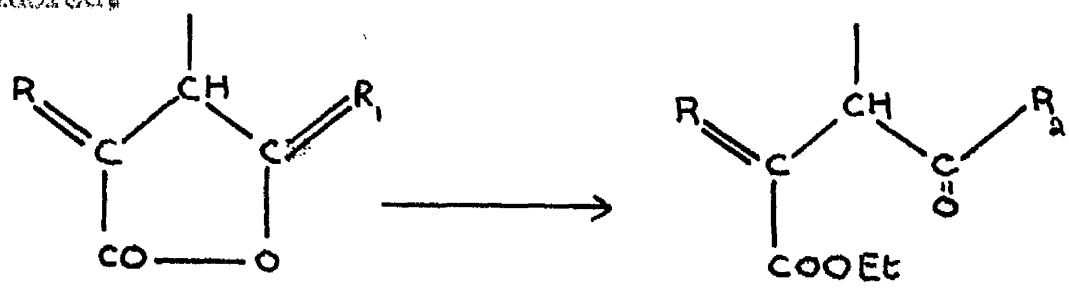
The optical changes caused by the action of alkali on artelolactone also indicated more complex alterations in the structure than simply opening of the lactone ring, and the rearrangement was interpreted as in scheme (LXXIV), being based upon analogous reactions of αβ- and βγ-unsaturated lactones[45,46,47,48].



(a) (b)

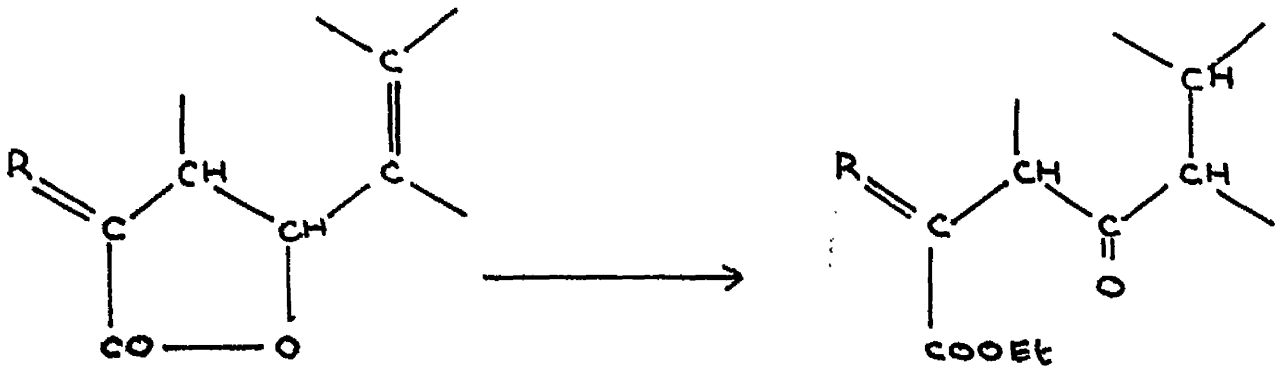
(LXXIV)

Other possible partial formulae (LXXV) and (LXXVI) were also considered.



(a) (b)

(LXXV)



(a) (b)

(LXXVI)

Hydrogenation confirmed the loss of one double bond in methyl oxoaristate but determination of the iodine value gave a high result (equivalent to 2.5 double bonds). This high value suggested that the fractional results obtained in iodine value determinations of aristolactone, were due to substitution in the molecule and not the slow addition to the postulated $\alpha\beta$-unsaturated double bond. The high iodine value also eliminated possible structures (LXXVa) and (LXXVIa), since, if the rearranged product still contained an $\alpha\beta$-double bond, then the iodine value would be about 1.5. It was therefore assumed that the $\alpha\beta$-double bond was eliminated in the rearrangement.

In carbon disulphide solution, methyl oxoaristate exhibited infrared bands at 1735 and 1150-1200 $cm.^{-1}$ (broad band carboxylic ester), and at 1720 $cm.^{-1}$ (ketone). Bands at 890 and 1650 $cm.^{-1}$ indicated the retention of the vinylidene system and this was subsequently confirmed by isolation of formaldehyde after ozonolysis. The peaks which occur at 782, 800 and 840 $cm.^{-1}$ in the spectrum of aristolactone, were still present although considerably reduced in intensity. A band appeared

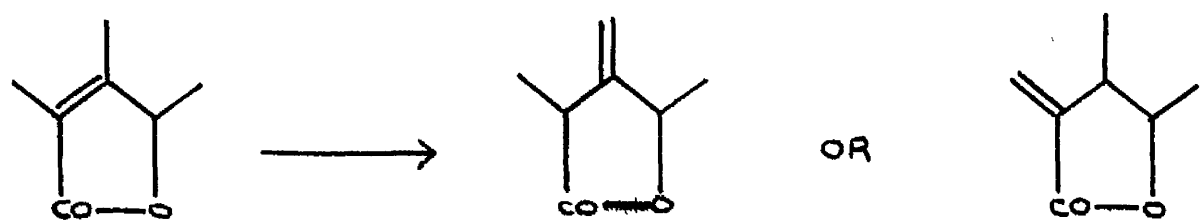
at 813 cm.$^{-1}$ (trisubstituted double bond) which is absent from spectra of aristolactone and it was therefore suspected that a second double bond might have shifted its position during the rearrangement already postulated (LXXIV). Finally, the two bands at 953 and 1010 cm.$^{-1}$ in the spectrum of hexahydroaristolactone are replaced by a single peak at 1100 cm.$^{-1}$ in the spectrum of the keto-ester. A band at approximately 1100 cm.$^{-1}$ has been ascribed to cyclohexanone derivatives[49] and this was thought to support the claim that a six-membered ring was present. This, however, must now also be reconsidered in the light of the possible influence of the oxygen substituents on absorption in this region.

Acid isomerization of aristolactone

It was found that prolonged boiling of aristolactone with glacial acetic acid produced an isomer of aristolactone, isoaristolactone, $[\alpha]_D$ -44°, in poor yield (approximately 15%). The same compound was isolated after refluxing with a solution of dry hydrogen chloride in ethanol, but the yield was even poorer. isoAristolactone had an ultraviolet spectrum which was very similar to that of aristolactone, showing a maximum at 209 mμ (ϵ, 11,200), but it also showed a low-intensity maximum at 272 mμ (ϵ, 640). No satisfactory explanation was advanced to account for this second maximum.

Since the lactone ring in isoaristolactone could only be saponified with difficulty (nearly 50% of starting material was recovered after thirty minutes reaction), the seat of the isomerization was deduced to be in or adjacent to the lactone ring. The very large change in the optical rotation during the formation of isoaristolactone (+155° to -44°)

could only be accounted for by a configurational change or by rearrangement of a double bond near an asymmetric centre. Hydrogenation of the two isomers afforded the same dihydro- and hexahydro- derivative and it was thus concluded that the change during isomerization was not a simple stereoisomeric one. During the hydrogenation of isoaristolactone, there was a definite break in uptake after one mol. had been absorbed and the product was dihydroaristolactone. Since aristolactone shows a definite break in uptake after two mols. of hydrogen have been absorbed, this supported the hypothesis that the isomerism involved shift of a double bond. The $\alpha\beta$-unsaturated double bond in structures (LXXVa) and (LXXVIa) is already in a stable position (50 (exocyclic to a five-membered ring) but in structure (LXXIVa) the $\alpha\beta$-double bond is a much less stable structure, being endocyclic to a five-membered ring. Consequently, this latter structure would tend to rearrange to either (LXXVII) or (LXXVIII), both of which are relatively more stable.



(LXXIVa) (LXXVII) (LXXVIII)

In the infrared region, isoaristolactone retained the typical vinylidene bands at 892 and 1656 cm.$^{-1}$, with the same intensity as in the spectrum of aristolactone. Bands at 787 and 842 cm.$^{-1}$ (tetra-

substituted double bond) are comparable in intensity with those of methyl oxoaristate, i.e., much reduced in comparison with those found in the spectrum of aristolactone at the same wavelength. A peak appeared at 815 cm.$^{-1}$ (trisubstituted double bond) which did not show in the spectrum of aristolactone. From comparison of the spectrum of methyl oxoaristate with that of isoaristolactone, and of their respective properties, it was deduced that the same double bond is involved in both acid and alkaline rearrangements.

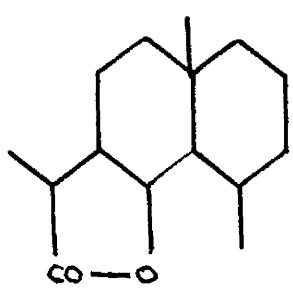
The broad maximum at 211 mμ exhibited by aristolactone is replaced by a much more sharply defined peak at 209 mμ in the ultraviolet spectrum of isoaristolactone. It was suggested that the broad maximum of aristolactone was due to summation of the contributions of an isolated tetrasubstituted ethylene (approximately 209 mμ) and of the αβ-unsaturated lactone function, at longer wavelengths (approximately 218 mμ), while the very sharp peak of isoaristolactone was the summation of the contributions of two similar systems at the same wavelength.

Dihydroaristolactone also had a very well defined ultraviolet maximum at 209 mμ (ε, 7,800) but its formation from both lactones was an apparent anomaly at first. Later, however, it was concluded that hydrogenation causes a shift of a double bond in exactly the same manner as heating with acid, and that the product is dihydroisoaristolactone and not dihydroaristolactone. The large change in specific rotation during the formation of the dihydro-derivative from aristolactone (+155° to −77°) favours this theory, since otherwise such a gross alteration is unaccountable. Finally the relative ease with which isoaristolactone forms the dihydro-compound compared with aristolactone also agrees with this deduction.

The theory that aristolactone contains an isolated tetrasubstituted double bond in addition to the $\alpha\beta$-tetrasubstituted double bond seems rather doubtful, since the intensity of absorption would almost certainly be much higher than it is. Too much stress appears to have been placed on the 'peaks' in the 208-212 mμ range, since these were almost certainly false maxima. The intensity of the end-absorption in this region can be useful but to deduce the presence of a certain conjugated system or systems because of the position of apparent maxima has obvious dangers.

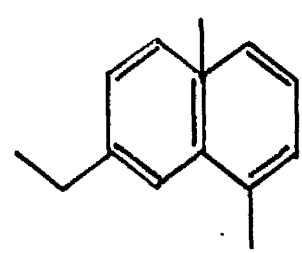
Dehydrogenation experiments

Attempts were made to demonstrate the presence of a six-membered ring in aristolactone by heating the fully saturated hexahydroaristolactone with palladium on charcoal and examining the distillate spectroscopically. To test the method, control experiments were carried out using tetrahydroalantolactone and cineole, since these compounds were known to yield naphthalenic (LXXIX) and benzenoid (probably p-cymene; LXXX) products respectively, as follows:-

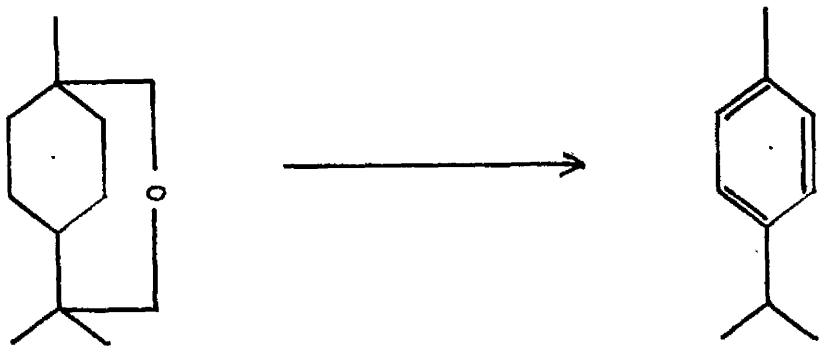


Tetrahydroalantolactone

(Based on the accepted structure at that time)



(LXXIX)



Cinnole (LXXX)

The product obtained from tetrahydroalantolactone exhibited typical naphthalenic absorption in the ultraviolet region ($\lambda_{max.}$ 230, 280 and 325mμ) while the product from cineole gave a more typically benzenoid spectrum. The liquid obtained in similar fashion from hexahydroisoalantolactone exhibited an ultraviolet spectrum which was very much like that of the product from cineole, but it was observed that this is not necessarily proof of a non-conjugated ring in the skeleton, since ring contraction during dehydrogenation is not unknown(51).

Although Stenlake and Williams(52) carried out a number of dehydrogenation experiments, they were unable to demonstrate the presence of either naphthalenic or azulenic products at all and only showed a possibly benzenoid product in one case (above). Later, using modified techniques, the author obtained violet azulenic material in several instances and, in one case, isolated a few crystals having the typical ultraviolet spectrum of a naphthalene derivative (page 69). Possible reasons for the results obtained by Stenlake and Williams in their experiments will be discussed later.

Oxidation with potassium permanganate in acetone

This mild oxidation procedure, carried out at 0 C, afforded reasonably good yields of dihydroxyaristolactone, $C_{15}H_{22}O_4$, $[\alpha]_D$ + 128°, $\lambda_{max.}$ 209 mμ (ε , 3,400). This crystalline compound was neutral and the retention of the lactone ring was shown by saponification (equivalent weight, found 246; $C_{15}H_{22}O_4$ requires 266) and recovery of the starting material on acidification. Determination of the iodine value indicated the presence of two double bonds. Collectively, these results can be explained if the compound is formed from aristolactone by addition of two hydroxyl groups to the αβ-double bond. Further oxidation of non-crystalline material from the reaction yielded succinic acid as the only identifiable product.

Oxidation with chromic acid

Only small fragments of the molecule were successfully identified after application of this method of degradation. Steam distillation of the reaction mixture produced 1.6 mols. of volatile acid, identified as mainly acetic acid, which was considered to indicate the presence of two C-methyl groups in aristolactone.

Ether extraction of the non-volatile residues gave a semi-crystalline product, from which only succinic acid was recovered. The production of succinic acid indicated the presence of fragment (LXXXI) or possibly (LXXXII) in aristolactone, although the former seems much more likely.

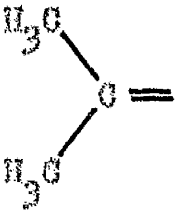
(LXXXI) (LXXXII)

A search for glutaric acid (which could arise from structure (LXXXII))

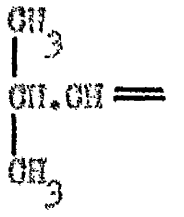
in the non-volatile portion was fruitless, no trace being found when using sensitive paper chromatographic procedures.

Ozonolysis experiments

Formaldehyde (60% of the theoretical for one vinylidene group) was the only volatile fragment obtained from ozonolysis of aristolactone. Similarly, ethyl ozoaristolate produced formaldehyde (40% of theory for one vinylidene group), indicating, as expected, that the methylene group was not involved in the alkaline rearrangement. The lack of other products, such as acetone or other simple carbonyl compounds, proved that terminal groups of type (LXXXIII) and (LXXXIV) are absent.



(LXXXIII)

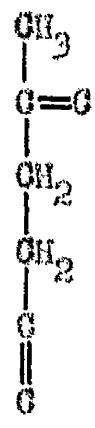


(LXXXIV)

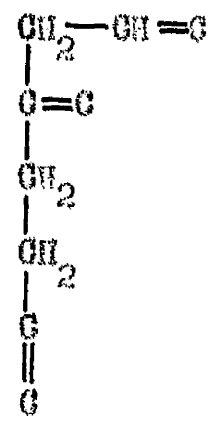
Ozonolysis invariably gave oily products, soluble in dilute alkali and which showed a strong reducing action with ammoniacal silver nitrate solution. The iodoform and alkaline sodium nitroprusside tests gave positive results, indicating the presence of at least one methyl ketone grouping. Since structures of type (LXXIVa) would be expected to yield pyruvic acid on ozonolysis, a careful search was made for this compound in the ozonolysis products, by paper chromatography. Using two different solvent systems for keto-acids, no trace of pyruvic acid was found but of the two spots which were evident, one had an R_F value identical with that of laevulinic acid in one system and slightly different from it in a

second system. Further evidence for the presence of laevulinic acid in the ozonolysis product from aristolactone was obtained by adding acid solution of 2:4-dinitrophenylhydrazine to the product and chromatographing the wide-range melting derivative on paper. Two spots were again obtained one of which had an identical R_F value to that of authentic laevulinic acid 2:4-dinitrophenylhydrazone. No trace of pyruvic acid 2:4-dinitrophenylhydrazone was evident.

The presence of laevulinic acid in the ozonolysis product would indicate that partial structure (LXXXV) or (LXXXVI) was contained in the molecule.



(LXXXV)

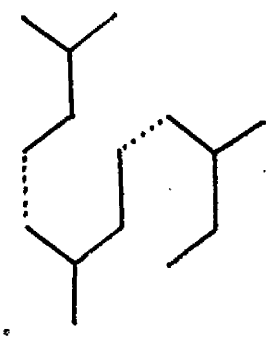


(LXXXVI)

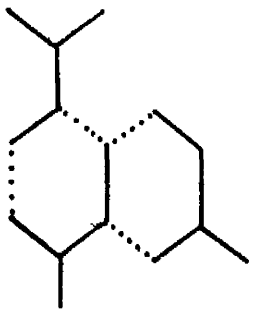
Consideration of possible structures for aristolactone

Since all known sesquiterpene compounds conform to the general isoprene rule, with the exception of ozomephilone[12,53], the proposed formulae were based on the supposition that this holds true for aristolactone. Further, since nearly all sesquiterpenes are found by head to tail arrangement of the three isoprene units, as in the farnesol type (skeleton LXXXVII) or contain the isoprene residues arranged irregularly, as in

the carotol type (skeleton LXXXVIII), possible formulae were limited to these two basic skeletons.



(LXXXVII)



(LXXXVIII)

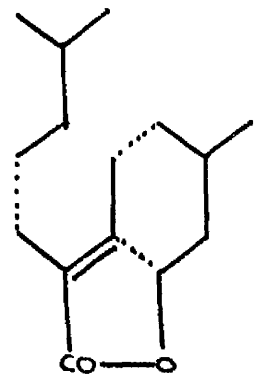
The points to be incorporated in any satisfactory formula were that aristolactone contained one ring only (apart from the γ-lactone ring), three double bonds, of which no two were in conjugation, and of which one was a vinylidene; a double bond must also be in conjugation with the lactone carbonyl. Since the presence of three ethylenic linkages was a further basic supposition, the evidence, satisfactory and unsatisfactory was summarised.

(1)	Analysis of hexahydroaristolactone	-	satisfactory.
(2)	Analysis of the hydroxy-acid derived from hexahydroaristolactone	-	satisfactory.
(3)	Quantitative bromination of aristolactone and isoaristolactone	-	inconclusive.
(4)	Uptake of hydrogen on complete hydrogenation	-	indicated three double bonds but not absolute conclusive.
(5)	Ethyl dihydro-oxoaristate was still unsaturated (tetranitromethane) although two double bonds had been removed	-	satisfactory.

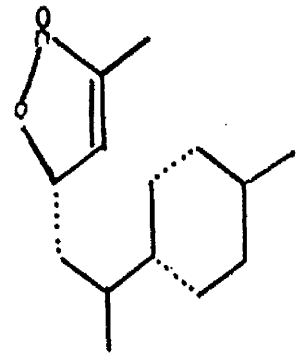
(6)	Quantitative bromination of dihydroxyarbutolactone indicated two double bonds	- inconclusive, since substitution is possible.

Considered together, the above facts provided strong evidence for the presence of three double bonds in arbutolactone.

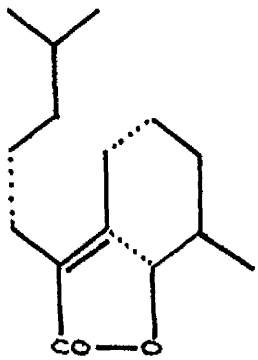
Application of the isoprene rule limited the basic skeletons to one of structures (LXXXIX - XCV), in which only the carbon atoms and the lactone ring are shown.



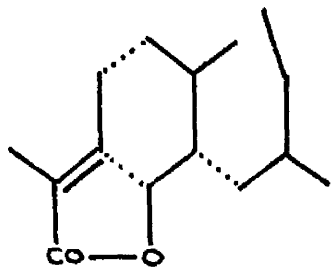
(LXXXIX)



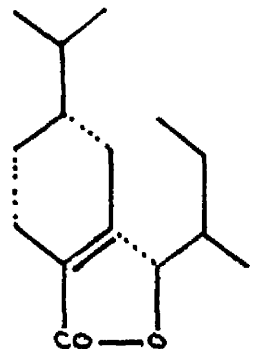
(XC)



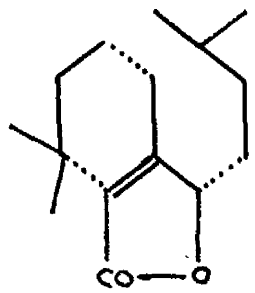
(XCII)



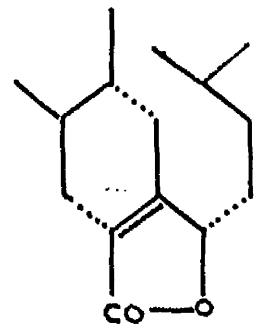
(XCI)



(XCIII)



(XCIV)

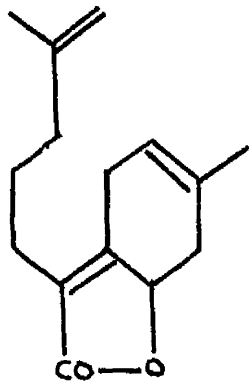


(XCV)

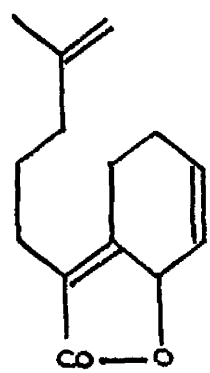
Structures (LXXXIX) and (XC) conform to the farnesol arrangement but all the others are based on carotol, having two isoprene units placed head to tail and the third unit attached irregularly. Since two of the structures (XC) and (XCI) would yield pyruvic acid on osonolysis, they were excluded at once.

Consideration of remaining structures

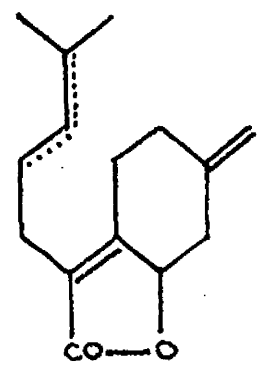
On the basis that any structure proposed must show no conjugation of the three double bonds (one of which is a vinylidene group), the following structures were derived from skeletons (LXXXIX), (XCII) and (XCIII).



A

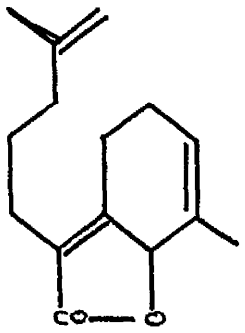


B

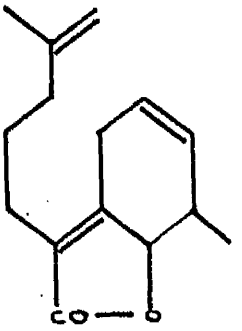


C

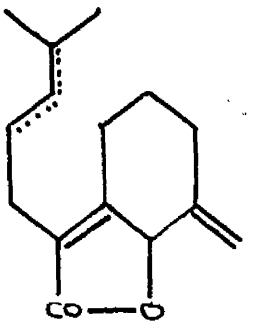
(LXXXIX)



A

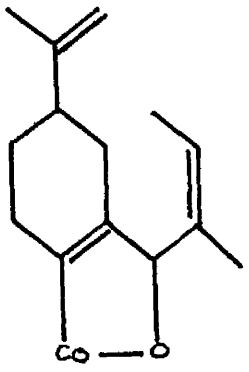


B

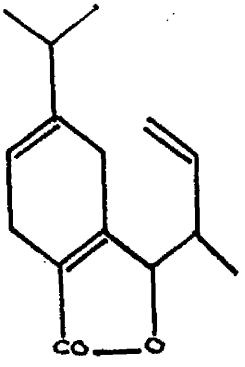


C

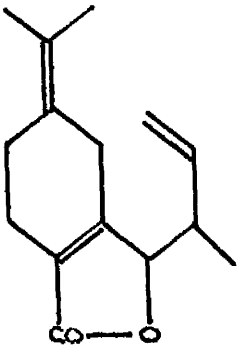
(XCII)



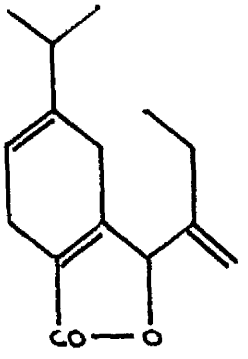
A



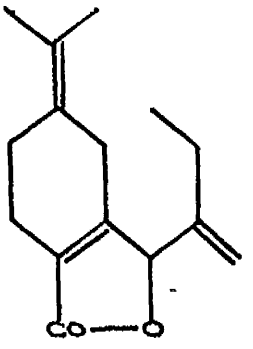
B



C



D



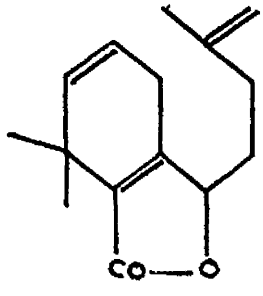
E

(XCIII)

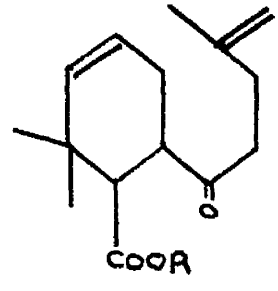
Since ethyl oxoarlotate does not contain a double bond in conjugation with the carbonyl group, structures which would give rise to such an arrangement (LXXXIXB; XCIIA and C; XCIIIA, D and E) were rejected. Similarly, structures (LXXXIXA and B; XCIIA, B and C) were ruled out since glutaric acid would be produced on chromic acid oxidation. Structures (LXXXIXC; XCIIC; XCIIIA, C and E) were also considered to be unsatisfactory because of the absence of volatile carbonyl compounds (other than

formaldehyde) after ozonolysis. The only remaining structure (XCIIIB) would not yield succinic acid after oxidation with chromic acid and is therefore unsuitable. Thus, even if no allowance is made for the second tetrasubstituted double bond, which is the least conclusive evidence, all of the derived structures (pages 47 and 48) are unsatisfactory.

Skeleton (XCIV) gives rise to one structure only (XCIVA), which was rendered unlikely on the grounds that the calculated absorption intensity of the keto-ester derived from it would be 300-600 ($\lambda_{max.}$ below 200 mμ), whereas the value found was 6,000-7,000 ($\lambda_{max.}$ 209 mμ).



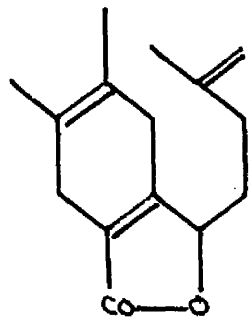
(XCIVA)



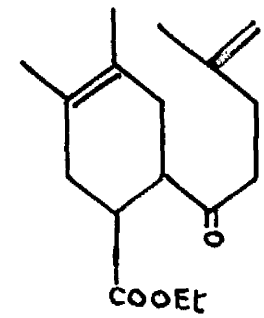
Keto-ester derived from (XCIVA)

Should this be a false maximum in ethyl cocarlotate, the argument holds good and the structure can be discarded.

Structure (XCVA), based on skeleton (XCV) is satisfactory on many points. It accommodates the known facts concerning the monocarbocyclic nature of artabolactone, the $\alpha\beta$-unsaturated lactone function, the vinylidene group, the tentatively proposed second tetrasubstituted double bond and it would be expected to yield succinic acid on chromic acid oxidation, but not glutaric acid.

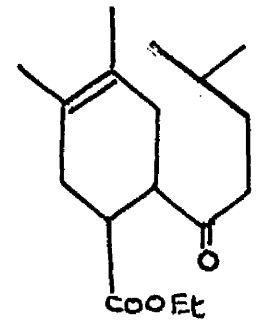


(XCVA)



(XCVI)

Ethyl oxoaristate derived from structure (XCVA) would have structure (XCVI), in which only the lactone ring had altered. Since ozonolysis of ethyl oxoaristate yields formaldehyde, whereas ozonolysis of the dihydro-derivative does not, ethyl dihydro-oxoaristate would be represented by structure (XCVII).

