A Thesis

entitled

"Studies in the Diterpenoid Series"

submitted to the

University of Glasgow

for the Degree of Doctor of Philosophy in the

Faculty of Science

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Chemistry Department.

May, 1958.

Preface

The writer wishes to express his deepest gratitude to Professor D.H.R. Barton, F.R.S., whose inspiring guidance and constant encouragement combined to make this a most valuable experience. Thanks are also due to Dr. K.H. Overton for much helpful discussion and invaluable advice.

This work was carried out from 1954 to 1957 during the tenure of a D. S. I. R. Maintenance Allowance.

CONTENTS

Introduction

Recent studies in diterpenoid chemistry have revealed several compounds which are of interest, not only on account of the nature of their functional groups, but perhaps even more so because they are non-isoprenoid. The biological isoprene rule of Ruzicka[1] which explains the carbon skeletons and stereochemistry of the entire triterpenoid family is daily being placed on a firmer experimental basis particularly by the rapid current developments in biological chamistry. The structure and stereochemistry of the irregular diterpenoids mentioned above therefore assume a new importance as a means of establishing the comprehensiveness of the Ruzicka hypothesis.

Before discussing the chemistry of the Colombo Root bitter principles a brief review* on the relevant aspects of diterpenoid chemistry would seem appropriate. Thus the structure and stereochemistry of the diterpenoids known to date are briefly described using the minimum of experimental evidence. This is followed by a short survey of the theoretical and practical developments in the current theory of terpenoid, particularly diterpenoid, biogenesis.

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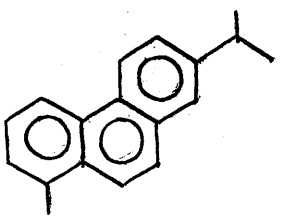
* The material on the earlier work of the diterpenoids is based extensively on a review by D. H. R. Barton. ('The Diterpenoids' Chemistry of Carbon Compounds, Vol. 11 B, Chapter 15, Rodd, Elsevier Publishing Co.)



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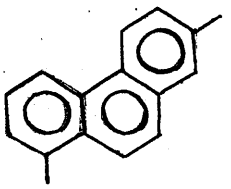

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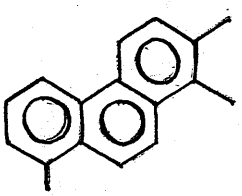

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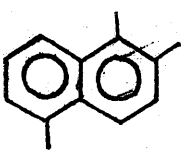


(IV).

1. Review of the Chemistry of the Diterpenoids

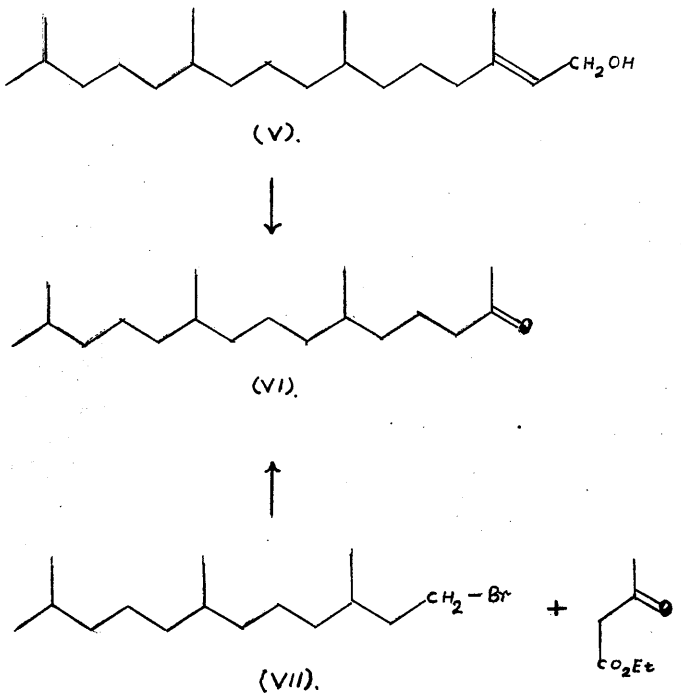
Introduction

The first diterpenoids to be isolated were impure specimens of abietic acid and the resin acids. Although these compounds have been the subject of investigation for the past hundred years it is only within the last thirty years that significant progress has been made.

The importance of the method of dehydrogenation in elucidating the skeleton of a condensed cyclohexane ring system has long been appreciated. It is indeed, therefore, interesting to note that the first use of this method was made in the diterpenoid field by Vesterberg[2] who obtained retene, 1-methyl-7-isopropylphenanthrene (1), from abietic acid by heating with sulphur.