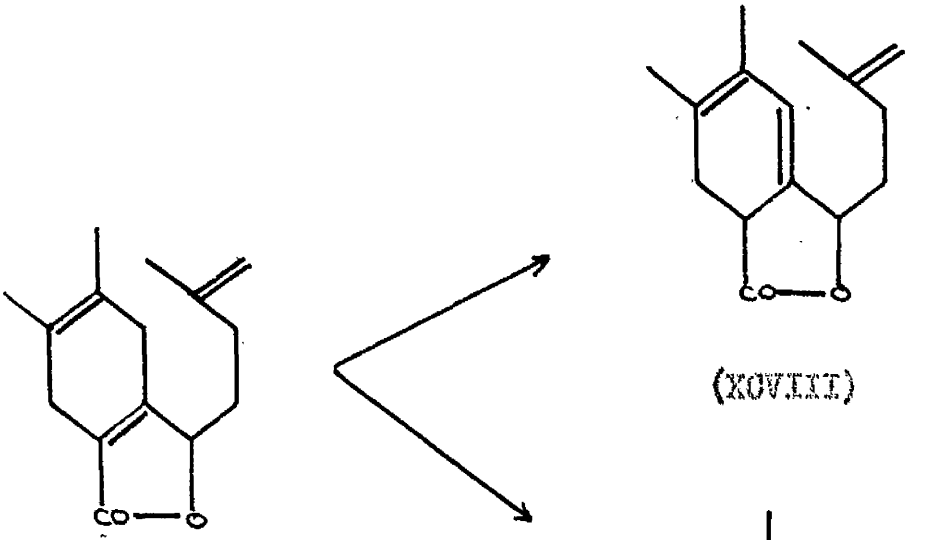


(XCVII)

On the other hand, ethyl dihydro-oxoaristate showed ultraviolet maxima at 208 mμ (ϵ, 3,600) and 287 mμ (ϵ, 52), and this decrease in absorption at 208 mμ compared with ethyl oxoaristate ($\Delta\epsilon$ approximately 3,000) is not in accord with the structure proposed, since the vinylidene in this environment would have very little absorption at that

wavelength. Another weakness of this postulate is that it does not explain the infrared absorption peak at 815 cm.$^{-1}$ in ethyl oxonristate.

It was also shown that acceptance of structure (XCVA) for aristolactone would lead to either (XCVIII) or (XCIX) for isoaristolactone, neither of which provided a satisfactory interpretation of the properties of this substance.



(XCVA)

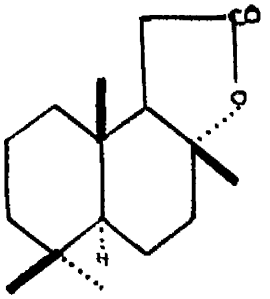
(XCVIII)

(XCIX)

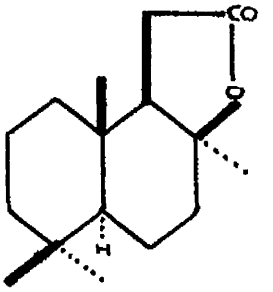
Structure (XCVIII) is unlikely since the calculated wavelength and intensity of the diene system shown (according to Woodward's rule[54]) would be 283 mμ (ε, approximately 6,000), and structure (XCIX) can be ruled out at once, since there is absolutely no evidence of triple conjugation in isoaristolactone. Thus the most suitable structure so far proposed was quite unsatisfactory on several counts.

It was suggested, as a final point in the discussion that the large

change in specific rotation ($+156^{\circ}$ to -44°) during the acid isomerisation might be interpreted as in solaresol[55] in which alteration of the method of fusion of the lactone ring occurs (trans fusion C, changing to cis fusion CI).



(C)



(CI)

The accompanying change in optical rotation in this case ($+117^{\circ}$ to -30°) is of the same order as in aristolactone. Klyne[55] has deduced that cis fusion of a five-membered ring to a six-membered ring is more stable than trans fusion and it was suggested that the acid isomerisation of aristolactone might be similarly explained as a result of a double bond shift.

In conclusion, it was observed that aristolactone might possibly be a mixture of closely related isomers, as in helenin, although this appeared unlikely. Complete explanation of the observed facts was therefore dependent on further research.

DISCUSSION

OF

EXPERIMENTAL

WORK

THE LIGHT PETROLEUM-SOLUBLE EXTRACTS FROM ARISTOLOCHIA SPECIES

A.reticulata

Aristolactone was isolated by crystallisation from the dark brown petrol-extract of the powdered root, as described by Williams[1]. It was later discovered that the yield of aristolactone could be almost doubled by chromatography of the residual extract on cellulose-charcoal (3:1) and seeding the appropriate fractions (Table 17, page 125). The chromatographic fractions were then bulked according to optical rotation into four residues (I-IV, Table 18, page 127). A small amount of free (-)-borneol was obtained (Table 20, page 129) from residue I, as well as a liquid fraction of doubtful purity. It appears therefore, that free (-)-borneol is present in A.reticulata, as well as bornyl formate[1]. Rao, Manjunath and Menon[56] have reported trace amounts of free camphor in A.indica.

Residue II (Table 18) was steam distilled. Fractional distillation of the more volatile portion produced a number of liquid fractions whose physical constants are compared with those of the corresponding fractions obtained by Williams (Table 21, page 130). 8g. of the liquid sesquiterpene reticulone[1] and about 5g. of the water-insoluble acid[1], $C_{10}H_{16}O_2$, were obtained from fractions 7 and 2-5 respectively. Residues III and IV (Table 18) were not examined.

Chromatography on alumina of the viscous brown unsaponifiable matter obtained from Williams yielded over 150 fractions, which were bulked into 11 large fractions (A-K, Table 22, page 132). On storage,

fraction A deposited a crystalline compound, m.p. 196° C, $\lambda_{max.}$ 211.5 mμ ($E^{1\%}_{1cm}$ 100), which analysed satisfactorily for $(C_{10}H_{14}O)_n$. The infrared spectrum showed a peak at 1750 cm.$^{-1}$ (potassium bromide disc) which could be due to either a γ-lactone function or to a ketone attached to a five-membered ring. This compound also showed peaks at 806 and 827 (trisubstituted double bonds), 1385 (C-methyl), 1643 and 1650 (unsaturation) and 914 cm.$^{-1}$ (vinylidene ?). It is possible that this compound is a diterpenoid lactone but further work was impossible due to the small quantity isolated.

Fractions D, E and F (Table 22, page 13) yielded β-sitosterol and a few crystals of coryl alcohol were obtained from fraction C. The β-sitosterol analysed satisfactorily for $C_{29}H_{50}O$, with one molecule of methanol of crystallisation (cf. Colombino and Kind[57]).

A. serpentaria

This species closely resembles A.reticulata but the rhizome is shorter and thinner and the roots are thin and wiry, being interlaced to form matted masses. The chief contaminant was A.reticulata and there were smaller amounts of Hydrastis canadensis rhizome, aerial parts of A.serpentaria and other vegetable material, possibly sweepings from drug-room floor. An extract was obtained, (equivalent to 4% of the powdered drug), which on crystallisation and (later) chromatography, afforded 7.8g. pure aristolactone (0.09%w/w).

A.indica

Extraction in the usual way yielded 1.83%w/w of dark green oil but no aristolactone was obtained from it either by seeding or chromatography. On standing at 0°-5° C., some white, waxy solid precipitated and was

purified by chromatography to give a white solid, m.p. 85-90° C. The nature of the crystals and the melting point suggest that this solid is probably a mixture of long-chain hydrocarbons.

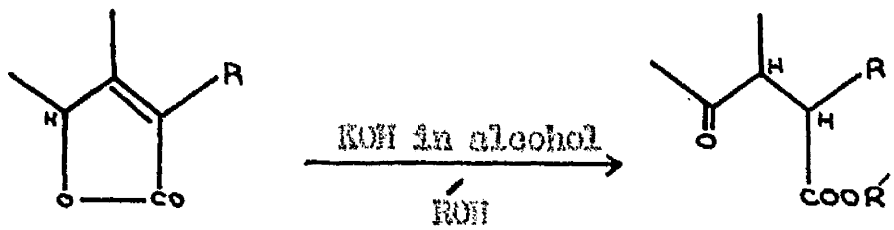
A.longa

Four species of Aristolochia have been represented as A.longa but three of those have been proved identical with other known species of the genus. The sample obtained was authenticated by reference to the Royal Botanic Gardens, Kew. The light petroleum extract represented 1% of the powdered root and no aristolactone was obtained from it.

A R I S T O L A C T O N E

THE REACTIONS AND STRUCTURE OF THE LACTONE RING

Stenlake and Williams[1,2] have satisfactorily established a number of facts concerning the structure of aristolactone. They showed that aristolactone was a sesquiterpene γ-lactone, and that a vinylidene group was present, probably in a location remote from the lactone ring. Treatment of aristolactone with acid produced an isomeric lactone, isoaristolactone, and the two lactones formed the same dihydro- derivative, shown to be dihydroisoaristolactone. The action of ethanolic potassium hydroxide on aristolactone undoubtedly yielded a keto-ester, but the partial structure (LXXIVa) proposed for aristolactone in order to explain the formation of this ester, seems open to serious criticism.

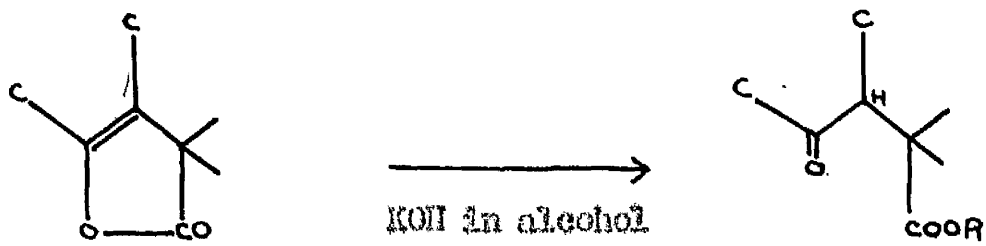


(LXXIVa) Keto-ester

An $\alpha\beta$-unsaturated lactone of this type would be expected to show an ultraviolet absorption maximum at 215-225 mμ[38], but Stenlake and Williams observed a maximum at 211 mμ (ϵ, 11,500). They attributed the position of the peak to summation of the maximum due to an isolated

tetrasubstituted double bond, at a wavelength less than 210 mμ, with the maximum due to an mμ -unsaturated γ -lactone function at longer wavelength (cf. Barton and de Mayo[58]). In the present work, the ultraviolet spectrum of aristolactone was re-examined, using a fused silica prism in order to extend the range of the instrument to shorter wavelengths. The absorption maximum was now observed at 205 mμ in absolute ethanol and at 198 mμ in 10% ethanol (Figs. 9 and 10, page 170), showing that the peak obtained by Stenlake and Williams was due to the effect of stray light. When this evidence is considered in conjunction with the fact that ozonolysis of aristolactone did not produce the pyruvic acid which would be expected from structure (LXXIVa), in which R=CH_3, there can be little doubt that aristolactone does not contain an αβ -unsaturated lactone function. This conclusion is substantiated by the failure of aristolactone to form an adduct with ammonia, since αβ -unsaturated lactones generally form such addition compounds.

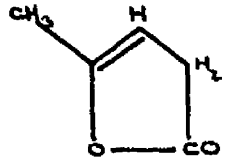
The formulation of aristolactone as a βγ -unsaturated lactone, however, is in complete agreement with experimental observations and does not conflict with the original interpretation of the alkaline rearrangement, which can now be illustrated as in scheme (CII).



(a) (b)

(CII)

A similar type of rearrangement was observed under acid conditions when α -angelicalactone (CIII) was treated with 2:4-dinitrophenylhydrazine in ethanolic sulphuric acid, the 2:4-dinitrophenylhydrazone of ethyl laevulinate (CIV) being formed.



(CIII)

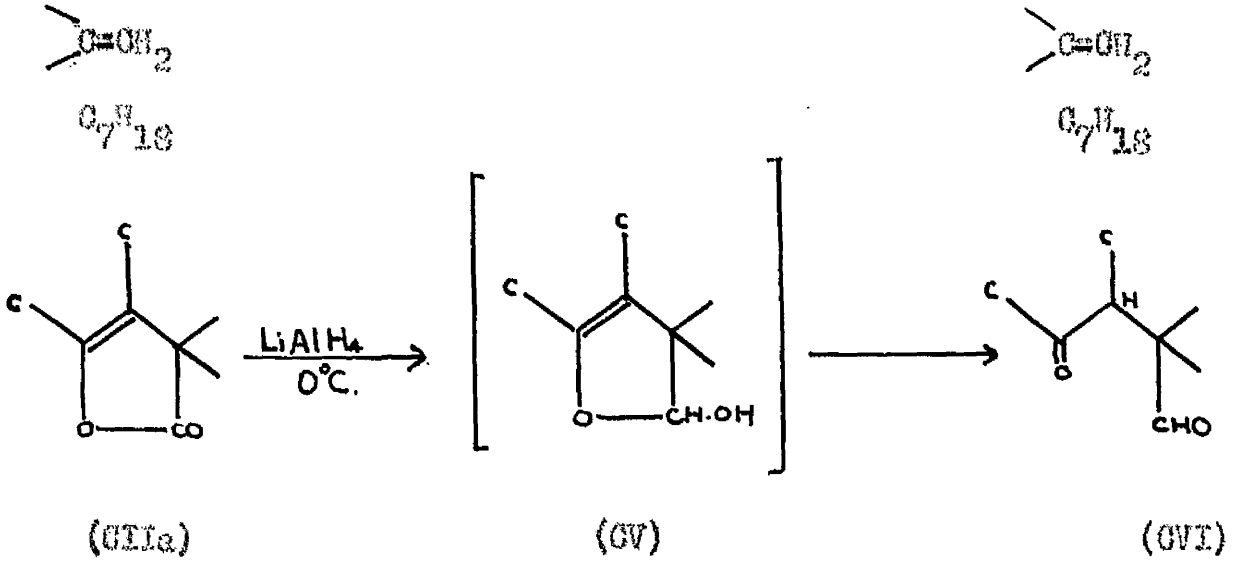
2:4-dinitrophenylhydrazine in ethanol/H_2SO_4 → $CH_3COCH_2CH_2COOC_2H_5$ (Isolated as 2:4-D.N.P.

(CIV)

The βγ -unsaturated lactone formulation provides a more satisfactory explanation of the fractional iodine values obtained for aristolactone since, according to Cavallito and Haskell[59], βγ -unsaturated lactones give fractional values whilst αβ -unsaturated lactones do not react. Structure (CIIa) also explains the failure of aristolactone to give a positive result in the Legal test for αβ -unsaturated lactones[52], although it must be said that the specificity of this test is doubtful since alantolactone (IIa) also failed to give a positive result.

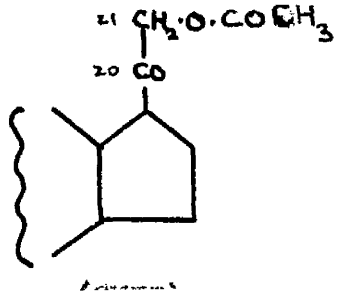
Stenlake and Williams observed in their early (unpublished) work on aristolactone that hydrogenation at an extremely active platinum catalyst in a mixture of acetic and hydrochloric acids showed an uptake equivalent to 4 mols. of hydrogen, the product being an acid. Hydrogenolysis in this way would be more readily explained if aristolactone contains a βγ -unsaturated lactone ring, rather than an αβ -unsaturated function[60,61].

Perhaps the most satisfactory single piece of evidence in support of structure (CIIa) was provided by lithium aluminium hydride reduction of aristolactone at low temperature (0°C). The crystalline product, a 1:4-ketal, $[\alpha]_D + 82°$, $\lambda_{max.}$ 284 mμ (ε, 58) and end-absorption (ε, 3234 at 210 mμ), was formed via the intermediate hemi-acetal[62] (CV), and is represented by partial structure (CVI).

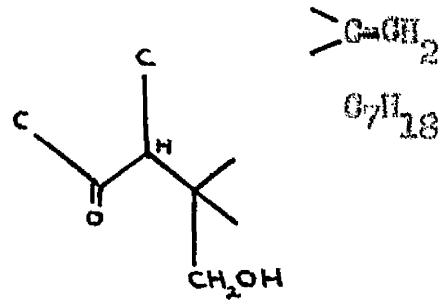


(CIIa) (CV) (CVI)

The ketal reduced ammoniacal silver nitrate solution and restored the colour to Schiff's reagent. The infrared absorption curve exhibited peaks at 904 and 1645 (vinylidene), 1752 (aldehyde carbonyl) and 1733 cm.$^{-1}$ (shoulder; ketone carbonyl). It is postulated that the abnormally high carbonyl frequencies are due to carbonyl interaction, since the 21-acetoxy-20-ketosteroids in which the two carbonyl groups are similarly spaced (CVII) show similar displacement to higher frequencies[63,64].

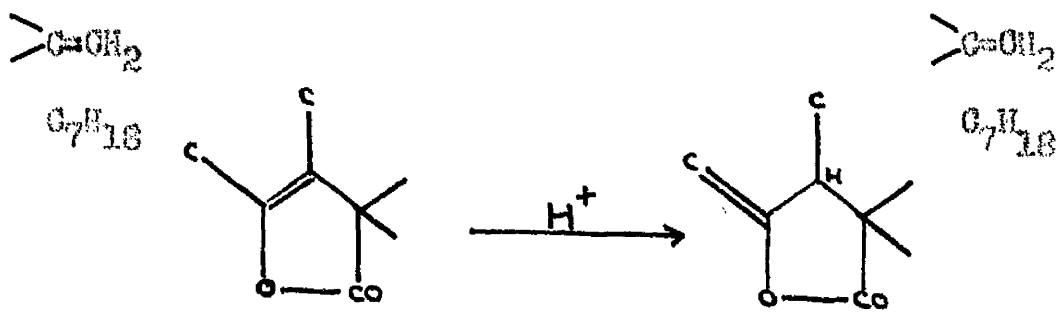


More vigorous reduction of aristolactone with lithium aluminium hydride[1] afforded the 1:4-ketol (partial structure CVIII), which showed end-absorption at 210 mμ and low-intensity absorption at 280 mμ (ketone).



(CVIII)

The acid isomerisation of aristolactone to isoaristolactone is known to involve a shift of the lactone ring double bond[1], and, in view of the proposal that aristolactone contains a double bond βγ - with respect to the lactone carbonyl, the isomerisation is now interpreted as rearrangement of a less stable βγ -endopentacyclic double bond (CIIa) to the more stable exopentacyclic position (CIX)[50].



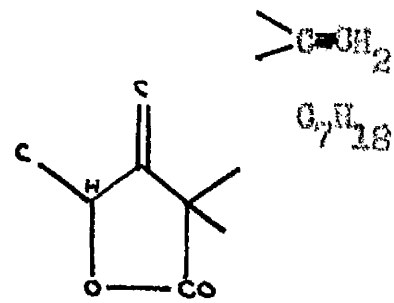
(CIIa) (CIX)

It has already been shown (Stenlake and Williams) that a similar rearrangement occurs as a hydrogen-catalyst induced migration of a

double bond during the partial reduction of aristolactone which results in the formation of dihydroisoaristolactone. The stereochemistry of such migrations has been discussed by Dreem, Eaton and Henbest[69] and it can be therefore concluded that approach to the catalyst is hindered at one face of the molecule.

Greatly improved yields of isoaristolactone were obtained (about 80%) by treating aristolactone with 10% sulphuric acid in ethanol (50%) at room temperature. This improvement followed an earlier modification of the original method[1] which increased the yield to 35% by heating with a sulphonic acid resin. The improved yields provided a much better supply of this interesting compound and facilitated the work upon it.

Since lithium aluminium hydride reduction of isoaristolactone yielded a second (isomeric) ketal, designated isoketal, $C_{15}H_{22}O_2$, the possible alternative partial structure for isoaristolactone (CX) can be confidently excluded.



(CX)

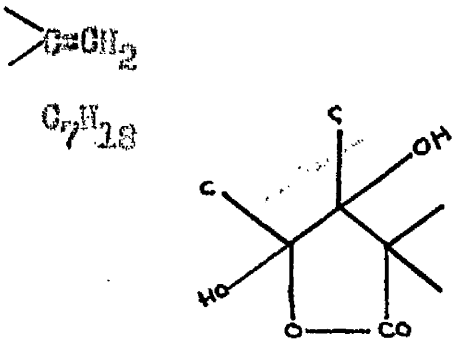
This 1:4 isoketal (CVI), $[\alpha]_D$ -50.1°, $\lambda_{max.}$ 290 mμ (ε, 30) and end-absorption (ε, 4,960 at 209 mμ), also reduced ammoniacal silver nitrate and gave a positive test with Schiff's reagent. Probably the only difference between the isomeric 1:4-ketals (CVI) is that they have

opposite configurations at the β-carbon atom (β- with respect to the the aldehyde function) since a new optical centre is introduced at this point in the formation of both compounds, existing optical centres being unaffected by lithium aluminium hydride.

THE DEGREE OF UNSATURATION

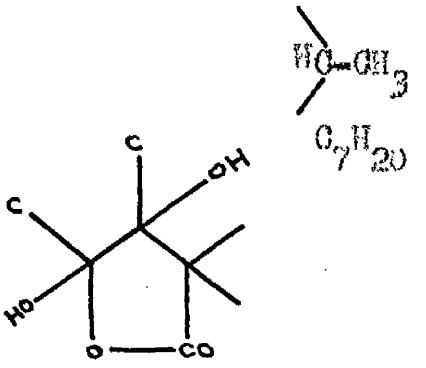
Since the argument that aristolactone has a monocarbocyclic skeleton is entirely dependent on the number of double bonds in the molecule, the evidence for three double bonds was reviewed and extended. The preparation of a hexahydro-derivative and methyl tetrahydro-oxo-aristate by Stenlake and Williams provided strong support for three double bonds. The question of iodine values (or bromine numbers) was reconsidered in detail. Stenlake and Williams carried out their iodine value determinations according to the pyridine bromide method of the British Pharmacopoeia, 1953, which uses carbon tetrachloride as solvent and a reaction time of 10 minutes. By this means the above authors obtained consistent values of approximately 2.5 double bonds for both aristolactone and isoaristolactone. It is now shown that much more accurate results can be obtained with known compounds by allowing the reaction to occur for only two minutes (Table 1). Under these conditions, aristolactone and isoaristolactone gave values equivalent to just over two double bonds.

Dihydroxyaristolactone (CXI), which is prepared by oxidation of aristolactone with potassium permanganate in acetone[1], and the isoketol (CVI) gave values equivalent to almost exactly two double bonds. The preparation of both compounds involves the elimination of a double bond so that these values provide further evidence for the third double bond in the parent lactone. This was further substantiated by isolation of tetrahydrodihydroxyaristolactone (CXII) on complete hydrogenation of dihydroxyaristolactone.



C_7H_{18}

(CXI)



C_7H_{20}

(CXII)

Table 1

Iodine Values

Compound	Number of double bonds	Time of reaction	Iodine value
Aristolactone	3	2 mins.	2.05; 2.35
"	3	10 mins.	2.58
"	3	2 mins.	2.54; 2.63#
"	3	10 mins.	2.56 #
Iso Aristolactone	3	2 mins.	2.03
"	3	10 mins.	2.35
"	3	16 hours	2.59
Dihydroxyaristolactone	2	2 mins.	1.90
"	2	10 mins.	2.25
Iso Kotal	2	2 mins.	1.90
"	2	10 mins.	1.98

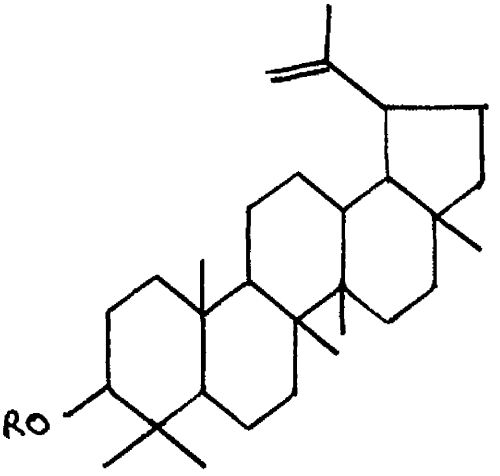
(cont'd. on page 65)

Table I (cont'd.)

Iodine Values

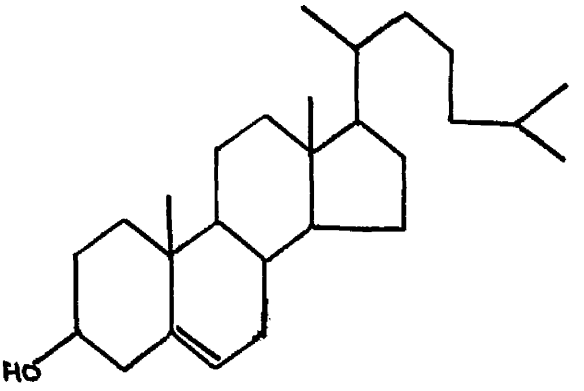
Compound	Number of double bonds	Time of reaction	Iodine value
Lupenyl acetate (page 66)	1	2 mins.	1.07
"	1	10 mins.	1.50
"	1	2 mins.	1.55 *
Cholesterol (page 66)	1	2 mins.	1.02
"	1	2 mins.	1.09 *
β-Sitosterol (page 66)	1	10 mins.	1.30
Hederagenin methyl ester diacetate (page 66)	1	2 mins.	0.65
"	1	10 mins.	0.90
α-Angelicalactone (CIII; page 58)	1	2 mins.	0.77
"	1	10 mins.	0.90
Reticulene[1] (structure unknown)	2	2 mins.	1.73
"	2	10 mins.	1.90

* Solvent, chloroform.

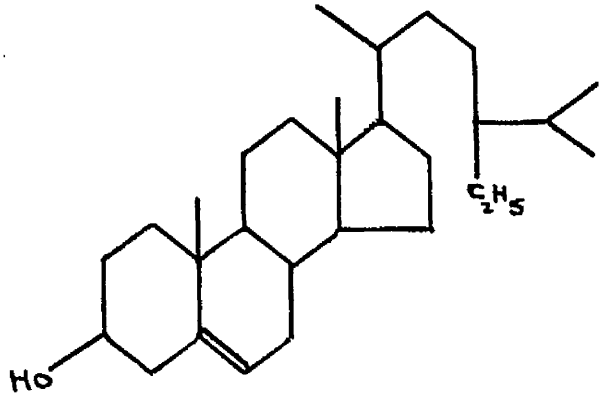


Lupenyl acetate (R=CH_3CO)

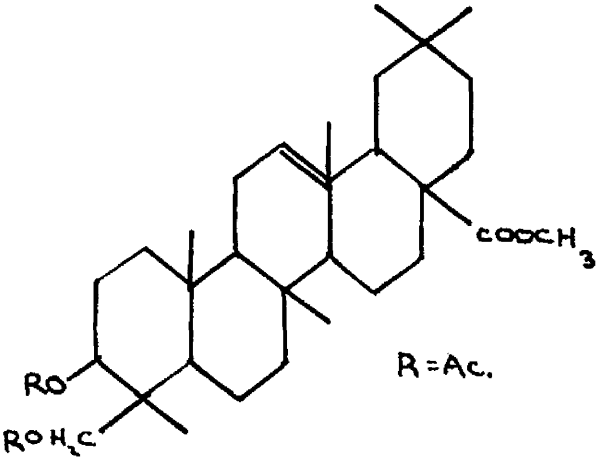
Lupeol (R=H)



Cholesterol



β-Sitosterol



Hederagenin methyl ester diacetate

The presence of the 1:2-glycol structure in tetrahydrodihydroxy-axistolactone was demonstrated by oxidation with sodium bismuthate[66], although the oily oxidation product gave an amorphous 2:4-dinitro-phenylhydrazone which was unstable and resisted characterization.

Oxidation of dihydroxyaristolactone with sodium metaperiodate[66a] did not yield formaldehyde, thereby establishing that the hydroxyl groups had not added to the vinylidene group; confirmation was provided by the infrared spectrum which exhibited a peak at 906 cm.$^{-1}$. It follows that the glycol link in dihydroxyaristolactone is as shown in partial structure (CXI) and further corroboration was provided by (a) its failure to rearrange in acid (compare with aristolactone) and (b) by a comparison of the end-absorption in the ultraviolet (ϵ , 3,200 at 210 mμ) with that of the ketal (CVI) (ϵ , 3,234 at 210 mμ) and the isoketal (CVI) (ϵ , 4,960 at 210 mμ).

With the confirmation of the third double bond in aristolactone, it follows that aristolactone is based on a monocarbocyclic system, as suggested by Stenlake and Williams.

DEHYDROGENATION EXPERIMENTS

At first, using the method of dehydrogenation adopted by Williams[1], on pure crystalline hexahydroaristolactone, no positive results were obtained. The liquid which distilled became solid on standing and was identified as unchanged starting material. Further heating yielded a liquid product which did not have an ultraviolet absorption spectrum typical of any aromatic system.

Later, using a sand-solid residue remaining from the preparation of hexahydroaristolactone (from pure aristolactone), and heating with 20% palladium-charcoal, a violet azulene was produced which was isolated and purified by a phosphoric acid separation. On two other occasions, a violet azulene was obtained in rather better yield (as judged by depth of colour, no actual weight of azulene being isolated) by a modified technique. The modification consisted of gradually heating the mixture of the material and palladium-charcoal catalyst to about 330°C. and maintaining it at this temperature until no more azulene was produced. The azulenic material was removed with a finely drawn test pipette as it distilled into a small bulb blown in the mill of the reaction tube and was washed out into a solvent, usually hexane or cyclohexane. The azulene-producing experiments are recorded in Table 2. The oils used in each case were obtained from pure aristolactone which gave no azulenic or naphthalenic product on similar treatment.

Table 2

Starting material	Method of dehydrogenation	$\lambda_{max.}$ of azulene product (mμ)
1. Semi-solid oil, from preparation of hexahydroaristolactone	Dry distillation over a micro-burner	279, 289, 306, 333, 348
2. Mixed oily residues from preparation of methyl oxoaristate, isoaristolactone and dihydroxyaristolactone	Dry distillation at 330°C.	280, 289, 306, 333, 348
3. Oily residue from preparation of isoaristolactone	"	279, 288, 306, 333, 348

It is quite certain from the shape of the three ultraviolet absorption curves and the positions of the various maxima that the three azulenic products are identical. Further, the clear definition of the peaks and the absence of shoulders or other irregularities (Fig. 1, page 70) indicate that the azulenes were pure. There was an additional maximum at approximately 245 mμ, but the hexane and cyclo-hexane used were nearly opaque at this wavelength and the exact position could not be determined accurately.

Two other dehydrogenation experiments (Table 3) afforded products having an absorption spectrum typical of a naphthalenic derivative, the product from one being a crystalline solid of sharp melting point but insufficient (yield less than 1 mg.) for characterisation.

Fig. 1

Ultraviolet spectrum of aristazulene
(hexane)

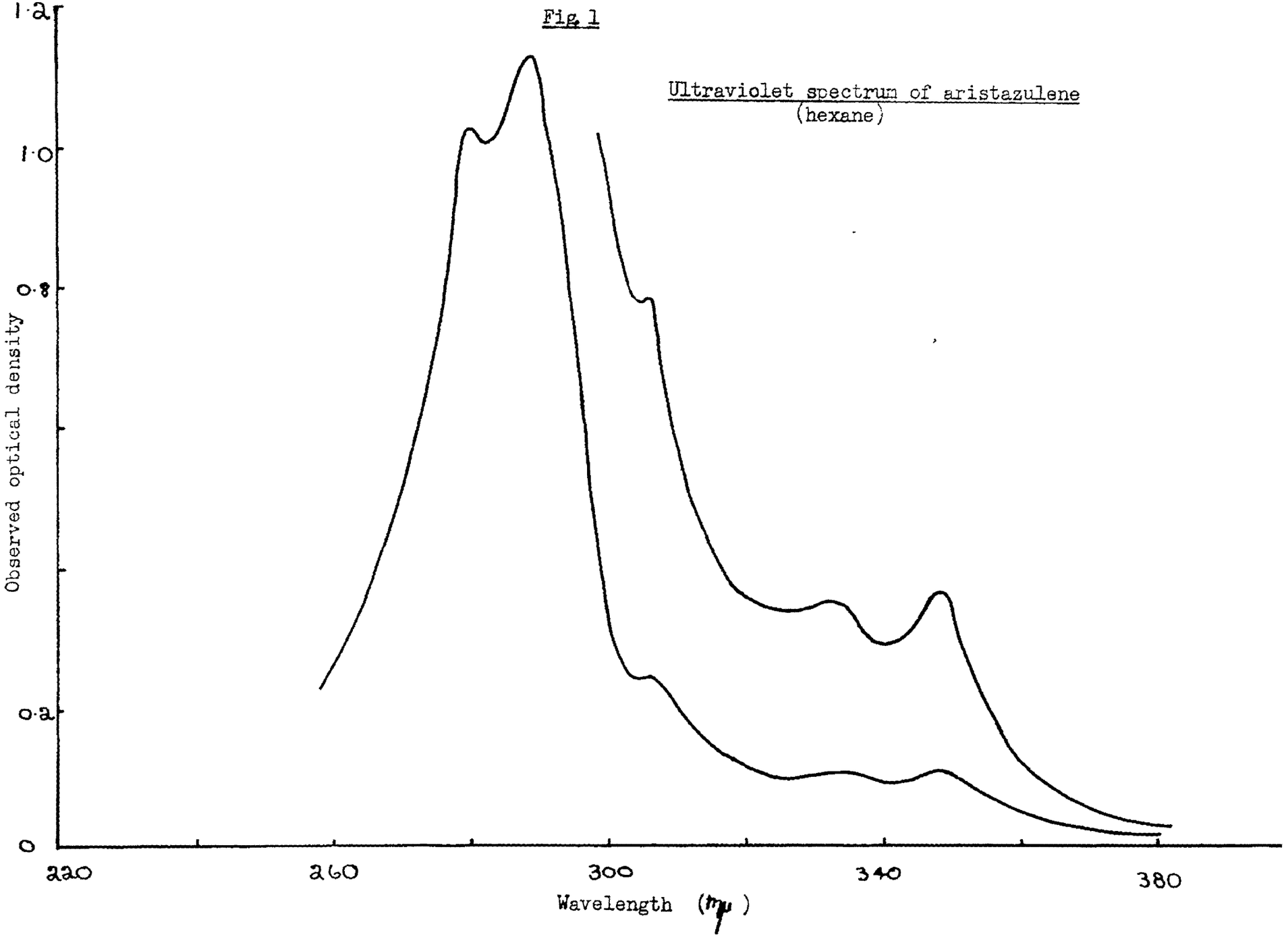


Table 3

Starting material	Method of dehydrogenation	$\lambda_{max.}$ of product (mμ)
1. Mixed oily residues as in Table 2	Dry distillation at 330° C.	229, 286, 318. (crystals, m.p. 144° C
2. Pure hexahydroaristolactone	"	228, 281, 312.

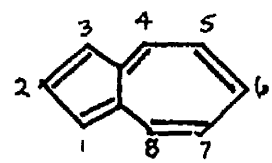
It is possible that use of oily starting materials in dehydrogenation experiments facilitated the formation of azulenes and naphthalenes, since one difficulty experienced in earlier work was that pure crystalline compounds tended to sublime very quickly and hence could not be dehydrogenated. It is worthy of note that no azulenes were obtained from pure crystalline starting material, although a naphthalene oil was isolated after dehydrogenation of pure hexahydroaristolactone. The use of a bath of Wood's metal was a further improvement in the method, since the more even and gradual heating permitted more prolonged interaction between catalyst and material. The constitution of the oils used is uncertain but the chance that they resulted from major structural alterations is remote, since the various preparations from pure aristolactone were carried out at room temperature or below. It is probable that the non-crystallisable residues used consisted of racemised, hydrogenolysed or partially reacted material.

Dehydrogenation of pure aristolactone with zinc dust gave a product which showed only end-absorption in the ultraviolet region and neither this, nor any of the palladium-charcoal catalysed dehydrogenations yielded

a product having a typical benzenoid absorption in the ultraviolet.

IDENTIFICATION OF THE AZULENE

Several generalisations can be made concerning azulenes and these help to narrow the field considerably. The azulene molecule, which consists of a seven-membered ring fused to a five-membered ring, as in structure (CXIII), possesses highly characteristic ultraviolet and visible absorption spectra. Substitution of the molecule with alkyl groups yields derivatives which have spectra different from azulene itself and these spectra are highly characteristic of the position to which the substituent is attached.



(CXIII)

Positions 1 and 3 are equivalent in the sense that substitution in these positions with the same alkyl radicle causes the same shift in the wavelengths of maxima absorption. Similarly, position 4 is equivalent to position 8 and 5 is equivalent to 7.

It has been found[67] that substitution in positions 1,3,5 or 7 causes a bathochromic shift of the absorption maxima and hence substitution in these positions produces blue azulenes. On the other hand, substitution in positions 2,4,6 or 8 causes a hypsochromic shift in the absorption maxima and produces violet azulenes.

A further generalisation can be made by inspection of published ultraviolet spectra, that, with the one exception of 1:4:8-trialkyl-

substituted azulenes (Table 6, page 76), all azulenes having a substituent in position 1 exhibit an absorption maximum between 360 and 370 mμ. The almost pure violet colour of the azulene obtained from aristolsetone derivatives and the lack of an absorption peak above 350 mμ indicate, therefore, that substituents are absent from positions 1 and 3.

The ultraviolet absorption maxima of the five monomethyl azulenes[68] are listed in Table 4 with those of the aristoluctone azulene (designated aristazulene for convenience). Examination of these shows that only the 2-methylazulene bears any close resemblance to aristazulene. The peaks of aristazulene, however, are generally a few millimicrons nearer the visible than those of 2-methylazulene, although the general shape of the two curves is almost identical. This positive shift of the maxima

Table 4.

Compound	Wavelengths of maxima (mμ)
1-methylazulene	240, 280, -, 299, 338, -, 347, 364.
2-methylazulene	240, 275, 284, 304, 333, -, 347, -.
4-methylazulene	241, 276, 284, 302, 332, 341, 346, 357.
5-methylazulene	238, 279, -, 298, 327, 338, 343, 353.
6-methylazulene	237, 283, -, 299, 331, -, 344, -.
aristazulene	(245), 279, 289, 306, 333, -, 348, -.

compared with those of 2-methylazulene suggests heavier substitution is present, but this is not simply an ethyl or isopropyl group in position

2, rather than a methyl, since the ultraviolet spectra of 2-ethylazulene and 2-isopropylazulene show negligible differences compared with 2-methylazulene.

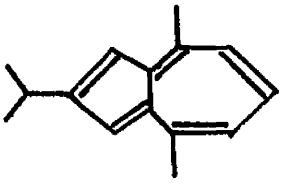
It was therefore assumed that other substituents must be present and available spectra of dialkylazulenes were examined (Table 5), omitting azulenes having substituents in position 1 for the reasons already stated. Only the spectrum of 2:6-dimethylazulene in any way resembled the curve of aristazulene but the low wavelength of the first peak (234 mμ) and the presence of a peak at 364 mμ showed that the two compounds were not identical.

Table 5

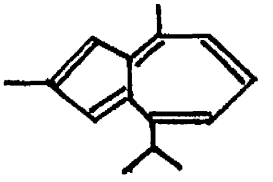
Compound	Wavelengths of maxima (mμ)	Reference
2:6-dimethylazulene	234, 278, 287, 307, 334, 350, 364.	69
4:5-dimethylazulene	244, 280, 306, 325, 333, 342, 353.	70
4:6-dimethylazulene	240, 278, 282, 288, 304, 333, 348.	69
4:8-dimethylazulene	246, 283, 331, 339, 344, 355.	68
aristazulene	(245), 279, 269, 306, 333, 348.	present work

Consideration of trialkylazulenes, however showed a very close resemblance between the spectrum of aristazulene and the spectrum of 2:4:8-trimethylazulene (Table 6). The shapes of the two curves are practically identical and there is only a very small discrepancy in the wavelengths of the maxima. The spectrum of vetivazulene (2-isopropyl-4:8-

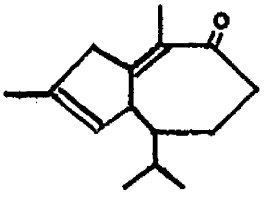
dimethylazulene CXIV), is also very like that of aristazulene although the entire absorption curve of vetivazulene seems to be about 3 mμ nearer the visible region, according to the only available modern spectrum[71]. Zierazulene[72] (CXV), obtained by dehydrogenation of zierone (CXVI) has been identified as 2:4-dimethyl-8-isopropylazulene but unfortunately no spectrum was available.



(CXIV)



(CXV)



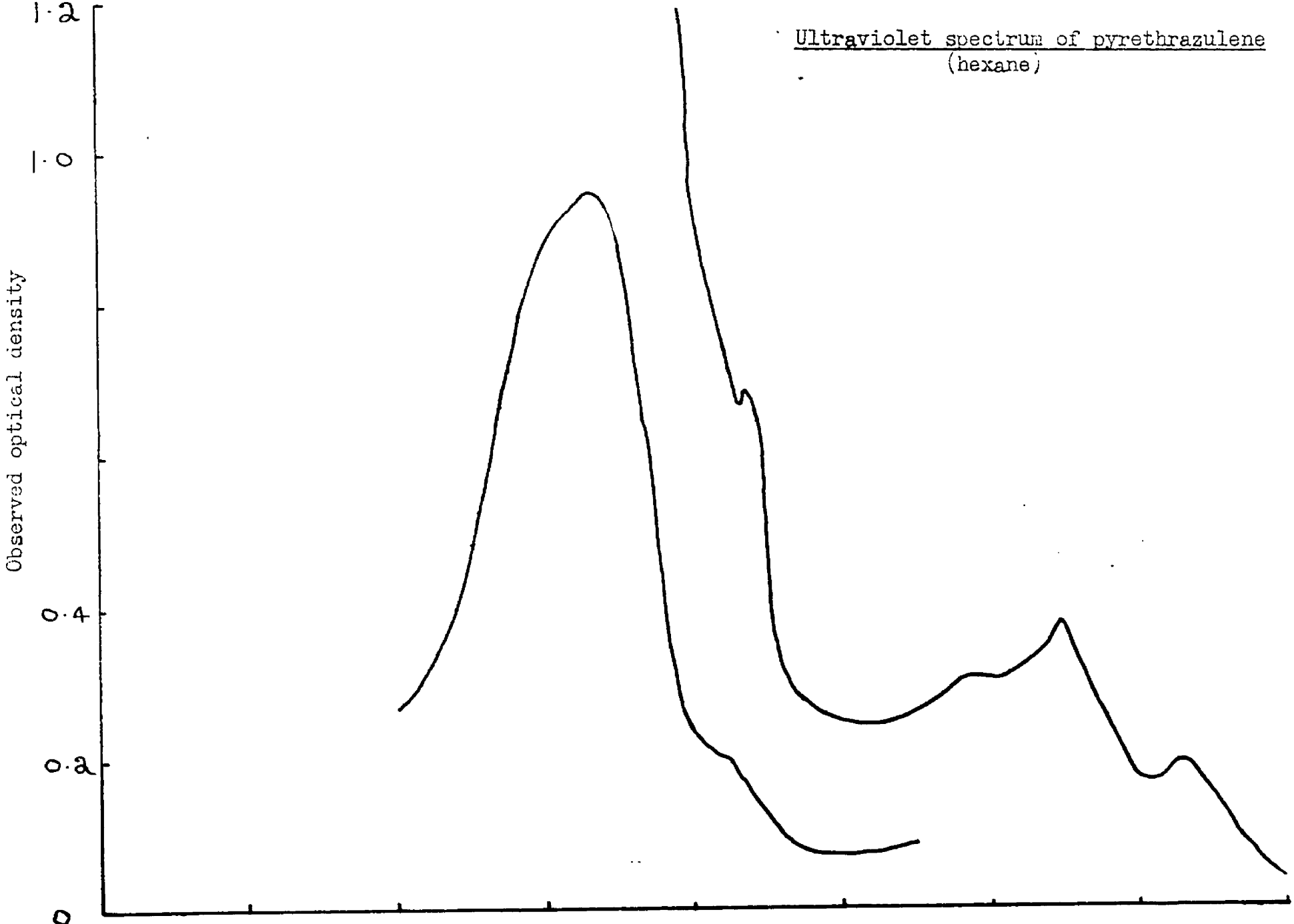
(CXVI)

Table 6

Compound	Wavelengths of maxima (mμ)	Reference
1:4-dimethyl-7-isopropyl-azulene (guaiazulene)	245, 290, 306, 336(inflect) 350, 368.	75
1:4:8-trimethylazulene	246, 286, 335, 348.	76
1:4-dimethyl-8-isopropyl-azulene	246, 287, 335, 348.	76
2:4:5-trimethylazulene	246, 279, 287, 305, 324, 331, 348.	70
2:4:8-trimethylazulene	248, 280, 288, 309, 335, 349.	73
2-isopropyl-4:8-dimethyl-azulene (vetivazulene)	290, 308, 336, 350* 251, 284, 292, 311, 335, 351*	74 71
aristazulene	(245), 279, 289, 306, 333, 348.	Present work

* Measured photographically.

Fig. 2


Ultraviolet spectrum of pyrethrazulene
(hexane)
1·2
1·0
0·4
0·2
0
Observed optical density

Pyrethrazulene, the intensely blue azulene obtained from pyrethrosin by Schechter and Haller[25] was thought by these workers to be 2:4:8-trimethylazulene. Present knowledge of the colour of azulenes in relation to constitution[67] renders this theory unlikely, as shown by Horn[73]. Confirmation of Horn' work was provided by zinc dust distillation of pyrethrosin in a bath of Wood's metal at 330°C. The blue azulene produced was purified and isolated (in solution) in the usual manner and the ultraviolet spectrum was determined (Fig. 2, facing page 77). The curve obtained showed peaks at 287, 307, 338 (inflect.), 349, and 366 mμ and this was practically identical in shape and wavelengths of the maxima with the ultraviolet spectrum of guaiazulene (Table 6). Since chamazulene (1:4-dimethyl-7-ethylazulene) was, in fact, isolated by Barton and de Mayo[7] from a pyrethrosin derivative (structure XVIII; R=Ac), it is therefore very likely that pyrethrazulene is really chamazulene. It is quite probable that the spectrum obtained by Schechter and Haller was poorly defined, due to the instruments in use at that time (1941), since Horn[73] (1951) was unable to demonstrate any marked difference between pyrethrazulene and 2:4:8-trimethylazulene, although he knew from the colours alone that they must be completely different.

Ultraviolet spectra of azulenes in 50% v/v sulphuric acid

In strong acid solution, azulenes form azulenium ions which exhibit characteristic ultraviolet spectra, quite different from those of the parent azulenes. In every case, three distinct maxima are shown and the wavelengths of these maxima are characteristic of the positions to which

Table 7

Compound	Wavelengths of maxima (mμ)
1-methylazulene	366, 266, 224.
4-methylazulene	351, 262, 227.
5-methylazulene	351, 261, 226.
2-methylazulene	370, 262, 225.
2-ethylazulene	372, 264, 223.
2-isopropylazulene	373, 264, 223.
1:2-dimethylazulene	382, 267, 226.
1:4-dimethylazulene	362, 271, 228.
1:8-dimethylazulene	360, 268, 227.
4:8-dimethylazulene	355, 267, 227.
chamazulene (1:4-dimethyl-7-ethyl)	358, 277, 232.
guaiazulene (1:4-dimethyl-7-isopropyl)	358, 277, 231.
pyrethrazulene (Structure unknown - Fig. 4)	363, 272, 230.
vetivazulene (2-isopropyl-4:8-dimethyl)	374, 272, 228.
4:8-dimethyl-6-isopropylazulene	353, 268, 237.
aristazulene (Fig. 3)	375, 268, 226. 374, 269, 227.

alkyl substituents are attached. The examples in Table 7 were obtained from the work of Chopard-dit-Jean and Heilbronner[77]. A number of azulenes already shown to be quite different from aristazulene, are included in this table in order to illustrate the differences induced by substitution. Again aristazulene (Fig. 3, page 80) and vetivazulene exhibit very similar spectra. (In sulphuric acid 50%V/v, pyrethrazulene does not resemble chamazulene so closely as it did in organic solvents, Fig. 4, page 81).

Consideration of visible absorption spectra

The majority of azulenes exhibit a characteristic visible spectrum, containing a number of well defined maxima which can be utilised in identification of unknown azulenes. Unfortunately, aristazulene showed only broad general absorption in this region, with a few small but definite peaks arising from it, as listed in Table 8. The absorption maxima of other 2:4:8-trisubstituted azulenes are also recorded there.

Table 8

Compound	Wavelengths of maxima (mμ)	Reference
2:4:8-trimethylazulene	545, 592.	73
2-isopropyl-4:8-dimethyl-azulene (vetivazulene)	546, 582, 592, 648.	78
2:4-dimethyl-8-isopropyl-azulene (sloerazulene)	546.	72
aristazulene	552, 560, 582, 590, 640. 559, 561, 585, 593, 642.	Present work

Fig. 3

Ultraviolet spectrum of aristazulene
(50% sulphuric acid)

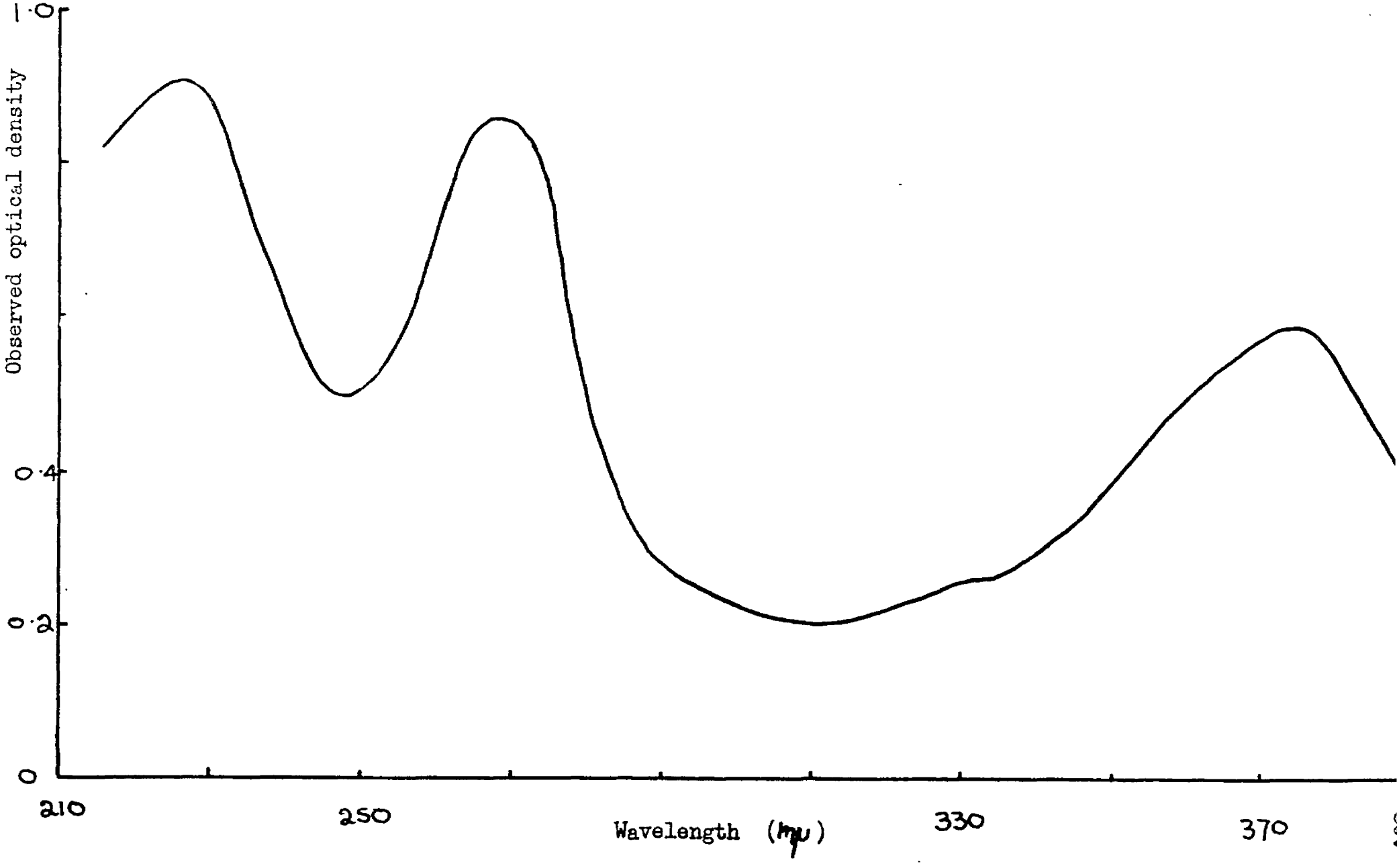
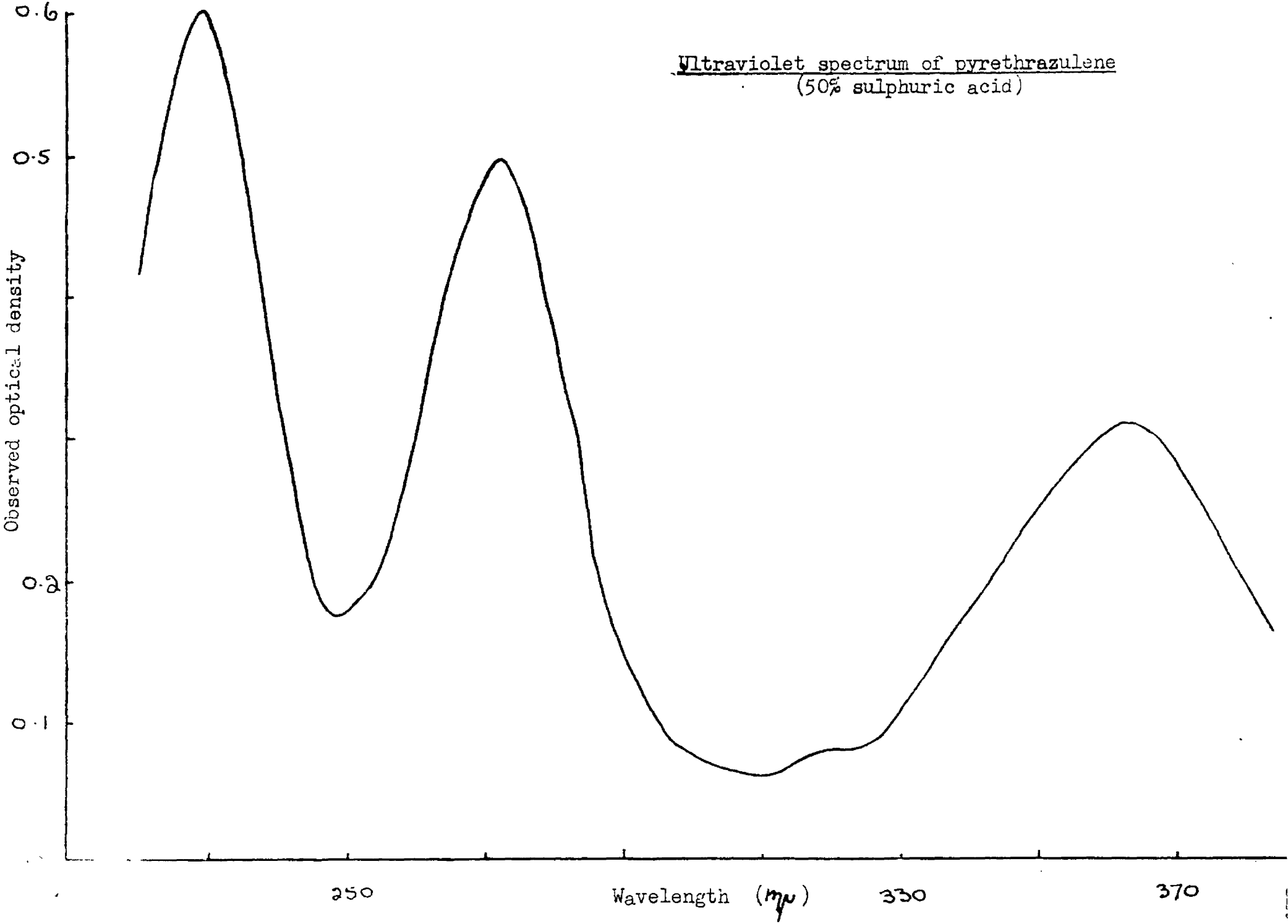


Fig. 4


Ultraviolet spectrum of pyrethrazulene
(50% sulphuric acid)
0·6
0·5
0·2
0·1
Observed optical density
250
Wavelength (mμ)
330
370

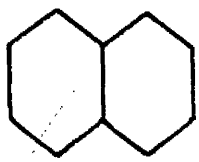
Sörensen and Hougen[71] showed that the azulene obtained by dehydrogenation of elemol (elemazulene) was in fact identical with vetivazulene and they stated that the visible spectrum gave no sharp maxima, as has been found with aristazulene.

Consideration of all the above evidence concerning aristazulene leaves very little doubt that it is a 2:4:8-trisubstituted azulene. No definite conclusions can be drawn as to the actual substituents in the respective positions but a possibility exists that aristazulene is identical with vetivazulene (CXII).

[69]
CXIV

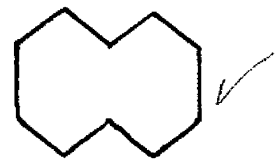
THE CARBON SKELETON OF ARISTOLACTONE

There are four carbon skeletons from which an azulene might conceivably be produced by dehydrogenation. These skeletons are perhydronaphthalene (CXVII), perhydroazulene (CXVIII), cyclodecane (CXIX) and the group consisting of a ring with a side-chain, as in xanthinin (CXX) and elemol (CXXI).

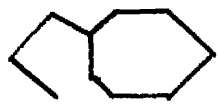


(CXVII)

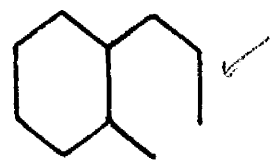
(CXVIII)



(CXIX)



(CXX)

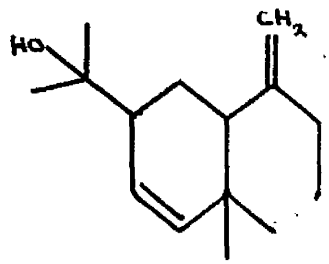


(CXXI)

Since aristolactone is known to be monocarbocyclic, skeletons (CXVII) and (CXVIII) can be rejected at once. Geissman and Sorm have not reported the presence of azulenic, naphthalenic or benzenoid products after dehydrogenation of xanthinin or xanthatin and a skeleton of this type (CXX) seems unlikely.

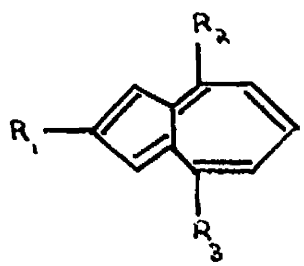
Although production of naphthalenic and azulenic materials from aristolactone derivatives does not necessarily mean rejection of skeleton (CXXI), since vetivazulene and a naphthalene derivative were isolated

from elemol[72] (CXXII), other evidence to be discussed later indicates that a cycloheptane skeleton (CXIX) offers the most satisfactory explanation of the properties and reactions of aristolactone.



(CXXII)

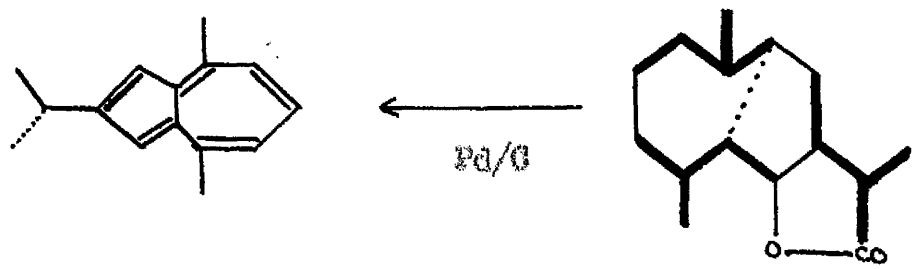
It has already been shown on spectroscopic evidence that dehydrogenation of various aristolactone derivatives affords a 2:4:8-trisubstituted azulene (CXXIII), and, although the nature of the individual alkyl groups has not been rigidly established, it is highly probable that one is either ethyl or isopropyl and that the remaining two are methyl groups.



(CXXIII)

Even in the unlikely event that all three substituents are methyl groups, the ensuing arguments are not affected, but on the assumption that the two possible structures for aristazulene are (CXXIV) and (CXXV),

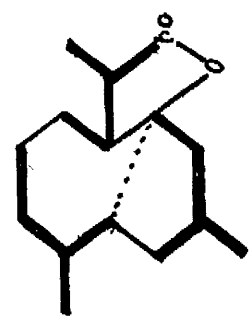
these could only arise from the carbon skeletons (CXXVI), (CXXVII) and (CXXVIII). This, however, disregards the possibility of methyl group migration during dehydrogenation.



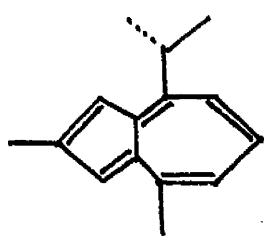
(CXXIV)

(vetivazulene)

(CXXVI)

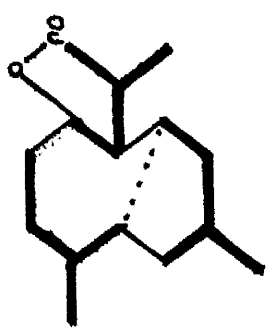


(CXXVII)



(CXXV)

(sesquiterpene)



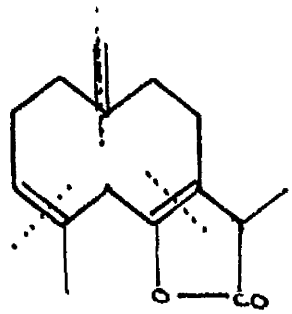
(CXXVIII)

As in other work of this nature, only structures which follow the isoprene rule are considered but on these grounds, structures (CXXVII) and (CXXVIII) must be rejected since the isoprene units, into which both can be divided, are joined in a very irregular manner (head-tail-tail-

head-head-tail) for which there is no precedent. Structure (CXXVI), on the other hand, follows the isoprene rule and is therefore regarded as the skeleton of aristolactone.

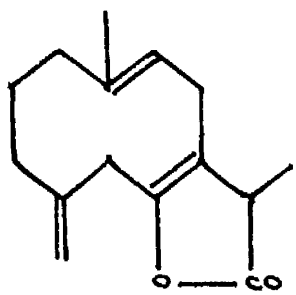
THE STRUCTURE OF ARISTOLACTONE AND METHYL OXOARISTATE

On the basis of skeleton (CXXVI) and partial structure (CIIa), aristolactone is formulated as in structure (CXXIX).

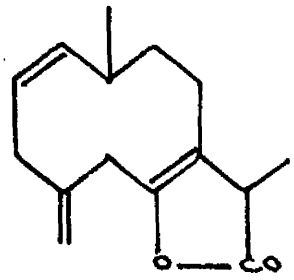


(CXXIX)

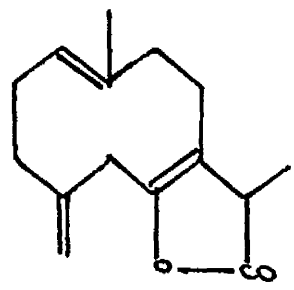
This is in complete agreement with the results of chromic acid oxidation reported by Stenlake and Williams[1], which gave acetic acid (1.6 mols.) equivalent to 2 C-methyl groups, and succinic acid as the only other large identifiable fraction, which could arise by cleavage of structure (CXXIX) as indicated by the dotted lines. These products clearly exclude such structures as (CXXX), and, if we omit all structures which contain conjugated double bonds, for which there is no evidence, only two possible alternatives (CXXXI) and (CXXXII) are left.



(CXXX)

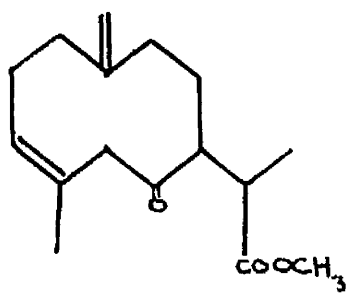


(CXXXI)

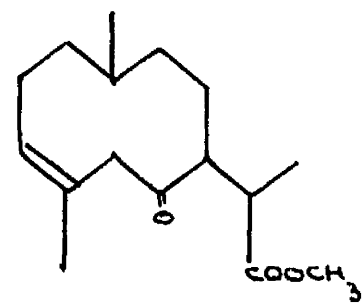


(CXXXII)

Confirmation of the position of the two non-lactonic double bonds as in structure (CXXIX) is provided by the action of alkali (described below) on methyl oxoaristate and methyl dihydro-oxoaristate, which are formulated as structures (CXXXIII) and (CXXXIV) respectively.



(CXXXIII)



(CXXXIV)

Treatment of methyl oxoaristate (CXXXIII) with warm methanolic potassium hydroxide solution gave a new crystalline acid, iso-oxoaristic acid, $C_{15}H_{22}O_3$, $[\alpha]_D$ -3.45°, which exhibited an ultraviolet absorption maximum at 243 mμ (ε, 6,780). The progress of this reaction was followed spectrophotometrically, by withdrawing samples at intervals from the reaction mixture, diluting as required and examining the ultraviolet spectrum, when a peak gradually appeared at 240-250 mμ, culminating in the maximum at 243 mμ; at the same time, the low-intensity carbonyl absorption at approximately 290 mμ (typical of methyl oxoaristate) slowly disappeared (Fig. 5). The wavelength of maximum absorption of iso-oxoaristic acid is in excellent agreement with the calculated wavelength (239 mμ) for an αβ-unsaturated ketone having two substituents on the β-carbon atom[40]. Formation of such a carbonyl compound by treatment with alkali confirms the presence of the

Fig. 5

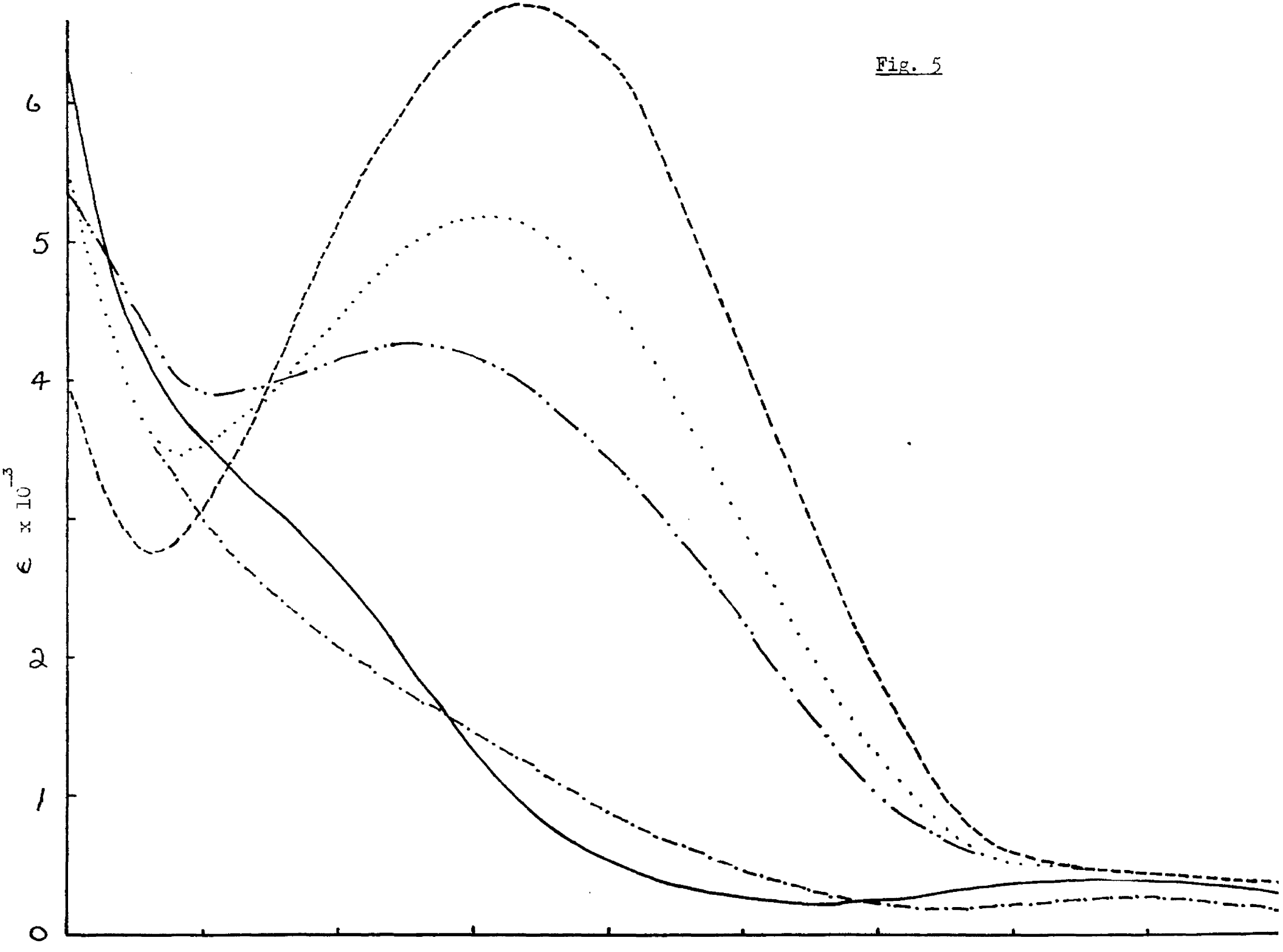


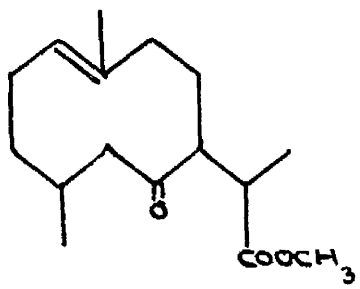
Figure 5

————	Curve I	Methyl oxoaristate.
·—·—·—·	Curve II	Methyl oxoaristate, after 11 days treatment with potassium hydroxide at room temperature.
··—··—··—	Curve III	Methyl oxoaristate, after 25 days treatment with potassium hydroxide at room temperature and warming on the water-bath for 2 hours.
·· ·· ·· ··	Curve IV	After an additional 2½ hours warming.
— — — — —	Curve V	Isooxoaristic acid.

The calculated intensities of curves II-V are based on the molecular weight of Iso-oxoaristic acid.

βγ-unsaturated carbonyl group in methyl oxoaristate but does not distinguish between structures (CXXXII), (CXXXI) and (CXXIX) for the parent lactone. The properties of methyl dihydro-oxoaristate, however, clarify this point.

It has already been established that partial hydrogenation of methyl oxoaristate to methyl dihydro-oxoaristate saturates the vinylidene group of the former, since the latter no longer yields formaldehyde on ozonolysis nor shows a band at approximately 900 cm.$^{-1}$ in the infrared. Also, methyl dihydro-oxoaristate shows end-absorption in the ultraviolet (ϵ, 3,600 at 210 mμ) characteristic of a trisubstituted double bond[79,80], in agreement with either of the alternative structures (CXXXIV) or (CXXXV), based on the two possible parent lactone formulations (CXXIX) or (CXXXII). This also excludes aristolactone structure (CXXXI), which would lead to a methyl dihydro-oxoaristate



(CXXXV)

containing a cyclic disubstituted bond, exhibiting only very low-intensity end-absorption at 210 mμ (ϵ, 200-300)[79,80,89].

Treatment of methyl dihydro-oxoaristate with ethanolic potassium hydroxide gave an oily product which also showed an ultraviolet

Fig. 6

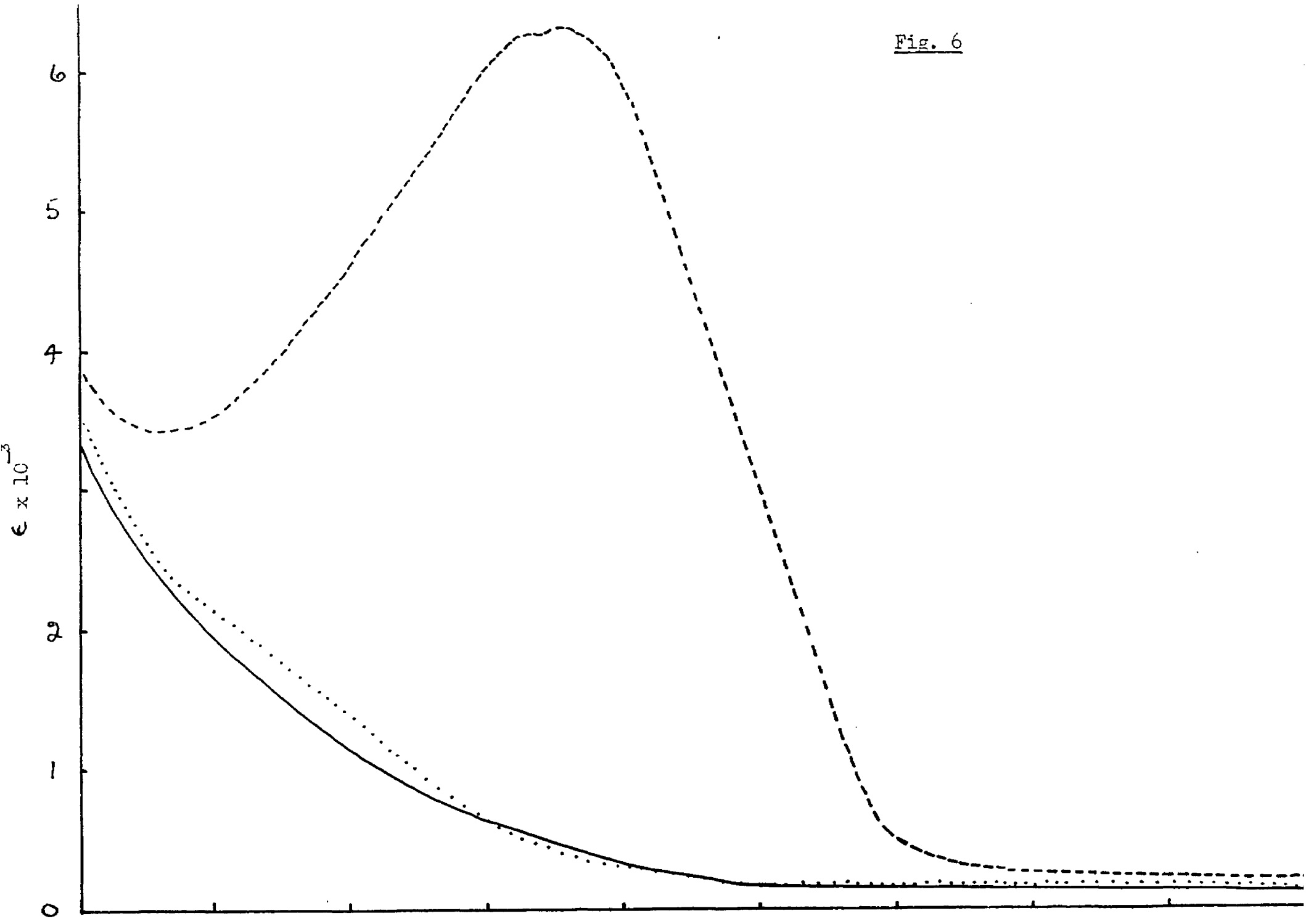


Figure 6

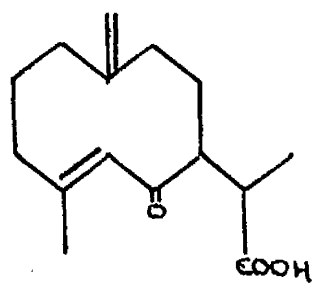
. . . . . Curve I Methyl dihydro-oxoaristate.

——— Curve II Methyl dihydro-oxoaristate after warming with potassium hydroxide for 4 hours.

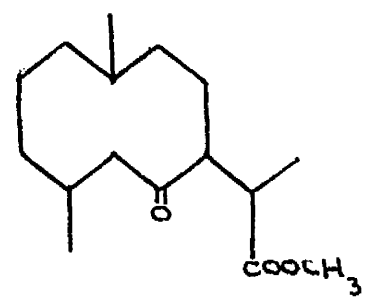
– – – – – – – Curve III Dihydroiso-oxoaristic acid.

The calculated intensities in Curves II and III were based on the molecular weight of dihydroiso-oxoaristic acid.

absorption maximum at 245 mμ (ε , 6,300) (Fig. 6) characteristic of an αβ-unsaturated ketone. Thus the trisubstituted double bond of methyl dihydro-oxoaristate is βγ- with respect to the carbonyl group, thereby establishing (CXXXIV) as the correct structure for methyl dihydro-oxoaristate, in preference to (CXXXV), and supporting structures (CXXIX) and (CXXXIII) for aristolactone and methyl oxoaristate respectively. On this basis, iso-oxoaristic acid must be represented by structure (CXXXVI).



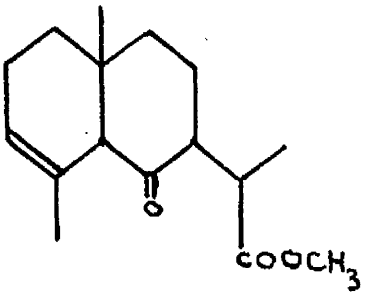
(CXXXVI)



(CXXXVII)

Evidence that methyl oxoaristate (CXXXIII), methyl dihydro-oxoaristate (CXXXIV) and methyl tetrahydro-oxoaristate (CXXXVII) are medium ring ketones as postulated, follows from the typical non-reactivity of the carbonyl group in these compounds. Treatment of methyl tetrahydro-oxoaristate with aluminium isopropoxide yielded only unchanged starting material and neither methyl oxoaristate, ethyl oxoaristate nor any of their hydrogenated derivatives formed a 2:4-dinitrophenylhydrazone. This lack of reactivity is due to the typical O-inside configuration of medium ring ketones[81] and not to steric hindrance, since the analogous bicyclic alantolactone derivative,

methyl-6-oxocanta-3-enoate (CXXXVIII), reacts readily with 2:4-dinitrophenylhydrazine[32].



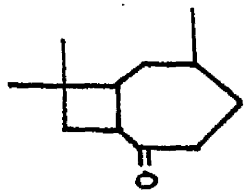
(CXXXVIII)

The carbonyl frequency in the infrared absorption spectra has been observed to vary with the size of the ring to which the carbonyl group is attached. Sorm, Dolejs and Pliva[30] found the carbonyl frequencies to vary as recorded in Table 9, and Büker and Günthard[28] published a similar set of results, Table 10.

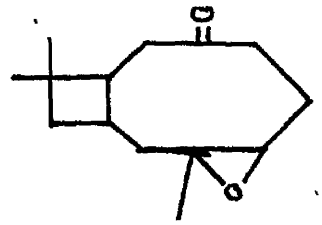
Table 9

Compound	Carbonyl frequency ($cm.^{-1}$)	
	Liquid	in $CHCl_3$
cyclohexanone	1712	1708
cycloheptanone	1703	1694
cyclo-octanone	1700	1692
[a]ketone, $C_{12}H_{20}O_2$ (CXXXIX)	1705	1693
[a]oxido-ketone, $C_{14}H_{22}O_2$ (CXL)	1698	1693

[a] Structures as given by Sorm et al.[30].



(CXXXIX)



(CXL)

Table 10

Compound	Carbonyl frequency ($cm.^{-1}$) in CCl_4
cyclohexanone	1718
cycloheptanone	1706
cyclo-octanone	1704
cyclononanone	1703
cyclodecanone	1705

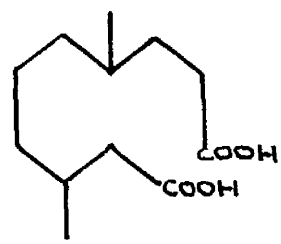
In comparison, the carbonyl frequencies found by Stenlake and Williams[1] and in the present work are recorded in Table 11.

Table 11

Compound	Carbonyl frequency ($cm.^{-1}$)	Solvent
Ethyl oxoaristate	1704	Paraffin mull.
Methyl oxoaristate (CXXXIII)	1720	CS_2.
Methyl dihydro-oxoaristate (CXXXIV)	1693	(KBr disc.)

Although the results in Table II are not of outstanding value, due to the different solvents and the lack of standards examined under the same conditions, there is nevertheless some indication that the carbonyl frequencies are rather lower than might be expected for a six-membered ring and in this respect they also support the proposed structures.

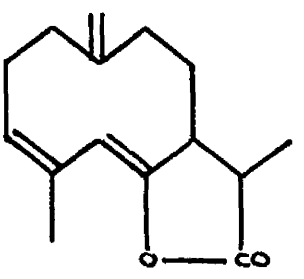
On the basis of structure (CXXXVII), the dibasic acid, $C_{12}H_{22}O_4$, obtained by nitric acid oxidation of methyl tetrahydro-cucurbitate and characterised as its silver salt, will be formulated as 4:8-dimethyl-sebacic acid (CXLI). Paper chromatography of the acid indicated only that it was of higher molecular weight than pimelic acid.



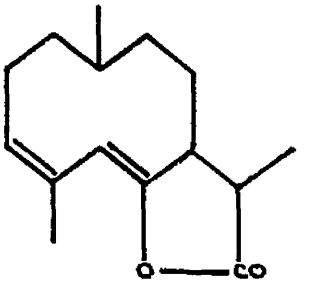
(CXLI)

THE STRUCTURE OF ISOARISTOLACTONE

Partial structure (CIX) [160] has already been advanced for isoaristolactone and the complete structure can now be represented by (CXLII) and dihydroisoaristolactone by (CXLIII).



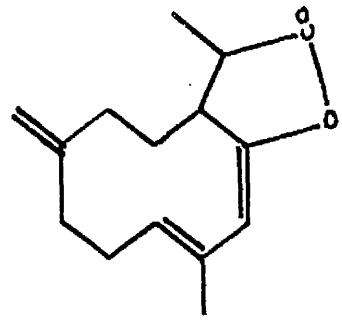
(CXLII)



(CXLIII)

Support for these structures comes from oxidation experiments with chromic acid, which, as in the case of aristolactone, yielded succinic acid as the largest identifiable fragment. One of the puzzling features of the ultraviolet spectrum of aristolactone was the low-intensity maximum at 272 mμ (ε, 640), which persisted almost unchanged in dihydroisoaristolactone (ε, 600 at 271 mμ). The structures now proposed for these compounds offer an explanation of this apparent anomaly. Examination of molecular models shows that the most likely conformation for a cycloöct-1:3-diene is one in which the double bonds are cisoid. Such a system appears to be most readily capable of taking up a strain-free conformation, in which the two double bonds, however, are not coplanar. This double bond system may therefore be regarded as a pseudo-homoannular diene (CXLIV) and may be expected to show an ultra-

violet absorption spectrum characteristic of such a system.



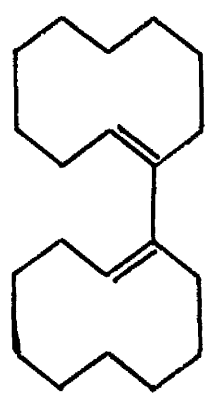
(CXLIV)

The effect of the oxygen atom of the lactone ring on the wavelength of absorption of the conjugated system is uncertain but if it is treated as having the same effect as an alkyl substituent, the theoretical wavelength of the diene system, according to Woodward[54], is 273 mμ (observed wavelength 272 mμ). The absorption intensities, however, are not typical, being markedly depressed, but this is readily explained by the non-coplanarity of the double bonds, as observed by Braude and Nachod[63] and as found for dicycloocta-1-onyl[64]. Braude and Nachod showed that if coplanarity of the conjugated groups was obstructed by steric interference, one of two effects could follow. Either (a) the intensity of maximum absorption ($\epsilon_{max.}$) was decreased while $\lambda_{max.}$ remained unaltered, if steric inhibition was relatively small, or (b) the $\lambda_{max.}$ was shifted to shorter wavelengths, if steric inhibition was severe.

It is clear that isoaristolactone falls within the first of these two categories, having $\lambda_{max.}$ unchanged and $\epsilon_{max.}$ decreased from a few thousands to just over 600. The retention of this peak in the common dihydro-derivative from both lactones is further evidence that the compound is dihydroisoaristolactone and not dihydroaristolactone. The

unchanged intensity of this peak in dihydroisoaristolactone (CXLIII)
unchanged intensity of this peak in dihydroisoaristolactone (CXLIII)
also substantiates the ozonolysis evidence (absence of formaldehyde)
that it is the isolated vinylidene group which has been eliminated during the hydrogenation. The structures proposed for isoaristolactone and dihydroisoaristolactone comply with all the known properties and reactions of the compounds. A further modicum of evidence for the conjugation of double bonds in isoaristolactone is provided by the end-absorption at 204 mμ (actually a false maximum) which has an intensity of 16,280, compared with aristolactone, which shows a false maximum at 205 mμ (ϵ, 12,000), both spectra being determined in absolute ethanol with a fused silica prism. In 10% ethanol, the relative intensities are almost the same, although both are considerably reduced (solvent effect), Fig. 9 and 10, page 170.

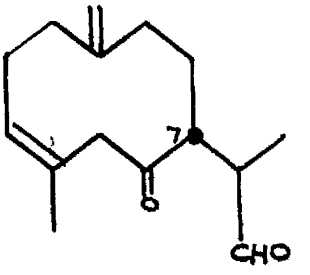
The absence of an absorption maximum at about 220 mμ in isoaristolactone may be explained by a certain amount of steric hindrance, as already discussed (page 97) since Braude and Gofton[84] have shown that dicyclodec-1-enyl exhibits little absorption above 210 mμ although a conjugated double bond system is present.



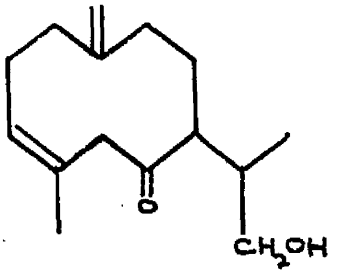
dicyclodec-1-enyl

OTHER DERIVATIVES OF ARISTOLACTONE

The two isomeric ketals formed by lithium aluminium hydride reduction of aristolactone and isoaristolactone, may now be represented by the common structure (CXLV), and as already established (page 62), they probably differ only in configuration about $C_{(7)}$.

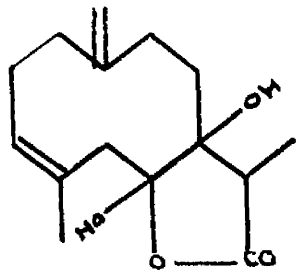


(CXLV)

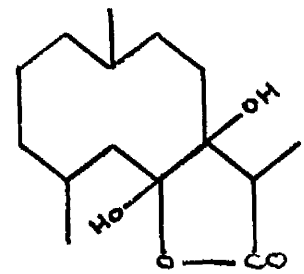


(CXLVI)

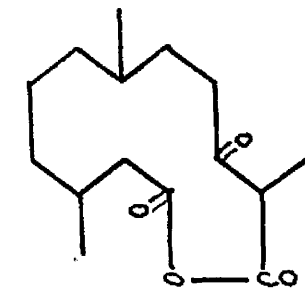
The 1:4-ketol isolated by Stenlake and Williams[1] from the lithium aluminium hydride reduction of aristolactone at higher temperature, has structure (CXLVI). It is significant that the carbonyl group in this compound is not reduced by lithium aluminium hydride to the corresponding diol. This is in accordance with the expected low reactivity of a ten-membered ring ketone. Dihydroxyaristolactone and tetrahydrodihydroxy-aristolactone are now represented by structures (CXLVII) and (CXLVIII) respectively. As already described, the latter is readily oxidised by sodium bismuthate (presence of 1:2-glycol), though the expected keto-anhydride (CXLIX) was only isolated as an amorphous unstable 2:4-dinitro-phenylhydrazone, which resisted characterisation.



(CXLVII)



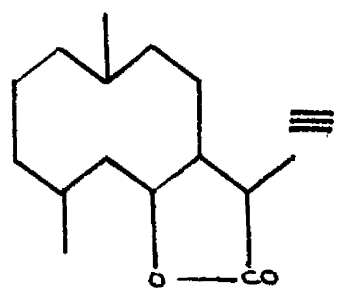
(CXLVIII)



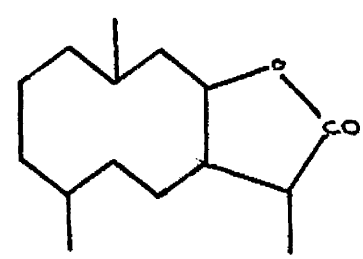
(CXLIX)

Hexahydroaristolactone

The fully saturated derivative of aristolactone has hitherto been referred to as hexahydroaristolactone but since it is undoubtedly formed by way of dihydroisoaristolactone, it is hereafter more correctly designated hexahydroisoaristolactone. The similarity between the infrared spectrum of this compound (CL) and that of tetrahydroalantolactone (CLI) has already been discussed[1] and this is now explained by the close similarity of the carbon skeletons of the two compounds.

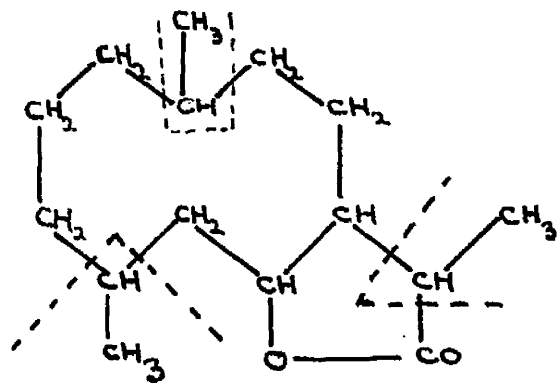


(CL)



(CLI)

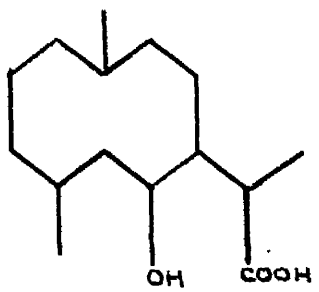
Chromic acid oxidation of hexahydroisoartelotolactone however, did not yield any trace of the glutaric acid which might be expected from the structure proposed (CL), only succinic acid being identified (paper chromatography). This Kuhn-Roth type oxidation is reputed to give good yields of acetic acid from C-methyl groups, and if the yield is quantitative, then it must be formed as indicated by the broken lines in structure (CLII); formation of glutaric acid is therefore impossible and even succinic acid is unlikely. Since yields of acetic acid are usually



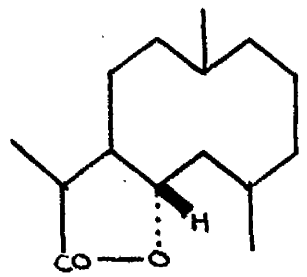
(CLII)

somewhat less than quantitative, we might expect some succinic acid but the amount of glutaric acid would be very small indeed, if any. These conclusions were supported by chromic acid oxidation of alantolactone-isoalantolactone mixture[65] (structures IIa and IIb) which also yielded only succinic acid, under identical conditions.

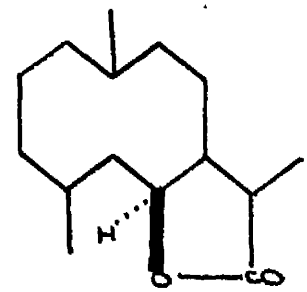
Comparison of the molecular rotation of hexahydroisoartelotolactone (CL; M_D + 7.3°) and that of the corresponding hexahydrohydroxyisoartelic acid (CLIII; M_D +41°) shows that ΔM_D for lactone-acid is -33.7°.



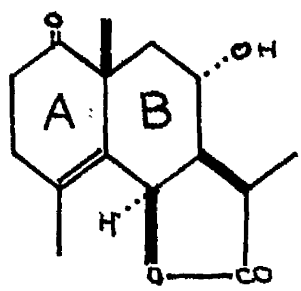
(CLIII)



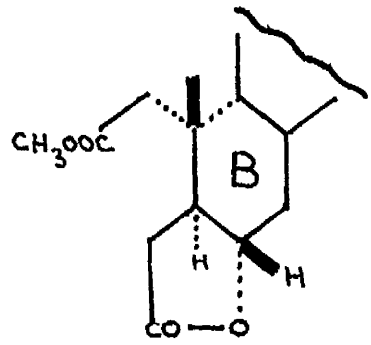
(CLIVa)



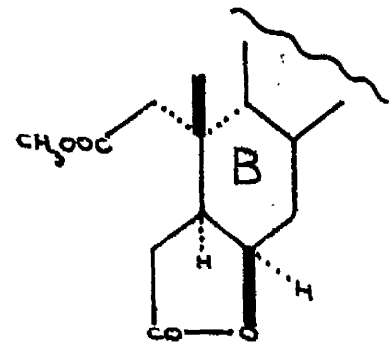
(CLIVb)



(CLV)



(CLVIa)



(CLVIb)

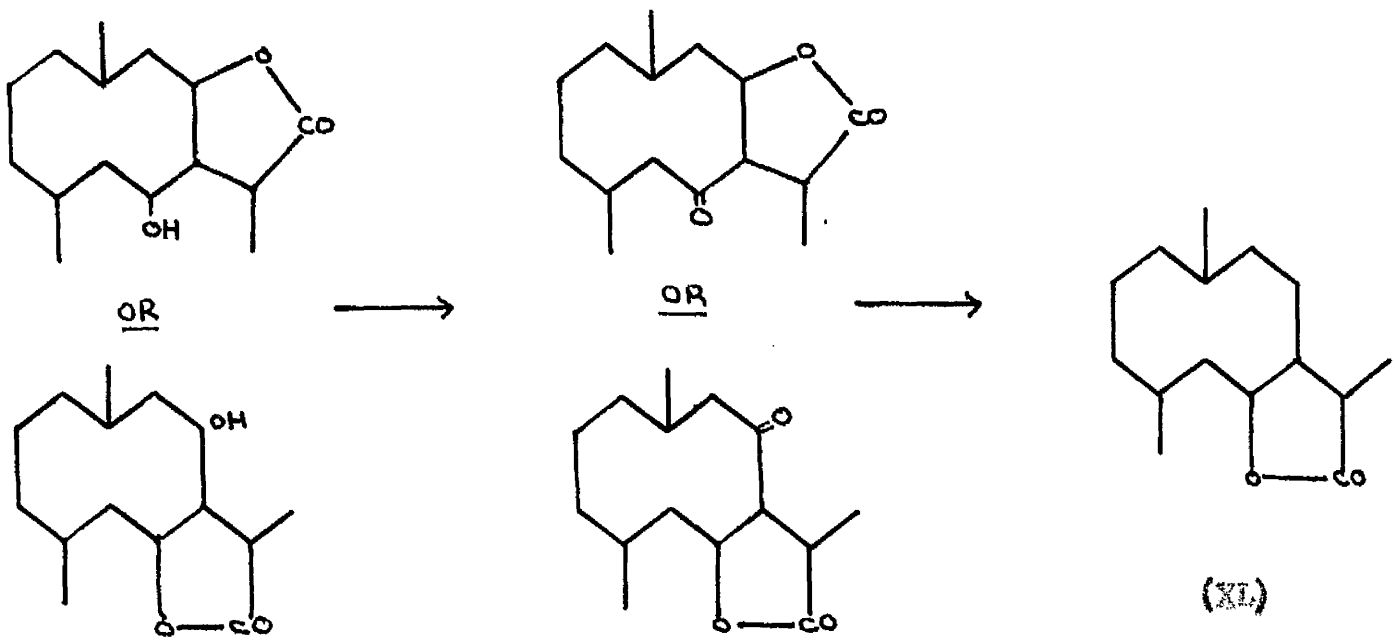
Application of the Klyne-Hudson lactone rule[86] in this example, where the ΔM_D value for lactone-acid is negative, leads to the conclusion that the hydrogen atom at $C_{(6)}$ in hexahydroinganistolactone, is above the plane of the ring. This is identical with the work of Jones and Shoppee[87] who found that the lactone derived from 6α-hydroxy-2:3-seco-cholestane-2:3-dioic acid (ΔM_D negative) had conformation (CLVIa) while the corresponding enantiomorph from 6β-hydroxy-2:3-seco-cholestane-2:3-dioic acid (ΔM_D positive) had conformation (CLVIb). Hexahydroinganistolactone can therefore be represented as either structure (CLIVa) or (CLIVb) but in view of the stereochemistry of γ-santonin[88]

(CLV), conformation (CLIVb) has been adopted.

It has already been shown[1] that hexahydrohydroxyisoaristolic acid is readily relactonised to hexahydroisoaristolactone. In compounds where a five-membered ring is fused with a six-membered ring, this ready relactonisation could provide evidence of cis-fusion of the two rings, but the non-rigidity of the cycloheane ring does not permit any further conclusion with regard to the stereochemistry at $C_{(6)}$ and $C_{(7)}$.

RELATIONSHIP WITH ARGEMONICIN

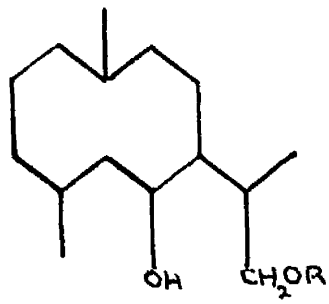
During their work on argemonicin (XLIV), Suchy, Horak, Herout and Sorm obtained by degradation, a fully saturated liquid lactone, $C_{15}H_{26}O_2$, of structure (XL)[45], which should be stereoisomeric with hexahydroisoparietolactone, if the structure of the latter (CL) is correct. The lactone (XL), a liquid, was derived from a crystalline keto-lactone precursor (XXXIX), but since this compound was obtained by oxidation of a crude mixture of stereoisomeric hydroxylactones (XXXVIII) (produced by hydrogenolysis and hydrolysis of argemonicin) and since also no optical rotations or yields are reported, there is no evidence by which to establish the stereo-homogeneity of the lactone product (XL).



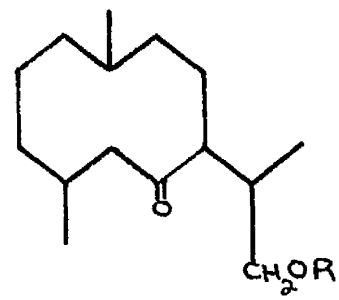
(XXXVIII) (XXXIX)

Reduction of this lactone (XL) with lithium aluminium hydride, however, gave (in unstated yield) the crystalline diol (XLII; R=H),

$C_{15}H_{30}O_2$, m.p. 117°C. from which a crystalline monobenzoate (XLII; R=C_6H_5CO-), m.p. 89°C. was obtained.



(XLII)



(XLIIa)

Oxidation of the latter gave a ketone (presumably XLIIa; R=C_6H_5CO-) for which no characters, other than infrared carbonyl frequency was stated. Thus, although hexahydroisogarrisiolactone is crystalline, in contrast to the arabiopicrin derivative (XL), comparison of the respective diols and their monobenzoates seemed desirable.

Reduction of hexahydroisogarrisiolactone with lithium aluminium hydride afforded an almost quantitative yield of the crystalline diol, $C_{15}H_{30}O_2$, m.p. 106-107°C., $[\alpha]_D$ +16.7°, isomeric with structure (XLII; R=H) and this gave an oily benzoate, $C_{22}H_{34}O_3$, $[\alpha]$ +1.9°, confirming the non-identity with the arabiopicrin derivative. The small quantity of monobenzoate available precluded the possibility of further oxidation to the corresponding keto-benzoate, but the present lack of characters for the arabiopicrin series of compounds renders this approach to a proof of the garrisiolactone skeleton unfruitful for the time being.

ULTRAVIOLET END-ABSORPTION OF VINYLIDENE GROUPS IN ARISTOLACTONE DERIVATIVES

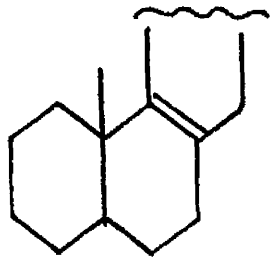
Saturation of the vinylidene group in the formation of methyl dihydro-oxoaristate (CXXXIV) from methyl oxoaristate (CXXXIII) is accompanied by a marked fall in the ultraviolet end-absorption intensity at 210 mμ (ϵ , 2,900, Table 12). The comparable $\Delta\epsilon$ values for the reduction of isoaristolactone (CXLII) to dihydroisoaristolactone (CXLIII) and ethyl oxoaristate to ethyl dihydro-oxoaristate are 2,850 and 3,401.

Table 12

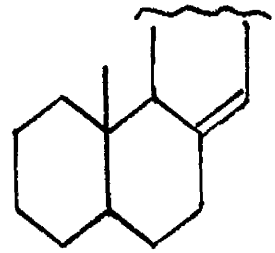
Compound	ϵ at 210 mμ	Vinylidene contribution
Isoaristolactone (CXLII)	10,430	2,850
Dihydroisoaristolactone (CXLIII)	7,570	
Methyl oxoaristate	6,500	2,900
Methyl dihydro-oxoaristate	3,600	
Ethyl oxoaristate	6,863	3,402
Ethyl dihydro-oxoaristate	3,462	

Extinctions of this order are abnormally high for a simple vinylidene group (ϵ at 210 mμ for 1-octene is only 400[89]) and it was decided therefore to probe for their significance. It is well established[79,80] that the end-absorption of ethylenic bonds in the 200-220 mμ region is increased by alkyl substituents and further enhanced when the double bond is in an exocyclic position. Thus, while the tetrasubstituted double

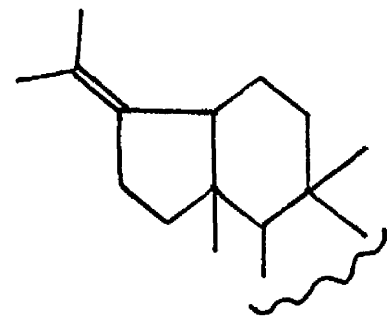
bond in cholest-8(9)-ene shows end-absorption of 4,400 at 210 mμ [80], this is increased to 10,100 at 210 mμ in cholest-8(14)-one [80] and to 6,400 at 210 mμ in γ-lupene [79] in both of which the double bond is exocyclic.



Cholest-8(9)-ene



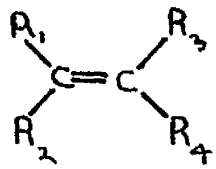
Cholest-8(14)-ene



γ-lupene

It seemed that the unexpectedly high vinylidene absorption of the aristolactone series might be similarly explained and a number of model compounds (with both acyclic and exocyclic double bonds) was therefore examined and vinylidene contributions recorded (Table 13).

This aspect of the work was unfortunately hampered by the difficulty of obtaining suitable compounds for examination and only limited generalisations are therefore possible. It is evident, however, that terminal acyclic vinylidene groups (CH_2:CHR) as in 1-hexene and 1-octene show little absorption, and that introduction of a second alkyl substituent, as in lupeol, leads to a considerable increase in absorption intensity. This is in accordance with prediction based on the observations of the effect of alkyl substituents in compounds of the type

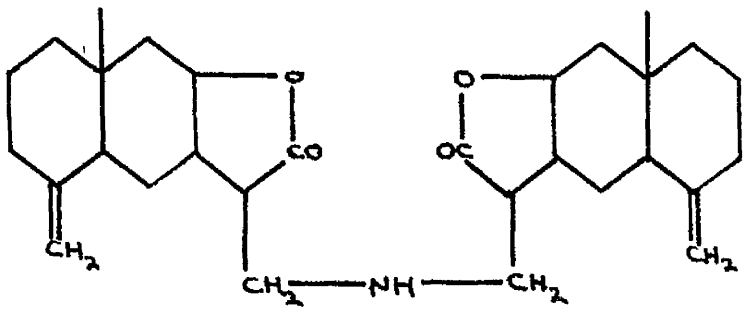


(79,80). The deflection of carbon-carbon valencies with

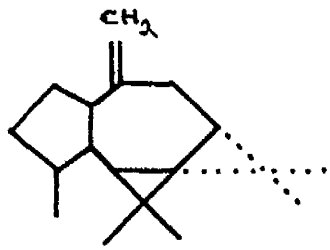
Table 13

	Compound	ε at 210 mμ	ε at 215 mμ	Vinylidene contribution at 210 mμ	Vinylidene location	Ref.
1.	1-Hexene	26	--	26	acyclic	*
2.	1-Octene	400	--	400	acyclic	89
3.	Lupeol (page 66; R=H)	2000 2850	-- 1185	2000 2850	acyclic(6)	89 *
4.	Taraxasteryl acetate (page 110)	4279	1954	4279	exocyclic(6)	*
5.	Methyl 14β-H-8(20)-en-15-oate (page 110)	4550	2457	4550	exocyclic(6)	*
6.	β-Pinene (page 110)	4600	--	4600	exocyclic (bridged 6)	89
7.	Camphene (page 110)	4000	--	4000	exocyclic (bridged 6)	89
8.	β-Eudesmol (page 110)	986	341	986	exocyclic(6)	*
9.	Iso-Alantolactone ammonia adduct (page 109)	1213	480	1213	exocyclic(6)	*
10.	Aromadendrene (page 109)	2562	1362	2255	exocyclic(7)	*
11.	Dihydroaromadendrene (page 109)	207	227		---	90
12.	Iso-apo-Aromadendrene (page 109)	1070	861	1070	exocyclic(8)	*
13.	Caryophyllene (page 109)	6700	5131	3160	exocyclic(9)	*
14.	Dihydroisocaryo-phyllene (page 109)	3540	2032		---	*

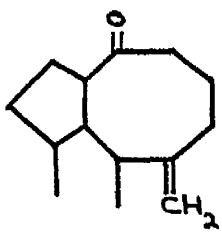
* present work



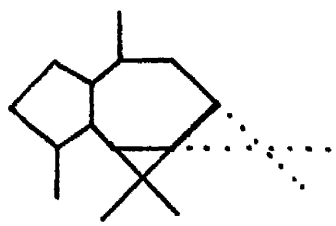
Iso-Alantolactone ammonia adduct



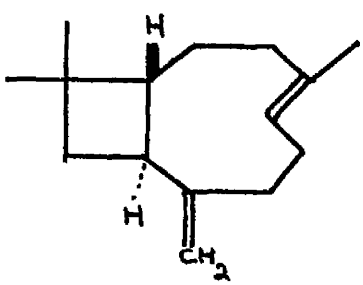
Aromadendrene (91)



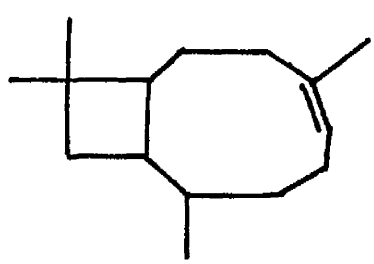
Iso-Aromadendrene (91)



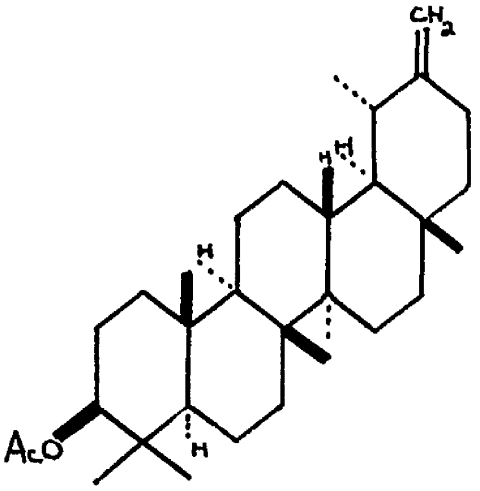
Dihydroaromadendrene



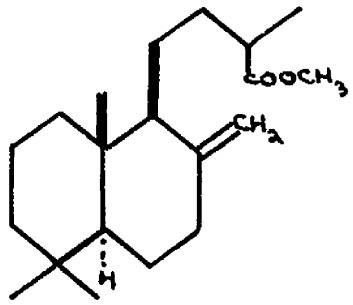
Gazyophyllene (92)



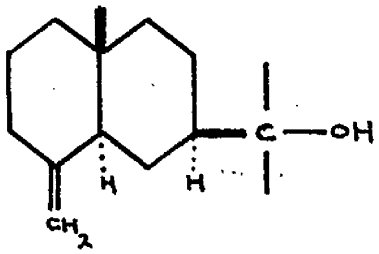
Dihydroisogazyophyllene (93)



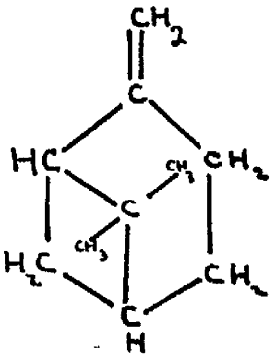
Taraxasteryl acetate (94)



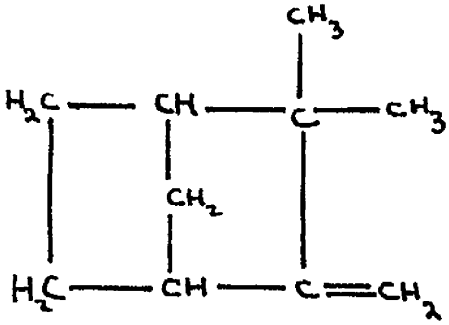
Methyl labd-8(20)-en-15-oate (95)



β-Rhodeanol (96, 97, 98)

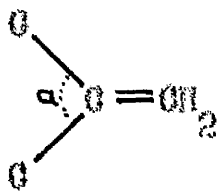


β-Pinene (99)



Camphene (100)

the increase in the angle a, which is imposed by the methylene group of an exocyclic vinylidene (CLVII), will introduce an element of strain into the otherwise strainless six-membered rings and we might expect this to be reflected in the end-absorption (at 210 mμ) of such compounds.



(CLVII)

Compounds 4, 5, 6 and 7 in Table 13 all show this effect, the absorption intensity being raised to 4,000 and above at 210 mμ . Two of these, however, (nos. 6 and 7) are bridged rings, whilst β-cudesmol (no.8) and isoalantolactone ammonia adduct (no.9) are apparently anomalous in that the end-absorptions are relatively low. Only one example of each of the 7-, 8- and 9-membered ring exo-methylene compounds (not necessarily typical) was available (Table 13) and conclusions are therefore only tentative. The pattern of observed end-absorption intensities appears to be in accord with the somewhat unusual properties of medium-sized rings[81] in which the lowered intensities could conceivably be ascribed to varying degrees of steric hindrance imposed by a vinylidene-inside configuration comparable with Prelog's[81] C-inside conformation of the cycloalkenes. Alternatively, the strain imposed by the increased valency angle a (CLVII) may be absorbed by the greater flexibility of the larger rings.

The rapidity with which end-absorption falls off between 210 and 220 mμ is often a better indication of substitution effects in isolated double bonds than measurement of the actual intensity at 210 mμ[79,80]. The average slope of the curve between 210 and 215 mμ was therefore found for each compound by drawing a straight line through the intensities at 210 and 215 mμ plotted against wavelength, on the same arbitrary scale, and calculating $\tan\theta_{215}^{210}$, where θ is the angle the line makes with the x-axis.

Table 14

Compound	No. of carbon atoms in the ring	$\tan\theta_{215}^{210}$
Taraxasteryl acetate	6	1.16
Methyl labd-8(20)-en-15-oate	6	1.05
β-Eudesmol	6	0.32
*iso*Alantolactone ammonia adduct	6	0.39
Δε-Ayonadendrone-dihydroayonadendrone	7	0.56
*iso*Ayonadendrone	8	0.11
Δε-Caryophyllene-dihydrolaccaryophyllene	9	0.03
Δε-*iso*Alantolactone-dihydro*iso*alantolactone	10	0.11

The values of the tangent obtained in this way (Table 14) were plotted as ordinate against the number of carbon atoms in the ring to which the vinylidene is attached (abscissa), as shown in Fig. 8, page 113.

Fig. 7

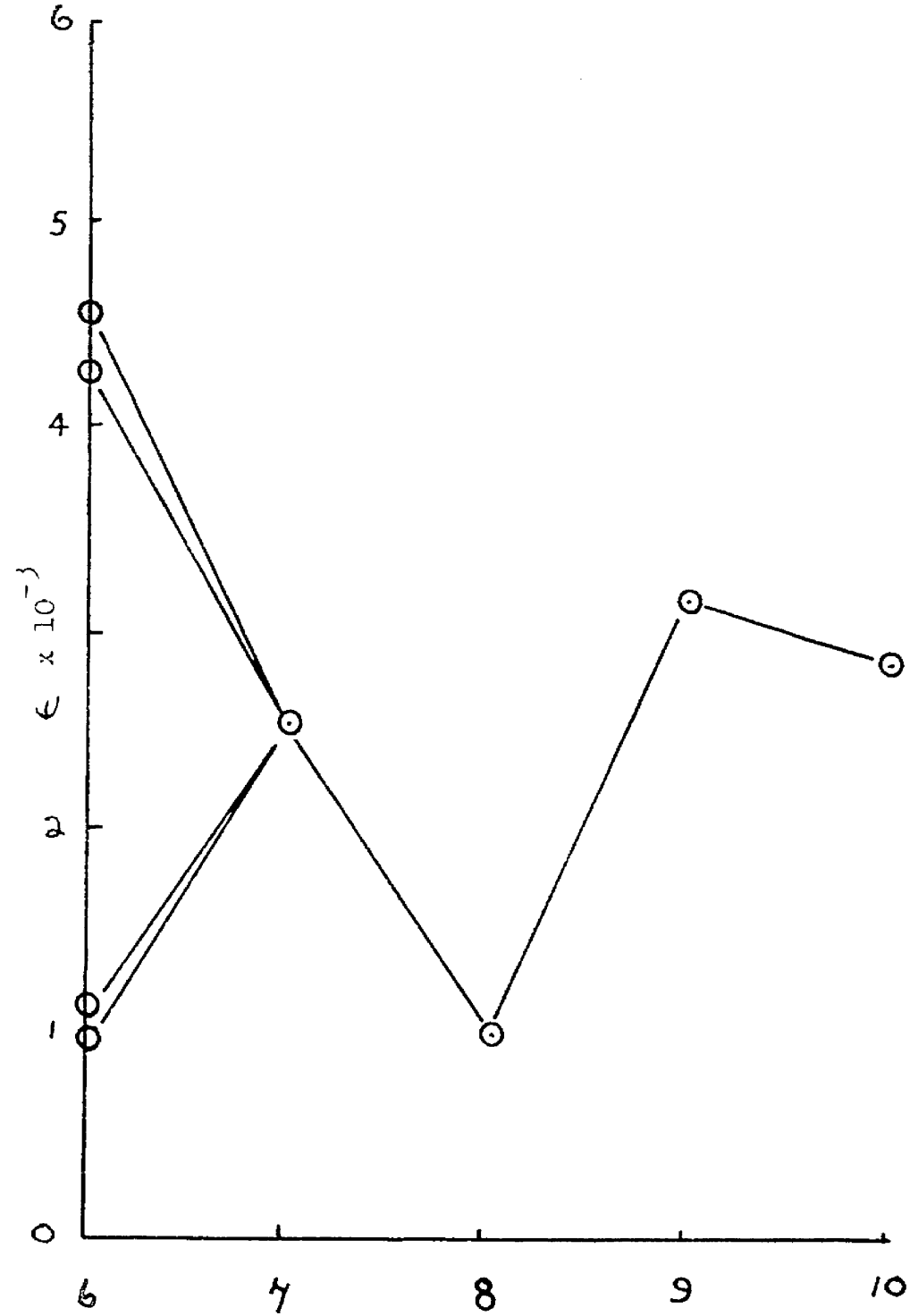
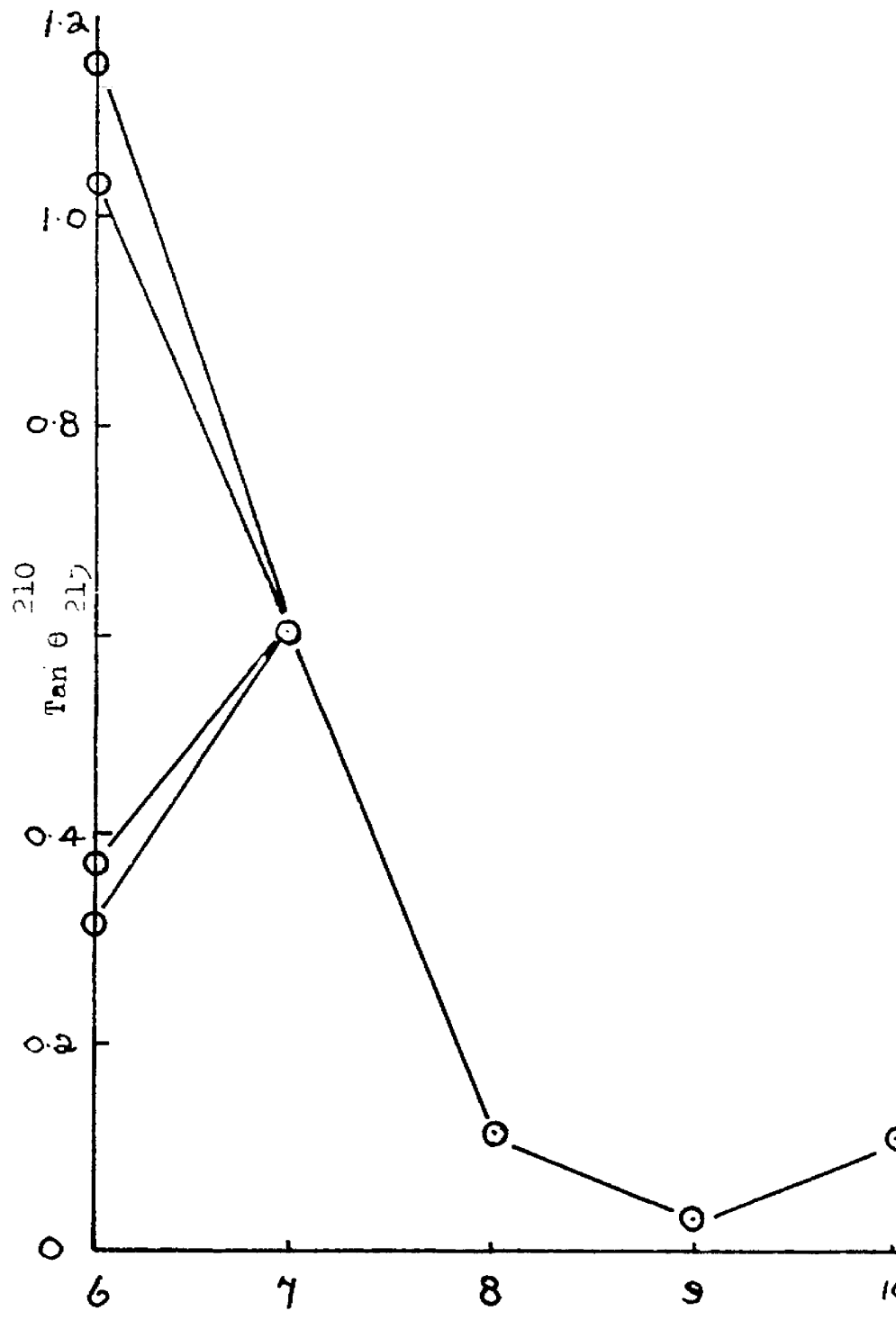


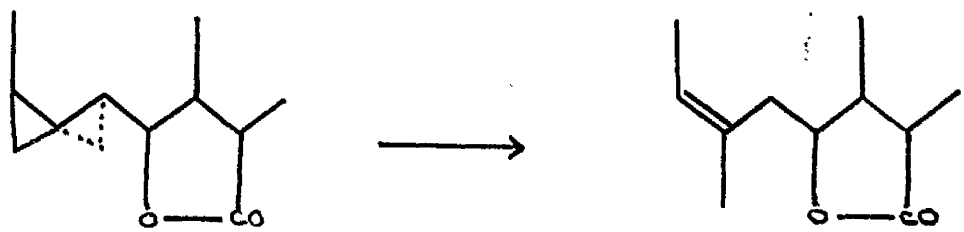
Fig. 8



Number of carbons in the ring.

The results confirm the pattern obtained from the plot of end-absorption intensities at 210 mμ against ring size (Fig. 7, page 113), with the slight difference that the minimum value corresponds to a nine-membered ring. Tan $\theta^{210}_{\lambda\varepsilon}$ based on $\Delta\epsilon$ values for ingenistolactone-dihydroisoaristolactone is, however, significantly lower than the corresponding figure for even the unusually low six-membered ring compound (Table 14), and in this, it provides some measure of support for the proposed ten-membered ring structure.

After these studies were complete, a further anomaly became apparent in the ultraviolet spectra of aristolactone derivatives, which at first could not be reconciled with the proposed structures. The ketal and isoketal (CXIV), methyl oxoaristate (CXXXIII) and dihydroxy-aristolactone (CXLVII) which all contain the same two double bonds (the cyclic trisubstituted double bond and the exocyclic vinylidene group), showed marked dissimilarity in end-absorption at 210 mμ, the respective values being 3,234, 4,950, 6,500 and 3,200. One explanation considered to account for the anomaly and later rejected, was that aristolactone contained a cyclopropane ring which was ruptured on treatment with acid or alkali to produce a trisubstituted double bond, as in partial structure (CLVIII), in ingenistolactone and methyl oxoaristate.



(CLVIII)

This theory was supported by the presence of the infrared band at 875 cm.$^{-1}$ in isoaristolactone and methyl oxoaristate, which is absent from the spectrum of aristolactone itself. Since neither acid nor alkali (except in traces) is used in preparation of the ketal (OKLV) and dihydroxyaristolactone, the cyclopropane ring should be retained, so that the principal contributor to the end-absorption of these compounds would be the vinylidene group. Hence, the absorption intensity would be less than that of methyl oxoaristate or the isoketal, which would both have the contribution of a trisubstituted double bond. Infrared spectral evidence for a cyclopropane ring, however, was inconclusive, since in those substances thought to contain a cyclopropane ring, the vinylidene group was also present and both functions absorb at the same frequency in the 3050 cm.$^{-1}$ region. Bands were also observed in the 1010 cm.$^{-1}$ region in every infrared spectrum but these are of little analytical value since the oxygen substituents in the molecule absorb at the same frequency(44).

Investigation of the iodine values of available compounds containing a cyclopropane ring showed that the cyclopropane ring remained practically intact under the conditions used (see page 167). The results are recorded in Table 15. It seemed, therefore, that the fractional values obtained for aristolactone could not be attributed to a cyclopropane ring.

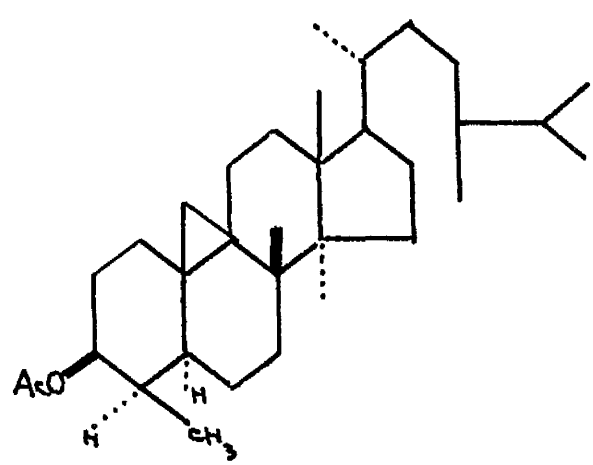
Table 15

Iodine values of compounds containing a cyclopropane ring

Iodine values of compounds containing a cyclopropane ring

Compound	Number of double bonds	Time of reaction	Iodine value
cycloEucalanyl acetate	∞	2 mins.	0.024
"	∞	10 mins.	0.076
"	∞	16 hours	1.110
"	∞	10 mins.	0.250 [35]
Dihydroaromadendrene	∞	2 mins.	0.03
"	∞	10 mins.	0.06
"	∞	16 hours	0.16

∞ solvent, chloroform.



cycloEucalanyl acetate

Conclusive evidence for the absence of a cyclopropane structure was provided by ketal and dihydroxyaristolactone which could not be isomerised by treatment with acid or alkali, although a cyclopropane ring would certainly have been ruptured by this treatment. The cyclopropane hypothesis was therefore abandoned, and it is accepted that the ketal and isoketal (CXLV), methyl oxoaristate (CXXXIII) and dihydroxyaristolactone (CXLVII) all contain the same two-double-bond system.

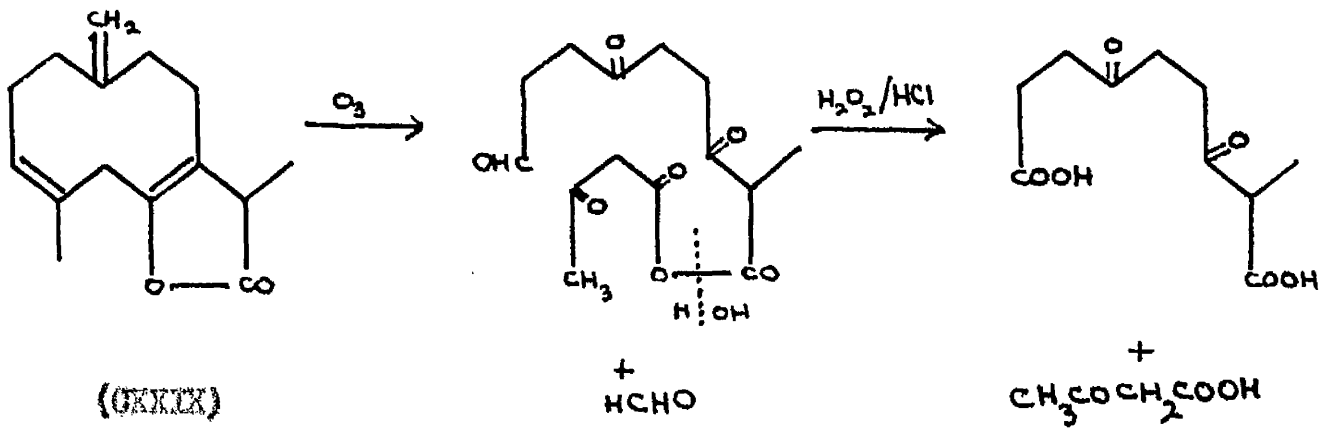
It follows that whereas the contribution of the vinylidene group, as already shown, is significant in methyl oxoaristate (CXXXIII) and also probably in the isoketal (CXLV), it is relatively small in the ketal (CXLV) and dihydroxyaristolactone (CXLVII). This was confirmed to some extent when an attempt to prepare dihydrodihydroxyaristolactone afforded a crystalline solid, m.p. 135-136°C., ϵ, 3,000 at 210 mμ, after hydrogenation, with the uptake of one mol. of hydrogen, but unfortunately in insufficient amount for satisfactory characterisation.

A decision must now be made as to whether the end-absorption intensity due to the vinylidene group is suppressed in the ketal and dihydroxyaristolactone or alternatively, whether this is normal for a ten-membered ring and the vinylidene absorption in methyl oxoaristate, the isoketal and isoaristolactone, is potentiated in some way. The balance of evidence would appear to favour the latter, since as observed, the end-absorption intensities of vinylidene groups exocyclic to medium rings appears, in general, to be lower than when the same function is exocyclic to a smaller ring.

Construction of molecular models shows that an O-inside conformation is highly probable in all ketone derivatives, in agreement with the non-reactivity of the carbonyl groups, since in this configuration, interaction with the methyl substituent on $C_{(4)}$ is at a minimum. In consequence, the carbonyl group and the $\Delta^{5,6}$ and $\Delta^{6,7}$ double bonds of isoaristolactone and aristolactone respectively, are all in much closer proximity to the vinylidene group than the $\Delta^{3,4}$ double bond and some measure of interaction is quite probable. This would explain the augmented vinylidene absorption of the isoketal, methyl oxoaristate, aristolactone and isoaristolactone, and the lack of potentiation in dihydroxyaristolactone. Further, it is established that the ketal and the isoketal differ only in configuration at $C_{(7)}$ and models show that while one isomer is capable of carbonyl interaction, the other is not. The anomalous carbonyl absorption already discussed for the ketal (page 59) suggests, in the absence of comparable spectra for the isoketal, that it is this isomer which is capable of carbonyl interaction. Reduction of typical ring carbonyl activity, possibly by hydrogen bonding with the aldehydic hydrogen, would also eliminate the possibility of vinylidene-carbonyl interaction, so that the ultraviolet end-absorption is not augmented, as it is in the isoketal (isomeric at $C_{(7)}$). Comparison of molecular rotations for methyl oxoaristate (CXXXIII; M_D + 904°) and the ketal (CXLV; M_D +192°) suggests that they have the same configuration at $C_{(7)}$ but interaction would be precluded in the former since the aldehydic hydrogen is replaced by the ester methoxyl group.

OZONOLYSIS OF ARISTOLACTONE AND METHYL DIHYDRO-OXOARISTATE

On the basis of structure (CXXX) for aristolactone, ozonolysis would be expected to proceed as shown in scheme (CLIX).



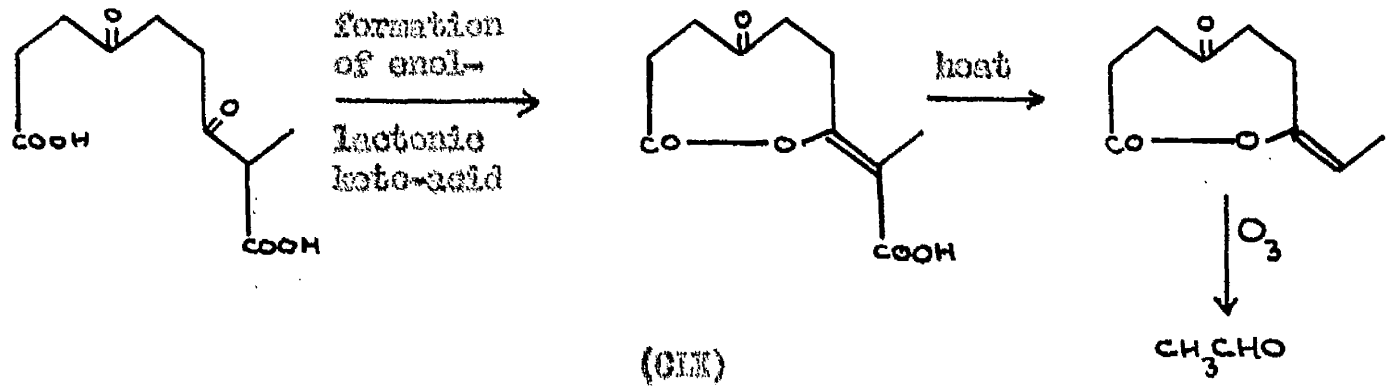
(CXXIX) (CLIX)

It has been established by Stenlake and Williams[2] that formaldehyde and two keto-acids are formed in accordance with this scheme. The latter were obtained as an acidic oil, which was separated into its components by paper chromatography with (a) butanol-pyridine-water-ethanol and (b) pyridine-water-ammonia. The keto-acid A had R_F 0.62 in solvent (a) and 0.80 in solvent (b), which is closely similar to but not identical with the values found for laevulinic acid (R_F, 0.62 in (a) and 0.76 in (b)), in reasonable agreement with its now suggested formulation as acetoacetic acid. Repetition of the ozonolysis in the course of the present work also failed to yield solid products, though methyl ketone reactions were shown by the more readily water-soluble acidic fraction.

A careful search, however, failed to yield any trace of acetone, which should have been produced by decarboxylation, but, in retrospect, it is evident that this may well have been due to the fact that the oxidative hydrolysis of the ozonide was conducted in the presence of only 0.1N acid.

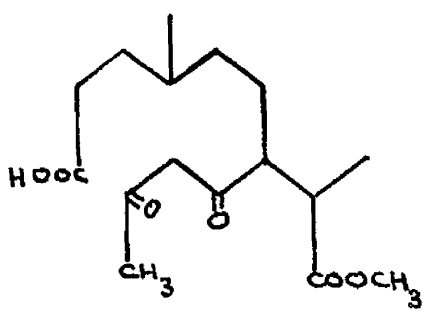
An interesting feature of the ozonolysis was the observation that extraction of the acidic products yielded an oily chloroform-soluble unsaturated keto-acid, which showed end-absorption in the ultraviolet ($E^{1\%}_{1cm.}$ 250, at 210 mμ) and saturated ketone absorption at 275 mμ ($E^{1\%}_{1cm.}$ 5.6). This product was obtained even following prolonged treatment with ozone up to three hours. Other chemical evidence gave no hint of a resistant double bond in aristolactone and the facile cleavage of this double bond on further ozonolysis therefore renders such an explanation unlikely. Re-ozonolysis of the redistilled unsaturated keto-acid yielded acetaldehyde identified as its 2:4-dinitrophenylhydrazone and as its dimedone derivative.

Although the analytical and other evidence is not entirely satisfactory, a tentative explanation of the above observations is given in scheme (CLX).

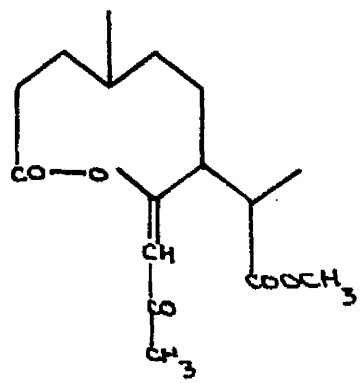


(CLX)

Ozonolysis of methyl dihydro-oxoaristolate (CXXXIV) also provided some unexpected results. The oily product, on distillation gave a yellowish, acidic oil which analysed satisfactorily for $C_{16}H_{24}O_5$. All sixteen carbon atoms of methyl dihydro-oxoaristolate are therefore retained in the product which proves conclusively that the one remaining double bond in methyl dihydro-oxoaristolate is situated in a ring system. The expected product, however, would have structure (CLXI), which has the formula $C_{16}H_{26}O_6$ and it seems that distillation results in removal of the elements of water.



(CLXI)



(CLXII)

The product, $C_{16}H_{24}O_5$, gave a yellow colour with tetranitromethane (unsaturation) and a strongly positive colour reaction with alkaline sodium nitroprusside (methyl ketone). The most feasible explanation seemed to be that the product was an enol-ketone which might be formulated as (CLXII), but the ultraviolet spectrum did not bear this out. Determination of the spectrum at various pH values up to pH 10.20

gave curves with two distinct maxima, but at pH 11.58, only one peak remained. The results are recorded in Table 16. No structure has so far been advanced which offers a completely satisfactory explanation of the spectra of the distilled oil.

Oxidation of this product with alkaline potassium permanganate, followed by treatment with diazomethane and distillation, gave a small amount of a clear oil, $C_{15}H_{24}O_4$, which could not be identified.

Table 16

Concentration	pH	$\lambda_{1\,max.}$ (mμ)	$E^{1\%}_{1cm.\,max_1}$	$\lambda_{2\,max.}$ (mμ)	$E^{1\%}_{1cm.\,max_2}$
0.0038	1.49	238	250	289	212
0.0038	5.80	240	200	289	184
0.0038	10.20	249	187	290	167
0.0038	11.58	249	184	--	--
0.00152	5.80	240	164	292	167

CONCLUSION

The reactions and properties of the derivatives examined are in substantial agreement with the structure (LXXIX) proposed for aristolactone but a few minor points remain incomplete, such as the chemical identification of the azulene obtained by dehydrogenation of various derivatives and the identification of the oxonolysis product from methyl dihydro-oxoaristate. Re-examination of the infrared spectrum of aristolactone by the potassium bromide disc method revealed a low-intensity peak at 812 $cm.^{-1}$, which could be attributed to a tri-substituted double bond, thus resolving one of the doubtful points, since no peak was evident at this frequency in the curves examined by Stenlake and Williams[1].

The work, as a whole, has been hampered by lack of an abundant supply of material and by the tendency of aristolactone and some of its derivatives (notably the keto-esters and their hydrogenation products) to resinify or otherwise decompose on prolonged treatment with almost all reagents. The structural information acquired, however, will permit the last remaining doubts to be resolved as soon as a further supply of material becomes available.

Several avenues of investigation suggest themselves for future work. On the structural aspect, high priority must be accorded to confirmation of the structure of dihydroxyaristolactone, whilst the study of the stereochemical features demand that such correlations as are postulated to exist, e.g. between the ketal and methyl oxoaristate, be unequivocably established.

EXPERIMENTAL

EXPERIMENTAL

Melting points are uncorrected except where stated otherwise. Rotations were determined in a 1 dm. tube in the solvent indicated. Ultraviolet absorption spectra were determined in the stated solvent, on a Hilger Uvispek photoelectric spectrophotometer H 700/303. The light petroleum used boiled over the range 40-60° C.

The author is greatly indebted to the following:- the Pharmacognosy staff for identification and removal of adulterants from the raw drugs; Smith, Kline and French Ltd., for determination of infrared absorption spectra; Dr. Syme and Mr. McGrindale for the micro-analyses; the Chemistry Dept. of the R.C.S.T. for use of apparatus; Dr. Laurie and Dr. Stevenson for samples of cycloeucalanyl acetate, lupeol, lupanyl acetate and taraxasteryl acetate; Dr. F.J. McQuillin for sample of β-eudesmol 3:5-dinitrobenzoate; Dr. T.G. Halsall for sample of methyl labdanolate; the Thornton Research Centre, Chester for hexane-1; Dr. M.D. Sutherland for sample of aromadendrene and isoaromadendrone; Dr. W. Mitchell for pyrethrosin.

THE LIGHT PETROLEUM-SOLUBLE FRACTION OF ARISTOLOCHIA RETICULATA[1]

The crude drug

9.5Kg. of the raw drug were obtained, in one lot, and it was practically free from adulteration. The root is described in Williams' thesis.

Preparation and extraction

The root was reduced to a fine powder (approximately 80 mesh) in a disintegrator, after a preliminary drying at 35°C. for two days. The powder was macerated overnight under light petroleum, in a large copper percolator and percolation was thereafter continued until the runnings were almost colourless. Evaporation on a water-bath yielded a dark-brown extract, having the same fragrant odour as the crude drug. The extract (4.52%w/w of the dried root) undoubtedly still contained some solvent.

Isolation of aristolactone

When the extract was seeded with a few crystals of aristolactone, a crystalline deposit appeared almost immediately. After three days in the refrigerator, 9.7g. of crude aristolactone were filtered off.

Chromatography of the residual oil

An extract obtained by Williams (approximately 300 ml.) and the extract obtained by the author were treated in the following manner.

About 75 ml. portions of the extracts were diluted with an equal volume of light petroleum and chromatographed on a column (4.5 centimetres diameter) containing 300g. of a mixture of activated charcoal

(1 part) and Whatman standard grade cellulose powder (3 parts). The optical rotation of each 50 ml. fraction was measured in a 1 decimetre tube. A large number of fractions were collected and the figures in Table 17 represent a typical set of results.

The highly dextrorotatory fractions (11-20, Table 17) were evaporated and seeded individually with aristolactone but later fractions were bulked in lots of three or six before seeding. In this way, a further 6.3g. of crude lactone were isolated from the author's extract of A.reticulata (9.5Kg.). The total yield of crude aristolactone was therefore 18g., which produced 15g. of pure material, m.p. 110-111°C., representing 0.158%w/w of the dried root.

Table 17

Fraction	Eluant	Optical rotation	Fraction	Eluant	Optical rotation
1	light petroleum	0.00	21	light petroleum	+0.85
2	"	0.00	22	"	+0.79
3	"	0.00	23	"	+0.71
4	"	0.00	24	"	+0.66
5	"	0.00	25	"	+0.60
6	"	0.00	26	"	+0.50
7	"	0.00	27	"	+0.45
8	"	0.00	28	"	+0.39
9	"	-0.03	29	"	+0.34
10	"	-0.35	30	"	+0.28
11	"	+6.83	31	"	+0.24
12	"	+12.82	32	"	+0.19
13	"	+11.91	33	"	+0.17
14	"	+9.15	34	"	+0.15
15	"	+5.70	35	"	+0.14
16	"	+4.22	36	"	+0.13
17	"	+2.79	37	"	+0.13
18	"	+1.71	38	"	+0.14
19	"	+1.25	39	"	+0.13
20	"	+0.98	40	"	+0.14

Table 17 (cont'd.)

Fraction	Eluant	Optical rotation	Fraction	Eluant	Optical rotation
41	light petroleum	+ 0.13	72	½% ethanol in light petroleum	0.00
42	"	+ 0.11			
43	"	+0.09	73	2% ethanol in light petroleum	0.00
44	"	+0.09			
45	"	+0.07	74	"	0.00
46	"	+0.05	75	"	0.00
47	"	+0.05	76	"	+ 0.04
48	"	+ 0.05	77	"	+ 0.05
49	"	+ 0.09	78	"	+ 0.04
50	"	+ 0.02	79	"	+ 0.05
51	½% ethanol in light petroleum	+ 0.01	80	"	+ 0.07
			81	"	+ 0.10
52	"	0.00	82	"	+ 0.11
53	"	0.00	83	"	+ 0.11
54	"	0.00	84	"	+ 0.05
55	"	0.00	85	"	+ 0.04
56	"	0.00	86	"	+ 0.04
57	"	+ 0.03	87	"	+ 0.05
58	"	+ 0.03	88	"	+ 0.05
59	"	+ 0.04	89	"	+ 0.11
60	"	+ 0.05	90	"	+ 0.09
61	"	+ 0.02	91	"	+ 0.04
62	"	+ 0.02	92	"	+ 0.01
63	"	0.00	93	"	+ 0.02
64	"	0.00	94	"	0.00
65	"	0.00	95	"	0.00
66	"	0.00	96	"	+ 0.02
67	"	0.00	97	"	+ 0.04
68	"	0.00	98	"	+ 0.04
69	"	0.00	99	"	+ 0.04
70	"	0.00	100	"	+ 0.03
71	"	0.00			

After removal of the lactone, the fractions were bulked into several large residues and evaporated to low volume (Table 18, page 127).

Table 18

Residue number	Fractions bulked	Description
I	9, 10	Colourless fragrant oil. Laevorotatory.
II	11-72	Clear, mobile brownish oil, with typical terpene odour. Dextrorotatory.
III	73-100	Small amount of brown viscous oil.
IV	Later eluates	Dark, resinous oil.

Investigation of residue I

A light petroleum solution of the bulked laevorotatory fractions was brominated by the dropwise addition of an ethereal solution of bromine, until a faint yellow colour persisted. The light petroleum and ether were removed until about 20 ml. solution were left. Fractional distillation on an oil-bath yielded two volatile liquids, A and B.

Fraction A was almost entirely light petroleum and was discarded.

Fraction B, (1.8g.), b.p. 110°C./18m.m., was purple/blue when first distilled but it later turned blue, then green and finally, brown. Some dehydrogenation was suspected and this fraction was also discarded.

The dark-coloured residue from the distillation was chromatographed on alumina and separated into three components (Table 19).

Table 19

Fraction number	Eluting solvent	Description
1	Light petroleum	Fragrant yellow oil (3.9g.).
2	Light petroleum	Trace of brown oil.
3	10% ethanol in light petroleum	Dark-coloured semi-solid oil (0.3g.).

Fraction 1. A further chromatography on alumina, from light petroleum removed some more brown material and left 2.5g. of a clear yellow oil, which was distilled under high vacuum. The main fraction collected, b.p. 139-141°C./1.5m.m., d_{16}^{16} 0.949, n_D^{16} 1.4977, $[\alpha]_D^{16}$ +8.38° (c, 1.985), contained no bromine. A microanalysis carried out on this oil indicated that it was probably a mono- or sesquiterpene hydrocarbon which was contaminated with an oxygen-containing compound. No further work was carried out on this oil.

Fraction 2 was discarded.

Fraction 3. The semi-solid oil was chromatographed on alumina and fractions were collected as shown in Table 20. Only fractions m, n and o were examined. The crystals from these three fractions were filtered off and washed, yielding 5-10mg., m.p. 196-198°C. (sealed tube). The shiny hexagonal plate crystals had a very camphoraceous odour and the mixed melting point with authentic (-)-borneol (m.p. 202°C.) was undepressed.

Table 20

Fraction number	Volume collected (ml.)	Eluting solvent	Description
a	100	Light petroleum	Trace of yellow oil.
b	100	"	"
c	100	"	"
d	100	"	"
e	100	1% benzene in Light petroleum	"
f	100	"	"
g	100	"	"
h	100	2% benzene in Light petroleum	Trace of brown oil.
i	100	"	"
j	200	5% benzene in Light petroleum	"
k	200	10% benzene in Light petroleum	"
l	200	50% benzene in Light petroleum	"
m	100	1% ethanol in Light petroleum	Hexagonal plate crystals contaminated with oil.
n	100	"	"
o	100	"	"
p	100	10% ethanol in Light petroleum	Brown oil (trace).

Investigation of residue II (Table 18)

This residue appears to be almost entirely composed of sesquiterpene material and steam distillation followed by fractional distillation, produced a number of liquids. The results obtained were in substantial agreement with those of Williams[1] and the physical constants of some of the author's fractions are compared with the data given by Williams (Table 21). The details concerning the fractional distillation are therefore not recorded here.

Residues III and IV (Table 18) were not examined.

Table 21

Williams[1]

Fraction number	Refractive index	Specific rotation	Density (d.)
A	1.4770	- 30	0.886
B	1.4775	- 50	0.882
C	1.4765	- 44.4	1.000
D	1.4841	- 23.3	0.961
E	1.4910	- 8.3	0.937
F	1.4971	0	0.942
Retinulene	1.4944-72	+1.1- +1.6	0.913

(cont'd. on page 131)

Table 21 (cont'd)

Present work

Fraction number	Refractive index	Specific rotation	Density (d.)
1	no comparable fraction		
2	1.4756	- 39.31	0.857
3	1.4759	- 44.57	0.980
4	1.4822	- 25.83	0.960
5	1.4913	- 4.29	0.933
6	no comparable fraction		
7	1.4978	+ 1.25	0.916

Chromatography of the unsaponifiable matter

This fraction, received from Williams, was obtained by saponification of the residue left after steam distillation of the light petroleum extract, and shaking out the alkaline reaction mixture with light petroleum. Ceryl alcohol, known to be present, was removed by seeding and cooling. The oil was dissolved in benzene (500 ml.) and chromatographed on a column of alumina (60 x 4.5 cm.), collecting over 150 separate fractions of 50 ml. each. The results are summarised in Table 22.

Fraction A. After several months, a small amount of rod-shaped crystals was deposited, m.p. 186-190°C. Decolourisation of this solid with charcoal and repeated recrystallisation from methanol raised the melting point to 196°C. (25mg.). The Liebermann-Burchard test gave

a pink colour and when mixed with two drops of concentrated sulphuric acid, the crystals became brick-red.

Table 22

Fraction number	Weight (g.)	Volume of eluate (ml.)	Eluting solvent	Description
A	30	2,000	Benzene	Viscous brown oil. Very like starting material. Deposited crystals on long standing.
B	0.7	500	0.5% ethanol in benzene	Tailings of Fraction A.
C	1.1	500	1% ethanol in benzene	Pale orange oil.
D	0.3	500	2% ethanol in benzene	Small discoloured residue, eventually depositing crystals.
E	6.2	500	"	Dark oil. Deposited needle crystals on cooling.
F	2.4	500	"	Dark oil but no crystals separated until much later.
G	0.5	600	5% ethanol in benzene	Golden yellow oil.
H	2.0	1,000	10% ethanol in benzene	Dark resinous oil.
I	1.3	500	25% ethanol in benzene	"
J	0.8	900	1% glacial acetic acid in benzene	"
K	1.3	500	"	"

Found C, 79.66, 80.53, 80.42; H, 9.08, 9.27, 8.86

$(C_{10}H_{14}O)_n$ requires C, 79.95; H, 9.39%.

$[\alpha]_D^{17}$ +136.2°, $\lambda_{max.}$ 211.5 mμ ($E^{1\%}_{1cm.}$, 100).

The infrared spectrum (in potassium bromide disc) showed peaks at 1,750 (γ-lactone or carbonyl attached to a five-membered ring), 1,643 and 1,650 (unsaturation), 1,385 (C-methyl group), 914 (possibly vinylidene) and 806 and 827 $cm.^{-1}$ (trisubstituted double bonds).

The residual oil of fraction A was not examined.

Fractions B,G,H,I,J and K were not examined.

Fraction C. After several weeks, a few waxy crystals were obtained, m.p. 75-76° C., which appeared to be ceryl alcohol, m.p. 78° C. No further tests were carried out on this fraction.

Fraction D. The crystals which appeared were filtered off and found to be identical with those from fraction E.

Fraction E. About 250mg. of impure crystals, m.p. 138-140° C. were obtained from fractions D. and E. Recrystallisation from methanol raised the melting point to 139.5-140.5° C., undepressed on admixture with authentic β-sitosterol, m.p. 140° C.

Found C, 81.22; H, 11.95.

Calculated for $C_{29}H_{50}O.CH_3OH$[43] C, 80.67; H, 12.16%.

Fraction F. Crystals which appeared after prolonged standing were also identified as β-sitosterol.

THE LIGHT PETROLEUM-SOLUBLE FRACTION OF ARISTOLOCHIA SERPENTARIA

The crude drug

The root was received in two lots of approximately 4.5Kg. each. The first lot was only roughly screened for the presence of grass impurities but the second batch was carefully hand-picked and all adulterants were removed (203g.).

Extraction of the root

The raw drug was powdered and extracted with light petroleum in exactly the same procedure used for A.reticulata, omitting only the drying stage. The petrol-soluble extract was a dark-brown oil (350g.) having the same terpene odour as the root itself.

Isolation of aristolactone

Seeding the extract afforded 3.7g. crude aristolactone and chromatography of the residual oil yielded a further 6.2g. of impure material. Recrystallization from light petroleum finally gave 7.8g. pure lactone, which represents 0.093%W/w of the powdered root. The chromatographic distribution of the fractions was an exact replica of those given in Table 17, pages 125 and 126 for the light petroleum-soluble fraction of A.reticulata. The individual 50 ml. eluates were bulked in similar fashion and set aside for later examination.

THE LIGHT PETROLEUM-SOLUBLE FRACTION OF ARISTOLOCHIA INDICA

The crude drug

The raw material was received in bundles of varying sizes, tied either with tough raffia-like strips or with a piece of stem from the plant itself. The bundles were made up of both root and aerial parts, as described by the British Pharmaceutical Codex, 1934. The reddish-brown root is tortuous, with occasional transverse constrictions or fissures and the aerial parts are yellow-brown, subcylindrical and twisted, larger pieces being warty. Thinner pieces are marked with longitudinal furrows and fine transverse fissures.

Extraction of the root

The raw drug was disintegrated, again without previous drying and the resulting powder was rather coarser than that from the other species, due to the fibrous nature of the root. A trial extraction of 3Kg. yielded 55g. of greenish-brown oil, with a distinct peppery odour, quite different from the fragrant smell of *A.serpentaria* and *A.reticulata*. The extract was cooled and seeded but no crystals precipitated after a month.

Chromatography of the extract

After several months in the refrigerator, a fine sludge was deposited. This precipitate was removed by decantation and the two portions, supernatant liquor and deposited solid, were chromatographed separately.

(a) Precipitate. The greyish solid was suspended in light petroleum and chromatographed on a 90g. column of activated charcoal (1 part) and Whatman standard grade cellulose (3 parts). The 50 ml. fractions were

bulked into four residues (I-IV), as shown in Table 23.

Table 23

Residue number	Fractions collected	Eluting solvent	Optical rotation	Description
I	1-17	light petroleum	(-)0.13	Trace of oil having odour of root.
II	18-38	1% ethanol in light petroleum	(-)0.56	Small amount of oil, deposited solid on long standing.
III	39-53	2% ethanol in light petroleum	-	White fatty-acid type solid.
IV	54-110	5% ethanol in light petroleum	-	Trace of oil.

Residues I and IV were not examined.

The white solid deposited in residues II and III was separated by filtration, with difficulty, due to the clogging nature of the small crystals, which matted into a solid mass. Some of the solid was obtained as white crystals, m.p. 85-90°C., after recrystallisation from methanol.

(b) Supernatant liquor. The greenish-brown liquid was chromatographed on a similar column of charcoal-cellulose powder, examining each 50 ml. fraction in a 1 dm. tube, for optical rotation (Table 24).

Elution with 20% ethanol in light petroleum yielded only a negligible trace of oil. Fractions 1-6 were bulked as residue I and fractions 7-9 were made residue II. Both residues are pale orange, sweet-smelling oils which have become more viscous on storage. No further examination has been carried out on the extract from A.indica.

Table 24

Fraction number	Optical rotation	Eluting solvent
1	0.00	Light petroleum
2	0.00	"
3	0.00	"
4	− 0.09	"
5	− 2.13	"
6	− 1.64	"
7	+ 0.13	"
8	+ 0.25	"
9	+ 0.20	"
10	0.00	"
11-25	0.00	"
26-44	0.00	1% ethanol in light petroleum
45-60	0.00	2% ethanol in light petroleum

THE LIGHT PETROLEUM-SOLUBLE FRACTION OF ARISTOLOCHIA LONGA

The crude drug

Botanically it consists of tawnish-brown root and root-stock, some of the larger pieces being split longitudinally. The crown bears scars of aerial parts and many of the pieces are wrinkled longitudinally and show warty root scars. Other apparently smooth pieces are finely wrinkled longitudinally and bear transverse markings and root scars. The fracture is short and the transversely cut surface displays a markedly radiate wood.

Extraction of the root

A trial extraction on 3½g. of powdered drug yielded 29g. of a dark-brown oil. No aristolactone was deposited after cooling and seeding.

ARISTOLACTONE

REACTIONS OF ARISTOLACTONE

Isomerisation with acid

(i.) Heating with a resin

Aristolactone (0.1g.) in ethanol (95%; 10 ml.) was refluxed with activated Zeo-karb-225(1g.) until the optical rotation had reached a constant negative value (about 9 hours). Evaporation of the solvent gave *iso*aristolactone, m.p. 90-91°C., (35mg. from light petroleum). Yield 35%.

(ii.) Heating with sulphuric acid

Aristolactone (50mg.) in ethanol (95%; 3 ml.) was mixed with a solution of sulphuric acid (10%) in ethanol (50%), warmed to 40°C. for two minutes and quickly cooled. The crystalline product obtained by adding a few drops of water was washed until free of sulphuric acid and recrystallisation gave *iso*aristolactone, m.p. 89.5-90°C. (block), (40mg. from aqueous ethanol), $[\alpha]_D^{20}$ -42°. Yield 80%.

(iii.) Treatment with cold sulphuric acid

Aristolactone (0.49g.) in ethanol (95%; 10 ml.) was mixed with a solution (40 ml.) of sulphuric acid (10%) in ethanol (50%) at room temperature. The optical rotation was observed by placing 5 ml. of the reaction mixture in a one decimetre tube. The results are summarised in Table 25, page 140.

Table 25

Time (mins.)	Specific rotation	Time (mins.)	Specific rotation
0	+ 156	82	+ 34
3	+ 151	114	+ 10
5	+ 146	142	− 4
8	+ 141	162	− 13
12	+ 134	186	− 23
20	+ 118	210	− 31
30	+ 97	240	− 39
50	+ 70	270	− 42
70	+ 46	300	− 43

When the optical rotation reached a constant negative value (5 hours), dilution with water gave white needle crystals, recrystallised to give isoaristolactone (0.43g. from aqueous ethanol), m.p. 90-91° C. (block). Yield 86%.

Infrared spectrum (potassium bromide disc): peaks at 1742 (γ-lactone), 824 (trisubstituted double bond), 902 and 1641 cm.$^{-1}$ (vinylidene).

Reduction with lithium aluminium hydride

Aristolactone (0.54g.) in sodium-dried ether (25 ml.) was cooled to 0° C. in an ice-bath, and lithium aluminium hydride (0.1g.) was added portionwise during ten minutes. The reaction mixture was allowed to reach room temperature (a further ten minutes) and the excess reagent was

then decomposed by addition of water from a teat pipette. Water (10 ml.) was added and the mixture was extracted with ether (3x25 ml.), the ethereal layers bulked, dried (sodium sulphate) and evaporated to yield a viscous oil (0.54g.). Partial crystallisation occurred on addition of dry methanol and one recrystallisation of the solid gave white needles of the kobal (OXLV), m.p. 197-198°C., (90mg. from methanol), $[\alpha]_D^{16.5}$ + 82° (c, 0.477 in ethanol), $\epsilon_{\lambda 10 m\mu}$, 3234 (end-absorption). There is also a low-intensity maximum at 284 mμ (ε, 58).

Infrared spectrum (potassium bromide disc): peaks at 1752 (aldehyde carbonyl), 904 and 1645 (vinylidene) and 1733 cm.$^{-1}$ (shoulder; ketone carbonyl).

Found	C, 76.90;	H, 9.30.
$C_{15}H_{22}O_2$ requires	C, 76.91;	H, 9.47.
$C_{15}H_{24}O_2$ requires	C, 76.25;	H, 10.24%.

The product reduced ammoniacal silver nitrate and restored the colour to Schiff's reagent.

Oxidation with potassium permanganate

Aristolactone (0.51g.) in acetone (50 ml.) was cooled to -7°C. in an ice-salt mixture and finely powdered potassium permanganate was added portionwise during 50 minutes, with stirring and shaking. The brown precipitate was filtered off and evaporation of the acetone filtrate and washings yielded a discoloured solid (40mg.). The inorganic precipitate was boiled with water (4x30 ml.) and the combined aqueous filtrates, after cooling, deposited off-white crystals (70mg.). The aqueous residue was then extracted with ether, the ether layer washed

with water, dried (sodium sulphate) and evaporated to yield a pale brown, crystalline solid (0.14g.). The solid fractions were bulked and boiled with charcoal in acetone solution. Recrystallisation gave white needles of dihydroxyaristolactone (0.15g. from petrol-acetone), m.p. 158°C., $[\alpha]_D^{20}$ +128°, $\epsilon_{210m\mu}$ 2,200 (end-absorption).

Attempted preparation of methyl oxoaristate

Aristolactone (0.1g.) in ethanol (95%; 10 ml.) was mixed with activated De-Acidite (0.5g.) and set aside at room temperature. No change occurred in the optical rotation of the solution and aristolactone was recovered.

Attempted dehydrogenation with zinc dust

Aristolactone (0.56g.) was intimately mixed with zinc dust (Analar grade; 4g.) and heated in a sand-bath while passing a current of hydrogen through the reaction vessel. A clear, colourless, mobile liquid (33mg.) which distilled at 220°C., gave a yellow colour with tetranitromethane and showed end-absorption in the ultraviolet region (ϵ, 7,240 at 210mμ). The ultraviolet spectrum gave no indication of the presence of benzenoid, naphthalenic or azulenic material.

Extraction of the residue with ether gave a mobile yellow oil (0.22g.) which was distilled, yielding a product which also showed end-absorption in the ultraviolet region (ϵ, 7,000 at 210mμ).

Preparation of iso-oxoaristolic acid

(1) Aristolactone (0.46g.) in methanol (20 ml.) was treated with cold potassium hydroxide solution (0.5N; 5 ml.) in methanol (90%), the rapidly increasing optical rotation of an aliquot being observed. On complete conversion to methyl oxoaristate (point of maximum optical

rotation[2]), potassium hydroxide solution (0.5N; 5 ml.) in methanol (90%) was added and the solution heated on a water-bath for 4 hours. Concentration, acidification with dilute hydrochloric acid and extraction with ether gave iso-oxoaristolic acid (CXXXVI) as colourless plates (44mg. from petrol-ether), m.p. 143-144°C. (block), $[\alpha]_D^{20}$ -3.45° (c, 0.87 in ethanol), $\lambda_{max.}$ 243 mμ (ε, 6,780).

Found	C, 72.01;	H, 8.86.
$C_{15}H_{22}O_3$ requires	C, 71.83;	H, 9.21%.

(11) The progress of this reaction was followed spectrophotometrically. Aristolactone (0.605g.) was dissolved in solution of potassium hydroxide (0.5N; 5 ml.) in methanol (90%) and diluted to exactly 25 ml. with methanol. A blank was prepared in the same way, but omitting the lactone. The optical rotation of an aliquot was observed and, at the point of maximum rotation (formation of methyl oxoaristolate), 1 ml. of the test and blank was removed, diluted (1:1000) with ethanol (spectrophotometric grade) and the ultraviolet spectrum determined (curve I, Fig. 5, facing page 69). As the optical rotation gradually diminished during a period of weeks, further samples were with-drawn, diluted and examined in the ultraviolet region (curves II, Fig. 5, facing page 69). After 25 days at room temperature, the blank and test solutions were warmed over a water-bath for varying periods, to speed up the reaction. The results are summarised in Fig. 5, in which the calculated intensities are based on the molecular weight of iso-oxoaristolic acid.

Ozonolysis

Aristolactone (1.64g.) was dissolved in dry, ethanol-free chloroform (30 ml.) and a stream of ozonised oxygen was passed in for 3 hours at 0°C. The chloroform was removed at room temperature, under vacuum and the glassy ozonide was decomposed by refluxing for 3 hours with dilute hydrochloric acid (0.1N; 10 ml.), hydrogen peroxide (30%^{w}/v; 10 ml.) and water (30 ml.). The resulting aqueous suspension of oil was continuously extracted with chloroform for 12 hours, the chloroform layer dried (sodium sulphate) and evaporated to give a pale yellow oil (0.83g.), slightly acid to litmus and which gave a green colour with alkaline solution of sodium nitroprusside. The oil exhibited an ultraviolet maximum at 275 mμ ($E^{1\%}_{1cm.}$ 5.6) and end-absorption at 210 mμ ($E^{1\%}_{1cm.}$ 250).

The aqueous residue from the continuous extraction process gave a strongly positive sodium nitroprusside test. The solution was freeze-dried until about 10 ml. were left, neutralised with sodium hydroxide (0.1N) and distilled. The distillate gave an immediate orange precipitate when mixed with acid solution of 2:4-dinitrophenylhydrazine in ethanol (50%) but chromatography from alumina and several recrystallisations failed to give constant-melting fractions. Two small fractions of a few milligrams each were recovered, m.p. 162-164°C. and 152-154°C., but they were not examined further.

Ozonolysis of the chloroform-soluble oil for 1½ hours, decomposition of the ozonide as before and continuous extraction with chloroform yielded a viscous oil (0.25g.). The oil in ethanol (5 ml.) gave a sticky, flocculent precipitate with acid solution of 2:4-dinitrophenyl-

hydrazine, which was discarded.

The aqueous residue from this second ozonolysis was distilled on an oil-bath and the distillate yielded a yellow-orange 2:4-dinitrophenylhydrazone, recrystallised from ethanol (70%) to m.p. 147°C.

Found	C, 42.73; H, 3.20; O, 29.15; N, 24.90.
$C_8H_8O_4N_4$ requires	C, 42.90; H, 3.60; O, 28.54; N, 24.99%.

The melting point of authentic acetaldehyde 2:4-dinitrophenylhydrazone was 149°C. when recrystallised from ethanol (70%), and mixed melting point with the derivative obtained from the ozonolysis was sharp and undepressed (cf. reference 101).

Some of the distillate from the aqueous residue was mixed with saturated aqueous solution of dimedone, when a small crystalline deposit was obtained, m.p. 131-134°C., mixed melting point with acetaldehyde dimedone derivative, 132-135°C.

REACTIONS OF ISOARISTOLACTONE

Chromic acid oxidation

isoAristolactone (0.5g.) was gently refluxed for 2 hours with potassium dichromate (4.5g.), water (10 ml.) and sulphuric acid (4 ml.). Steam distillation of the reaction mixture gave acetic acid, with traces of formic acid. The residue was extracted with ether, the ether layer dried (sodium sulphate) and evaporated to give the non-volatile products. Paper chromatography (phenol-formic acid-water, 80:1:19) indicated the presence of two dibasic acids, one of which was succinic acid, R_F 0.75. The other spot, R_F 0.97, appeared to be an acid containing approximately 8-10 carbon atoms. Succinic acid was confirmed by addition of chloroform which precipitated a small amount of crystals, m.p. 183-185°C. In a second solvent system, (butanol-formic acid (90%)-water, 10:3:10), two acids again showed up, R_F 0.75 and 0.90. The slower running acid had the same R_F value as authentic succinic acid.

The dibasic acid mother liquors were partitioned in chloroform on a buffered (pH 7.4) silica gel column (20x1 cm.). The column was prepared by mixing silica powder with half its weight of aqueous buffer solution and packed as a crumbly powder. The chloroform solution of the mixture was placed on top of the column and washed through with chloroform (collecting in weighed vessels). Several fractions were collected and run on paper in the butanol-formic acid-water system already mentioned, showing that the eluates contained only the faster running dibasic acid. The fractions were combined and evaporated to give an oil (36mg.) which distilled at about 150°C. and which subsequently, on cooling became semi-

solid. The product was insufficient for characterisation.

Reduction with lithium aluminium hydride

IsoAristolactone (0.4g.) in sodium-dried ether (25 ml.) was cooled to 0°C. in an ice-bath and lithium aluminium hydride (60mg.) was added portionwise during 10 minutes. The reaction mixture was diluted with water (10 ml.) (after decomposing the excess reagent with one drop of water) and extraction with ether (3x25 ml.), drying of the ethereal layers (sodium sulphate) and evaporation gave a clear oil (0.36g.). Addition of a little methanol resulted in partial crystallisation and the precipitated solid was filtered off and recrystallised to yield colourless needle crystals of Isohotol (OHIV) (40mg. from methanol), m.p. 211-213°C., (block; corrected), $[\alpha]_D^{20}$ -50.1° (c, 0.40 in chloroform), $\lambda_{max.}$ 209 mμ (ε, 4,960) and 290 mμ (ε, 30).

Found	C, 77.42;	H, 9.29.
$C_{15}H_{22}O_2$ requires	C, 76.91;	H, 9.47.
$C_{15}H_{24}O_2$ requires	C, 76.25;	H, 10.24%.

The Isohotol reduced ammoniacal silver nitrate solution on warming and restored the colour to Schiff's reagent in less than 5 minutes. A blank in both cases was negative. The Iodine value was 1.93 in 10 minutes, Table 1, page 64.

Ozonolysis

IsoAristolactone (0.153g.) was dissolved in dry chloroform (15 ml.) and a stream of ozonised oxygen was passed in for 45 minutes at -7°C. The solution was allowed to reach room temperature and glacial acetic acid (2 ml.) and zinc dust (Analar; 0.4g.) were added during 10 minutes.

After a further 30 minutes, with periodic shaking, the reaction mixture was filtered into a separator, the filter was washed with chloroform and the mixed filtrate and washings were extracted with water (4x20 ml.). The bulked aqueous extracts were neutralised (20% NaOH), slightly acidified (acetic acid) and a solution of dimedone (0.35g.) in ethanol (20 ml.) was added. After one hour in the refrigerator, formaldehyde dimedone derivative (0.103g.), m.p. 183-187°C. was filtered off. The mixed melting point with authentic formaldehyde dimedone derivative was 185-188°C. Yield, 54% of the theoretical weight for one vinylidene group.

REACTION OF DIHYDROISOARISTOLACTONE

Ozonolysis

Dihydroisoaristolactone (0.114g.) was ozonised and the ozonide was decomposed as described under isoaristolactone, (page 147). The boiled aqueous extracts gave no precipitate with solution of dimedone, after 2 hours in the refrigerator.

REACTIONS OF METHYL OXOARISTATE

Preparation of methyl dihydro-oxoaristate

Methyl oxoaristate (1g.) in ethanol (25 ml.) was shaken with platinum oxide in the presence of hydrogen until one mol. was absorbed. Filtration and evaporation gave colourless needles of methyl dihydro-oxoaristate (CXXXIV) (0.9g. from 60% ethanol), m.p. 68-68.5°C. (block), $[\alpha]_D^{15}$ +152° (c, 0.788 in ethanol), $\lambda_{max.}$ 290 mμ (ε, 160) and end-absorption at 210 mμ (ε, 3,600).

Infrared spectrum (potassium bromide disc): peaks at 815 (tri-substituted double bond), 1732 (ester carbonyl) and 1693 cm.$^{-1}$ (ketone carbonyl).

Found	C, 71.82%	H, 9.77.
$C_{16}H_{26}O_3$ requires	C, 72.13%	H, 9.84%.

The product gave a yellow colour with tetranitromethane.

Preparation of methyl tetrahydro-oxoaristate

Methyl oxoaristate (2g.) in ethanol (30 ml.) was shaken with platinum oxide (0.19g.) in the presence of hydrogen until two mols. were absorbed. Filtration and evaporation gave a colourless oil which could not be induced to crystallise.

REACTIONS OF METHYL DIHYDRO-OCOANISTATE

Ozonolysis

Methyl dihydro-ocoanistate (1.37g.) in dry chloroform (35 ml.) was cooled in an ice-bath and a stream of ozonised oxygen was passed through the solution for 2 hours. Evaporation of the chloroform at room temperature under reduced pressure left a glassy ozonide which was decomposed by heating with water (25 ml.) at 60°C. for 1 hour. Steam distillation gave a faintly acid distillate which was neutralised and steam distilled again. The second steam distillate did not react with acid solution of 2:4-dinitrophenylhydrazine.

The non-volatile residue was extracted with chloroform (4x25 ml.), the chloroform layers bulked, dried (sodium sulphate) and evaporated to yield a yellowish oil which was distilled to give a clear, pale yellow oil (0.58g.), b.p. 220-240°C./0.4m.m.Hg.

Found	C, 66.34;	H, 8.60.
$C_{16}H_{24}O_5$ requires	C, 64.84;	H, 8.20%.

The product gave a yellow colour with tetranitromethane and deep red colour with alkaline solution of sodium nitroprusside. The ultra-violet spectrum was studied in detail and the results are summarised in Table 16, page 122.

The oil (0.27g.) was dissolved in solution of sodium carbonate (2%; 20 ml.) and potassium permanganate solution (3%) was added slowly during 2 hours at 50°C., when the supernatant was faintly pink. The brown precipitate was removed by filtration, the filtrate was acidified

(dilute hydrochloric acid), extracted with ether, the ether layer dried (sodium sulphate) and evaporated to yield a pale yellow oil (0.18g.). Treatment with diazomethane and distillation gave a clear mobile liquid (76mg.), which could not be identified.

Found	C, 67.20;	H, 9.4.
$C_{15}H_{24}O_4$ requires	C, 67.10;	H, 9.0%.

Reaction with alkali

Methyl dihydro-oxoaristolate (0.22g.) was dissolved in solution of potassium hydroxide (0.5g; 5 ml.) in methanol (90%) and made up to exactly 25 ml. with methanol. A blank was made up in exactly the same way but omitting the sample. Both solutions were warmed over a water-bath for 4 hours, being cooled at intervals, one ml. aliquots withdrawn, diluted suitably with ethanol (spectrophotometric grade) and the ultra-violet spectrum determined. Very little change had occurred at the end of the 4 hours heating (curve IV, Fig. 6 facing page 91) but when some of the methanol was removed, thus concentrating the alkali, a profound change was observed in the ultraviolet spectrum. The results of the spectra are summarised in Fig. 6. Concentration of the solution, acidification (dilute hydrochloric acid) and extraction with ether gave an oily acid (0.16g.) which did not solidify, $\lambda_{max.}$ 245mμ (ε, 6,300), $[\alpha]_D^{20}$ - zero, n_D^{23} 1.5025. This oily acid is presumably dihydroiso-oxo-aristolic acid.

REACTIONS OF METHYL TETRAHYDRO-OXOARISTATE

Attempted reduction with aluminium isopropoxide

Methyl tetrahydro-oxoaristate (1.5g.) in dry isopropanol (25 ml.) was refluxed for 6 hours with aluminium isopropoxide (1g.). A small portion of the distillate was removed at intervals but no evidence of acetone could be detected at any stage and methyl tetrahydro-oxoaristate (1.4g.) was recovered.

Oxidation with nitric acid

Methyl tetrahydro-oxoaristate (0.7g.) in a little ether was warmed with concentrated nitric acid (5 ml.) at 60°C. until evolution of nitrous fumes ceased. The reaction mixture was extracted with ether, the ether layer dried (sodium sulphate) and evaporated to yield a viscous oil. This residue was treated with ammonia, boiled until no more ammonia was evolved and solution of silver nitrate (5%) was added. A slightly discoloured precipitate of silver salt which appeared was filtered off, washed thoroughly with water and dried under vacuum.

Found	C, 32.61;	H, 4.68;	Ag, 44.6.
$C_{12}H_{20}O_4Ag_2$ requires	C, 32.45;	H, 4.54;	Ag, 48.6%.

Paper chromatography in butanol-formic acid-water (10:3:10) indicated that the silver salt was derived from a dibasic acid (R_p, 0.97) of higher molecular weight than pimelic acid.

REACTIONS OF HEXAHYDROISOARISTOLACTONE

Reduction with lithium aluminium hydride

Hexahydroisoaristolactone (0.47g.) in sodium-dried ether (40 ml.) was mixed with lithium aluminium hydride (0.23g.), added portionwise during 15 minutes, at room temperature. The reaction mixture was kept at room temperature for a further 90 minutes, when the excess of lithium aluminium hydride was decomposed by dropwise addition of dilute hydrochloric acid. Water (20 ml.) was added and the reaction mixture was extracted with ether (4x20 ml.), the ethereal layers bulked, dried (sodium sulphate) and evaporated to give a solid crystalline mass, which was recrystallised to give long needles of the diol (XLII; R=H) (0.34g. from petrol-ether), m.p. 106-107°C. (block; corrected), $[\alpha]_D^{20}$ +18.7° (c, 0.3592 in chloroform).

Found C, 74.51; H, 12.53.

$C_{15}H_{30}O_2$ requires C, 74.35; H, 12.53%.

Preparation of the diol monobenzoate

The diol from hexahydroisoaristolactone (61mg.) was dissolved in dry pyridine (1 ml.) and a few drops of re-distilled benzoyl chloride were added. After 1 hour at room temperature, solution of sodium hydrogen carbonate (5%; 3 ml.) was added. Oily globules separated out but cooling in the refrigerator (24 hours) failed to yield a solid product. The reaction mixture was diluted with water (10 ml.) and extracted with ether (3x25 ml.); the ethereal

layers were bulked and washed successively with solution of sodium hydrogen carbonate (5%), water, dilute hydrochloric acid and water, in that order. The ether solution was dried (sodium sulphate) and evaporated to yield a colourless viscous oil (68mg.) which did not solidify, $[\alpha]_D^{23}$ +1.9° (c, 2.41 in chloroform).

Found	C, 75.37;	H, 10.10.
$C_{22}H_{34}O_3$ requires	C, 76.20;	H, 9.90%.

Dehydrogenation

(1) Semi-solid residues (0.3g.) from the preparation of pure hexahydrojuggaristolactone were mixed with 20% palladium on charcoal (30mg.) and gently refluxed over a micro-burner. The reaction vessel in this and subsequent dehydrogenation experiments consisted of a piece of glass tubing (internal diameter 7m.m.) having a small bulb blown at one end and a second much smaller bulb or depression blown in the wall of the tube about 5cm. from the larger bulb.

After a few minutes heating, a violet liquid began to reflux on the sides of the tube and this distillate was collected in the small bulb. The liquid was removed by means of a finely drawn test pipette and washed out into cyclohexane. This process was repeated until no more violet liquid collected in the small bulb. The cyclohexane solution was shaken out with phosphoric acid (90%), when the azulene material went into the acid layer, leaving the non-azulenic material behind. The acid layer was diluted with water to approximately 25% and re-

extracted with cyclohexane, when the violet colour was restored to the organic solvent. After washing with water (2x5 ml.), the cyclohexane solution was dried (sodium sulphate) and diluted suitably with cyclohexane for determination of the ultraviolet spectrum. Peaks showed at 244, 279, 289, 306, 333 and 348 mμ, but the visible spectrum showed only general absorption with no marked maxima. In oxygen-free sulphuric acid (50%), the amulone showed three characteristic maxima at 227, 269 and 374 mμ. The oxygen-free sulphuric acid was prepared by diluting a pure sample of sulphuric acid with an equal volume of distilled water, refluxing the mixture for 1 hour, while passing a stream of nitrogen through and storing the cold solution under nitrogen. Immediately before use, nitrogen was again passed through the sulphuric acid for 30 minutes.

The non-amulonic fraction which was separated by phosphoric acid extraction was also examined in the ultraviolet region but it showed only end-absorption.

(ii) Pure hexahydroisoaristolactone (0.4g.) was mixed with 20% palladium on charcoal (0.2g.) and heated in a bath of Wood's metal at 330°C. for 6 hours. No amulonic material distilled and the reaction mixture was thoroughly extracted with ether. The ethereal solution was filtered and evaporated to yield a brownish oil (30mg.) which showed well defined maxima at 228 and 281 mμ and a low-intensity maximum at 312 mμ.

Chromic acid oxidation

Hexahydroisoaristolactone (0.2g.) was warmed on the water-bath for 2 hours with a mixture of potassium dichromate (1g.), water (4 ml.) and

concentrated sulphuric acid (1 ml.). The reaction mixture was diluted with water (10 ml.) and extracted with ether (2x30 ml.). The ether extract was washed with several portions of water, dried (sodium sulphate) and evaporated to give a semi-solid oil (0.14g.). Paper chromatography of the product (phenol-formic acid-water, 80:1:19) indicated the presence of succinic acid, R_F 0.75 and a very large fragment, R_F 0.98.

The product was re-oxidised under the same conditions for a further 6 hours and extracted as above. Only a trace of product was isolated, R_F 0.87, (butanol-formic acid-water, 10:3:10), and no sign of succinic acid was found. Glutaric and adipic acids had R_F values of 0.85 and 0.89 respectively in the same system.

REACTIONS OF DIHYDROXYARISTOLACTONE

Hydrogenation

(i) Dihydroxyaristolactone (0.1g.) in ethanol (5 ml.) was shaken with 20% palladium on charcoal (13mg.) in the presence of hydrogen until one mol. was absorbed. Filtration and evaporation left a solid residue, recrystallised to give needle crystals (55mg. from petrol-acetone), m.p. 135-136°C., $\epsilon_{210m\mu}$ 3,000 (end-absorption). The melting point was raised to 139-140°C. on admixture with starting material.

The end-absorption in the ultraviolet spectrum was thought to indicate incomplete hydrogenation of even one double bond and the crystals were rehydrogenated, this time until no further uptake was observed. Isolation of the product in the usual manner gave only a few milligrams of solid, m.p. 120-125°C., while the remainder of the product was an oil.

(ii) Dihydroxyaristolactone (90mg.) in ethanol (10 ml.) was hydrogenated to completion as above, using platinum oxide (12mg.) as catalyst. Filtration and evaporation gave a slightly discoloured residue which was boiled with charcoal in ether and then recrystallised to give fine needle crystals of tetrahydrodihydroxyaristolactone (CXLVIII) (60mg. from petrol-ether), m.p. 123-124°C. (blocks; corrected), $[\alpha]_D^{20}$ +32.2° (c, 0.360 in chloroform).

Found	C, 66.59;	H, 9.47.
$C_{15}H_{26}O_4$ requires	C, 66.65;	H, 9.69%.

Oxidation with sodium metaperiodate[66a]

Dihydroxyaristolactone (21.3mg.) was suspended in water (2 ml.) and mixed with solution of sodium hydrogen carbonate (N/5 1.5 ml.) and solution of sodium metaperiodate in water (7%; 2 ml.). The soluble metaperiodate was prepared from the sparingly soluble paraperiodate by recrystallisation of 100g. from a mixture of concentrated nitric acid (45 ml.) and water (150 ml.).

The dihydroxyaristolactone gradually went into solution and the reaction was allowed to proceed for 1½ hours. A small precipitate which settled out was inorganic and was removed by decanting. The following reagents were then added in turn to the decanted liquid: hydrochloric acid (N/5 3 ml.), aqueous solution of disodium hydrogen arsenate (20.4%; 2 ml.), solution of sodium acetate (N/5 2 ml.) and saturated aqueous solution of dimedone (20 ml.). After three hours, no precipitate was obtained.

Control experiments using glucose (15-20mg.) were carried out previous to the above experiment and quantitative yields of formaldehyde dimedone derivative, m.p. 187-189° C., were obtained from the reaction mixture.

Saturated aqueous solution of dimedone was found to be more sensitive than the more usual alcoholic solution, when dealing with minute quantities of formaldehyde.

Attempted saponification

Dihydroxyaristolactone (0.2g.) in methanol (5 ml.) was refluxed with solution of potassium hydroxide (0.5N; 3 ml.) in methanol (90%) for 3½ hours. At this point the uptake of alkali was found to be less

than half of the theoretical amount required and extraction of the neutralised solution with ether yielded unchanged starting material (130mg.). The aqueous residue was acidified and extracted again with ether, this time giving 70mg. of crystals, m.p. 153-154°C., raised to 159°C. on chromatography from ether on a column of charcoal (5%) and cellulose powder.

Longer refluxing with alkali caused some decomposition and some dihydroxyaristolactone was recovered by ether extraction of the acidified reaction mixture, having m.p. 160°C., $[\alpha]_D^{18}$ +130°.

Attempted isomerisation

Dihydroxyaristolactone (55mg.) in ethanol (2 ml.) was warmed with a solution of sulphuric acid (10%) in ethanol (50%) for 2 minutes. The solution was cooled, diluted with water (10 ml.) and extracted with ether (30 ml.). The ether solution was dried (sodium sulphate) and evaporated, leaving a semi-solid product, recrystallised to give needle crystals of dihydroxyaristolactone (45mg. from petrol-ether), m.p. 159-160°C. (block corrected), $[\alpha]_D^{18}$ +127°, $\epsilon_{210m\mu}$ 3,300 (end-absorption).

When the attempted isomerisation was repeated, heating under reflux for 15 minutes, the solution became very dark and no product was isolated.

REACTION OF TETRAHYDRODIHYDROXYARISTOLACTONE

Oxidation with sodium bismuthate

Tetrahydrodihydroxyaristolactone (33mg.) in aqueous dioxan (30% 6 ml.) was mixed with sodium bismuthate (36mg., containing 80% $NaBiO_3$) and phosphoric acid (30% 2 ml.) and shaken for 3 hours, when the reaction mixture had become white and the reaction was regarded as complete. The milky solution was extracted with ether (3x20 ml.), the ether layers bulked, dried (sodium sulphate) and evaporated to yield a pale yellow oil. The oil, in ethanol, gave an amorphous yellow precipitate with acid solution of 2:4-dinitrophenylhydrazine but attempts to recrystallise caused decomposition.

REACTION OF KETAL FROM ARISTOLACTONE

Attempted isomerisation

The ketal (90mg.) was dissolved in a solution (6 ml.) of sulphuric acid (10%) in ethanol (50%) and the optical rotation was observed. After 6 hours, no change was observed and the starting material was recovered, m.p. 197-198°C.

MISCELLANEOUS EXPERIMENTS

DEHYDROGENATION EXPERIMENTS

Mixed oily residues

In the preparation of isoaristolactone, methyl oxoaristate and dihydroxyaristolactone from pure aristolactone, some oily residue invariably remained after isolation of the solid products. The oily fractions from a number of experiments were bulked in ethanol (25 ml.) and shaken with platinum oxide in the presence of hydrogen until no further uptake occurred. Filtration and evaporation gave a yellow oil (0.6g.) which was dehydrogenated as described under hexahydroisoaristolactone, page 155, in a bath of Wood's metal at 330°C., for 1 hour. A violet distillate appeared within a few minutes and it was removed with a finely drawn teat pipette as it collected in the upper bulb, and washed out into hexane. On standing in hexane overnight, clusters of tiny white crystals were deposited and these were isolated by decanting the violet supernatant and washing the crystals with a little hexane. The crystals (less than 1mg.), m.p. 144-145°C. (block; corrected), showed ultraviolet absorption maxima at 229, 287 and 318 mμ .

The violet azulene was separated from non-azulenic matter in the usual way (page 155) and it showed absorption peaks at 280, 289, 305, 333 and 348 mμ (in hexane), suggesting that it was identical with the azulene isolated previously (page 156). The spectrum of this azulene was not obtained at wavelengths below 260 mμ , due to the opacity of the hexane used.

Oily isoarlebolactone

The oily residue (0.15g.) from the isolation of isoarlebolactone was dehydrogenated as before, but without preliminary hydrogenation. The violet azulene obtained showed absorption peaks at 279, 288, 306, 339 and 348 mμ and, since it is typical of all azulene products derived from arlebolactone derivatives, the complete ultraviolet spectrum is reproduced in Fig. 1, page 70. The visible spectrum again showed only a broad general absorption with no marked peaks.

The ultraviolet spectrum in sulphuric acid (50%) showed peaks at 226, 268 and 374 mμ (Fig. 3, page 80).

PYRETHROSIN

Dehydrogenation

Pyrethrosin (0.5g.), m.p. 196°C. was intimately mixed with zinc powder (Analar; 2g.) and dehydrogenated by heating at 330°C. for 45 minutes, in a bath of Wood's metal, as described under hexahydroisoaristolactone, page 155. The bright blue azulene which distilled was separated from the non-azulenic material by a phosphoric acid separation. The ultraviolet absorption spectrum showed maxima (in hexane) at 287, 307, 349 and 366 mμ with a shoulder at approximately 336 mμ (Fig. 2, facing page 77). In sulphuric acid (50%), peaks showed at 230, 272 and 363 mμ (Fig. 4, page 81).

The non-azulenic material which was recovered had an ultraviolet absorption spectrum typical of a naphthalene derivative, as would be expected.

Attempted dehydrogenation of pyrethrosin with 20% palladium-charcoal also produced a blue distillate but the blue colour was quickly destroyed by continued heating and no azulenic material was recovered.

Hydrogenation

Pyrethrosin (0.5g.) in glacial acetic acid (15 ml.) was shaken with platinum oxide (69mg.) in presence of hydrogen until two mols. were absorbed. The catalyst was removed by filtration and the solvent was evaporated from the filtrate, under reduced pressure. The residue, a very viscous oil, was recrystallised from carbon tetrachloride (twice) and from petrol-ether (three times) to yield white micro-crystals of tetrahydropyrethrosin (83mg.), m.p. 225-227°C., (block; corrected).

The literature melting point is 230-232°C.[25]

This compound was used for comparison of its infrared spectrum with that of hexahydrodiepoxyisobolactone. (The spectra are unfortunately not available.)

DETERMINATION OF IODINE VALUES

All iodine values were determined by the pyridine bromide method of the British Pharmacopoeia, 1953, and the reagent was freshly prepared at not more than four-week intervals.

The determinations were carried out in a dry glass-stoppered bottle of approximately 30 ml. capacity, into which the substance under test (2-10mg.) was weighed and dissolved in carbon tetrachloride (1-2 ml.) or, occasionally, in chloroform. The reagent (2-5 ml.) was added by pipette, draining for a carefully measured time (1 minute) and the bottle was then stoppered and placed in a dark cupboard for a measured period. Solution of potassium iodide (10%) was added and the stopper was washed with a little distilled water. The liberated iodine was titrated to a very pale yellow colour with sodium thiosulphate solution (0.02N), two drops of an almost transparent starch (AnalaR grade) mucilage were added and titration was continued to a colourless end-point. A blank was carried out simultaneously with every determination, omitting the sample but otherwise identical. The difference between the blank and test titrations represents the uptake of halogen and the number of double bonds was calculated according to the formula:-

$$\text{No. of double bonds} = \frac{(a-b) \times m}{v \times 2000 \times 50}$$

where a = blank titration (ml. of 0.02N thiosulphate).

b = test titration (ml. of 0.02N thiosulphate).

m = molecular weight of substance under test, in milligrams.

v = weight of substance taken, in milligrams.

The results of a number of determinations are given in Table I, page 64 and Table 15, page 116.

ULTRAVIOLET SPECTRA IN THE RANGE 190-220 mμ.

By replacing the more usual quartz prism with a fused silica prism, the useful range of the spectrophotometer was extended to approximately 200 mμ. Although the silica prism is advertised as extending the range to 185 mμ, this ability was found to be very much dependent on the solvent used and no readings were possible below 203 mμ when using absolute ethanol (spectrophotometric grade) in a 1 cm. cell. It was discovered that stable readings were obtainable to 190 mμ with distilled water as solvent and 195 mμ was reached with 10% ethanol but other solvents, even when specially purified, were not satisfactory below 205 mμ. Attempts to increase the sensitivity of the instrument by using cells of path width 1m.m. were unsuccessful due to the rapid loss of liquid between the faces of the two parts of the cells available and all spectra in the 190-220 mμ region were therefore determined in 1 cm. cells.

The curves obtained from aristolactone and isoaristolactone are given in Figs. 9 and 10, page 170.

In absolute ethanol, dihydroxyaristolactone showed a maximum at 204 mμ (ε, 4,600) and isoketal at 205 mμ (ε, 6,500).

Fig.9

----- Aristolactone

_____ iso Aristolactone

Ultraviolet spectra in absolute ethanol

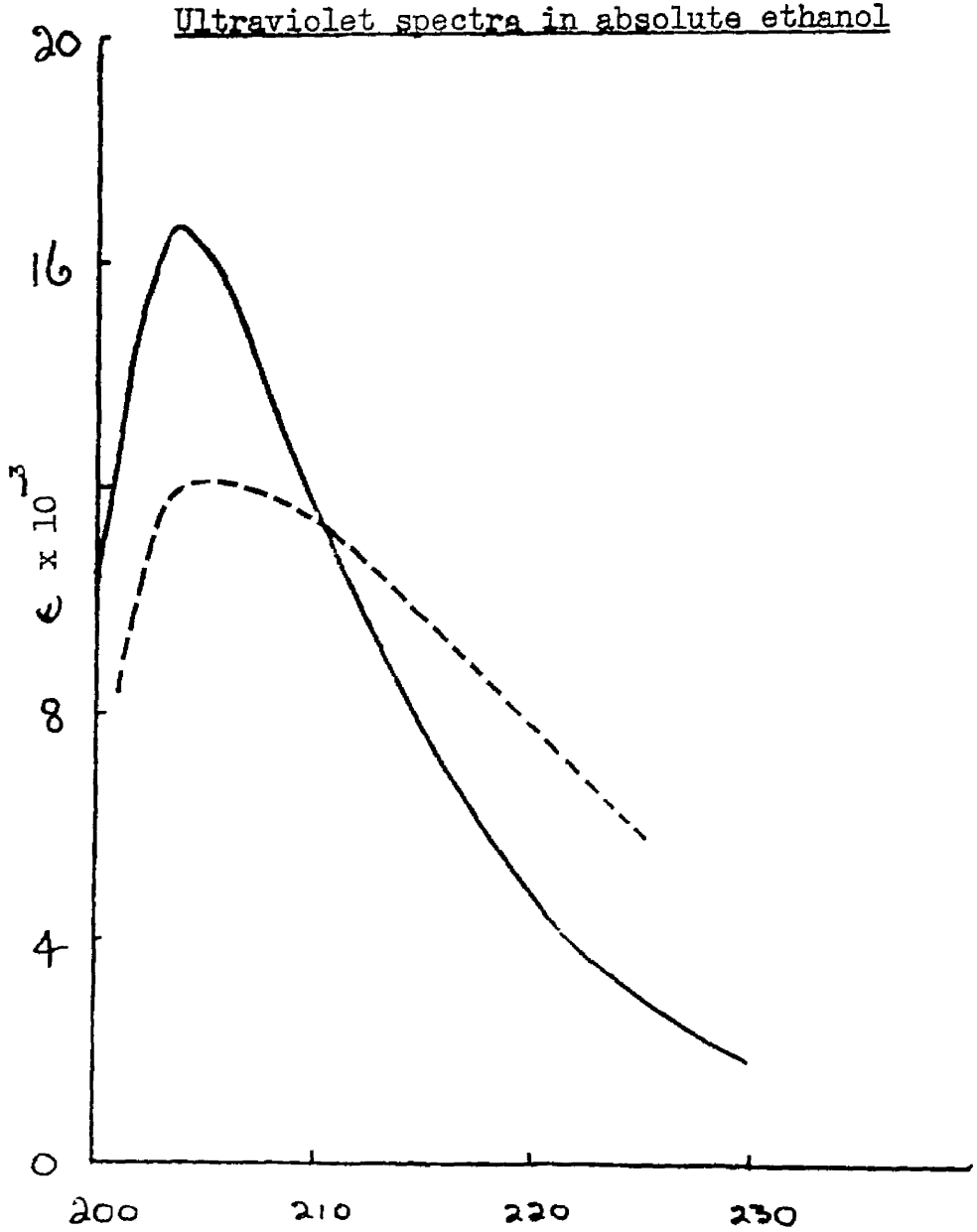
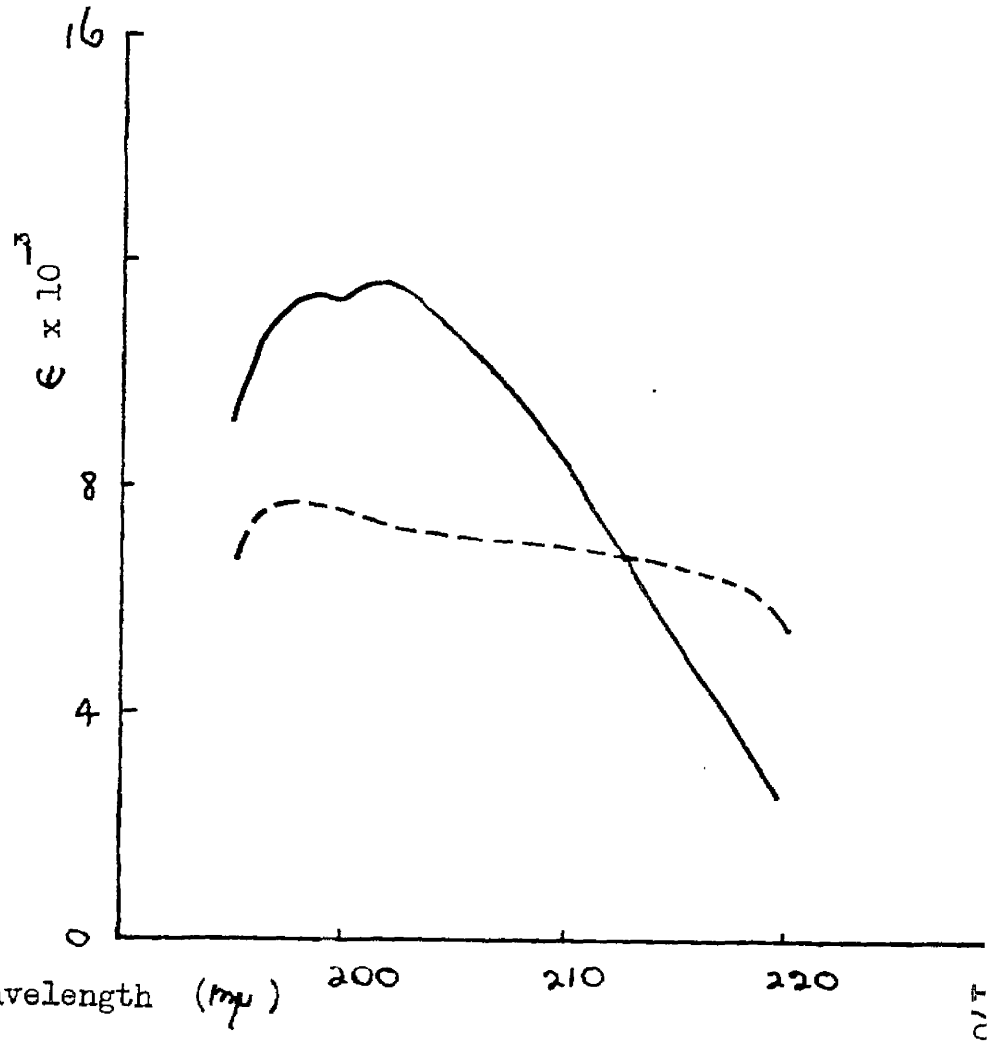


Fig. 10

----- Aristolactone

_____ iso Aristolactone

Ultraviolet spectra in 10% ethanol



CONTRIBUTION OF THE VINYLIDENE GROUPING TO THE ULTRAVIOLET ABSORPTION SPECTRUM IN THE 210-215 mμ REGION

All readings were determined in 1 cm. cells using absolute ethanol (spectrophotometric grade) as solvent. In three cases, the contribution of the vinylidene grouping was assessed by subtraction of the absorption of a hydrogenated derivative from the absorption of the parent compound, at the same wavelength. The intensities of absorption (ϵ) are listed in Table 13, page 108, and plotted against the number of carbon atoms in the ring, Fig. 7, page 113.

The slope of each curve was found by plotting the intensity of absorption (ϵ) against the wavelength between 210 and 215 mμ and joining the intensities at 210 and 215 mμ by a straight line. The tangent of the angle which this line makes with the horizontal is a measure of the slope of the curve. In this case, one large square on the graph paper (one inch) was chosen as equivalent to an intensity of 1,000 (ordinate) and two large squares represented 5 mμ (abscissa) but any convenient scales may be chosen provided that the same scale is used for comparison with the slopes of the curves of other compounds. The slope values for some of the compounds in Table 13 are listed in Table 14, page 112 and illustrated graphically in Fig. 8, page 113 in which the tangent of the angle is plotted against the number of carbon atoms in the ring to which the vinylidene is attached.

Preparation of β-cudesmol from β-cudesmol 3:5-dinitrobenzoate[102]

β-Cudesmol 3:5-dinitrobenzoate (0.2g.) in benzene (6 ml.) was placed on top of a column of alumina (40g.), which had been previously

treated with potassium hydroxide (4g.) in water (3 ml.). The column was eluted with 50 ml. of a mixture of ether and benzene (1:3). Evaporation of the solvent gave a semi-solid product, m.p. 68-75°C., which was purified by sublimation under vacuum to yield α-eudesmol (40mg.), m.p. 78-78.5°C. (block), $[\alpha]_D^{17}$ +63.2°. Literature constants[96], m.p. 76°C., $[\alpha]_D$ +63.8°.

Preparation of methyl labd-8(20)-en-15-oate from methyl labdanolate[95]

Methyl labdanolate (0.2g.) in pyridine (3 ml.) was mixed with phosphorus oxychloride (0.5 ml.) and left overnight at room temperature (16 hours). The reaction mixture was then added slowly, with stirring, to ice-water (25 ml.). The resulting solution was extracted with ether (3x20 ml.), the ether layers bulked, dried (sodium sulphate) and evaporated to yield an oil with a faint odour of pyridine. The oil was adsorbed on alumina (2g.) from light petroleum and eluted with benzene/light petroleum (1:4). Removal of the solvent afforded a very thick, pale yellow syrup (0.18g.), $[\alpha]_D^{18}$ +16°, n_D^{18} 1.4972. Literature constants[95], n_D^{20} 1.4969, $[\alpha]_D$ +27°.

Caryophyllene

A sample of caryophyllene obtained from Dr. G. Buchanan of the Chemistry Department, Glasgow University, was fractionally distilled and the middle fraction was collected, n_D^{18} 1.4926. Literature constant, n_D^{20} 1.4995[103].

Preparation of dihydroisocaryophyllene

Caryophyllene (1.35g.) in absolute ethanol (10 ml.) was shaken with 2% palladium-barium carbonate (0.43g.) in the presence of hydrogen until one mol. was absorbed. Filtration and evaporation yielded a

pale yellow oil, redistilled at 84°C./0.8m.m. Hg. to give a colourless fragrant liquid (0.7g.), n_D^{21} 1.4880, d_{21}^{21} 0.8876-0.8880. These constants compare favourably with those recorded by Naves and Perottet[103], b.p. 116-125°C./10m.m. Hg., n_D^{20} 1.486-1.490, d_{20}^{20} 0.887-0.889.

THE ALANTOLACTONES

The commercial product known as helenin (a mixture of alantolactone and isoalantolactone with a little dihydroisoalantolactone) was used in these experiments and the sample obtained melted over the range 66-78° C.

Ammonia adducts

Helenin (1.07g.) in ethanol (95%; 10 ml.) was saturated with ammonia gas, tightly stoppered and left overnight. The solid (0.7g.) which deposited was filtered off and fractional crystallisation gave four fractions (Table 26).

Table 26

Fraction number	Weight (mg.)	Melting point range (°C.)
I	102	236-240
II	56	227-233
III	276	213-215
IV	124	205-208

Fraction I, crystallised from ethanol (charcoal) yielded isoalantolactone ammonia adduct (38mg.), m.p. 248-249° C. (decomp.). Molecular weight (by Rast method) 345, theoretical 461, $\epsilon_{210 m\mu}$ 1213 (end-absorption).

Found C, 74.74; H, 8.64; N, 2.92.

Calculated for $(C_{15}H_{20}O_2)_2NH_3$, C, 74.84; H, 9.00; N, 2.91%.

Fraction IV, crystallised from ethanol (charcoal) yielded a few milligrams of colourless needle crystals of alantolactone ammonia adduct, m.p. 205-205° C. (decomp.), $\epsilon_{210\,m\mu}$ 2770 (end-absorption).

Found C, 74.82; H, 8.30; N, 2.86.

Calculated for $(C_{15}H_{20}O_2)NH_3$ C, 74.84; H, 9.00; N, 2.91%.

Dimethylamine adducts

Helenin (0.5g.) in absolute ethanol (10 ml.) was mixed with an excess of dimethylamine in a tightly stoppered flask. On standing overnight, long needle crystals, m.p. 148-150° C., separated out and were filtered off. Partial evaporation of the filtrate gave a second crop of crystals, m.p. 148-150° C. The two lots of crystals were bulked and recrystallised to yield colourless needles (42mg. from aqueous ethanol), m.p. 150° C. (block), $\epsilon_{210\,m\mu}$ 1218 (end-absorption).

Found C, 73.41; H, 9.25; N, 4.89.

$C_{15}H_{20}O_2NH(CH_3)_2$ requires C, 73.55; H, 9.80; N, 5.05%.

No attempt was made to isolate a second dimethylamine adduct from the mother liquors.

Reversed phase partition chromatography of helenin

Kieselguhr (15g.) which had been previously treated with dimethyl silane, was mixed with hexane (13 ml.), b.p. 68.5-69° C., and packed into a column of 1 cm. diameter. Helenin (152mg.) was mixed with a very

small quantity of the silicone- and hexane-treated kieselguhr and placed on top of the column. The column was eluted with aqueous ethanol of gradually increasing strengths, (previously saturated with hexane), 25 ml. fractions being collected. The results are summarised in Table 27. Polarimeter readings were taken in a 4 dm. tube.

Table 27

Fraction number	Eluting solvent	Polarimeter reading	Fraction number	Eluting solvent	Polarimeter reading
1-4	25% ethanol	0	17	40% ethanol	+0.37
5-8	30% ethanol	0	18	"	+0.53
9-12	35% ethanol	0	19	"	+0.55
13	40% ethanol	0	20	"	+0.16
14	"	0	21	"	0
15	"	+ 0.16	22-26	"	0
16	"	+0.26	27-40	50% ethanol	0

Fractions 15-20 were evaporated individually but the melting point of the residue from each fraction did not differ significantly from that of the starting material.

α-ANGELICALACTONE

Oxidation with potassium permanganate

α-Angelicalactone (6.6g.) in acetone (50 ml.) was immersed in an ice-salt mixture and finely powdered potassium permanganate (24.1g.) was added gradually, during 90 minutes, with constant agitation. The brown precipitate was filtered off and the filtrate evaporated to yield a mobile, pale yellow liquid (2.1g.), having the same odour as the starting material. Distillation of the product gave a colourless liquid (0.95g.), b.p. 98° C./2m.m. Hg., n_D^{14} 1.4490, d_{14}^{14} 1.104. The sample of angelicalactone used had the constants, d_{17}^{17} 1.100, n_D^{17} 1.4570, and since both starting material and product solidified on strong cooling, the liquid product is almost certainly unchanged α-angelicalactone.

The brown precipitate was acidified (dilute hydrochloric acid) and boiled with water (4x50 ml.), filtered and the filtrate extracted with ether. The ethereal layer was dried (sodium sulphate) and evaporated to give a thick, brown oil (0.72g.), having a strong acetic odour. Paper chromatography (butanol, saturated with ammonia-ethanol, 20:1), confirmed the presence of acetic acid, R_F 0.22, as its ammonium salt, but no other acid fragment was detected. Addition of 2:4-dinitrophenylhydrazine solution precipitated a flocculent derivative which could not be recrystallised and was discarded.

Reaction with solution of 2:4-dinitrophenylhydrazine

α-Angelicalactone (0.1g.) in ethanol, was mixed with acid solution of 2:4-dinitrophenylhydrazine in ethanol (50%) and an orange precipitate

appeared almost immediately. Recrystallisation give orange needle crystals (from aqueous ethanol), m.p. 103°C. (capillary tube).

Found	C, 48.07%; H, 5.13.
Calculated for $C_{13}H_{16}O_6N_4$	C, 48.14%; H, 4.97%.

The literature melting point of ethyl levulinate 2:4-dinitrophenylhydrazone is 101-102°C.

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