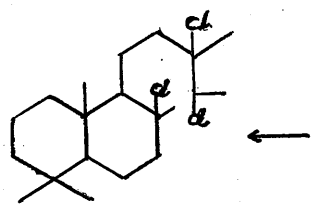
Since that date this method has been continually used as a powerful tool in the elucidation of the structures of the diterpenoids. In addition to (1) the other most commonly isolated hydrocarbons are pimanthrene, 1:7- dimethylphenanthrene (11), 1:7:8-trimethylphenanthrene (111) and 1:2:5- trimethylnaphthalene (1V). The diterpenoids have been classified[3,4] on a system based on dehydrogenation results. This system distinguishes three main classes, which on dehydrogenation afford hydrocarbons (1),(11) and (111) respectively. In this survey, however, an attempt is made to classify the material on biogenetic considerations.

(A) Acyclic Diterpenoid.

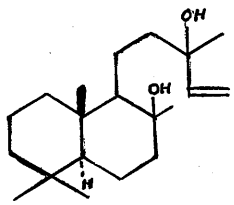


Phytol, $C_{20}H_{40}O$, (V) the alcoholic moiety of the chlorophyll molecule[5], is a mono-unsaturated aliphatic diterpenoid alcohol.

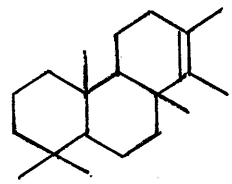
Ozonolysis[6] of phytol, which was known to be a primary alcohol, gave a saturated ketone (Vl) and glycollic aldehyde thus proving that the olefinic linkage was in the $\alpha:\beta$-position to the hydroxyl function. The ketone was shown to be 2:6:10- trimethyl-pentadecan-14-one by its synthesis from hexahydrofarnesyl bromide (Vll) as shown above.



(XII).



(VIII).



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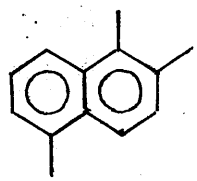
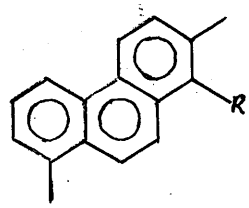


(IX).



(XI).

(B) Bicyclic Diterpenoids

(a) Alcohols and related compounds

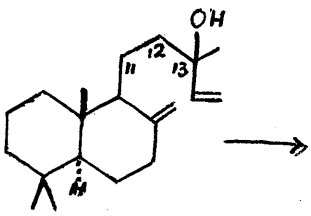
Sclareol[4,7], a constituent of the leaves of Salvia Sclarea L., is an unsaturated, ditertiary glycol, $C_{20}H_{36}O_2$, of constitution (VIII).

Catalytic hydrogenation of sclareol (VIII) affords the saturated dihydrosclareol $C_{20}H_{38}O_2$ indicating that it must be bicyclic. This is confirmed by dehydrogenation which yields 1:5:6- trimethylnaphthalene (IX). When dihydrosclareol was dehydrated using potassium hydrogen sulphate it furnished, amongst other products, dihydrocyclosclarene (X). The latter on dehydrogenation afforded 1:7:8- trimethylphenanthrene (XI;R=Me) and pimanthrene (XI;R=H).

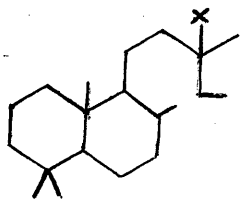
The presence of a methylene grouping in sclareol was demonstrated by the high yield of formaldehyde on ozonolysis, and by the formation of a C(19) dihydroxy-acid on permanganate oxidation.

These experimental facts, particularly the dehydrogenation results, indicate a carbon skeleton similar to agathenedicarboxylic acid (p. 15). Since the two hydroxyl groups are tertiary they must be placed as indicated in (VIII) which is in agreement with the formation of (X) on dehydration.

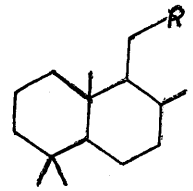
Manoöl (p. 7) and sclareol form the same trichloro- compound (XII). It follows therefore that the correlation of manoöl and abietic acid (p. 7) confirms the constitution and absolute configuration of sclareol as shown (VIII).



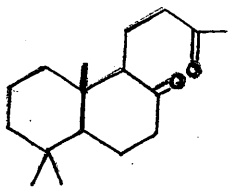
(XIII).



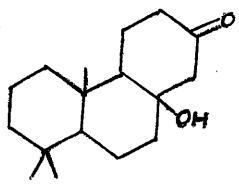
(XIV).



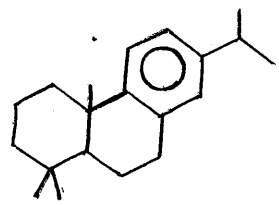
(XV).



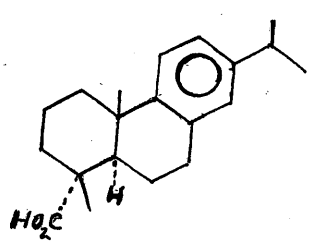
(XVI).



(XVII).



(XVIII).



(XIX).

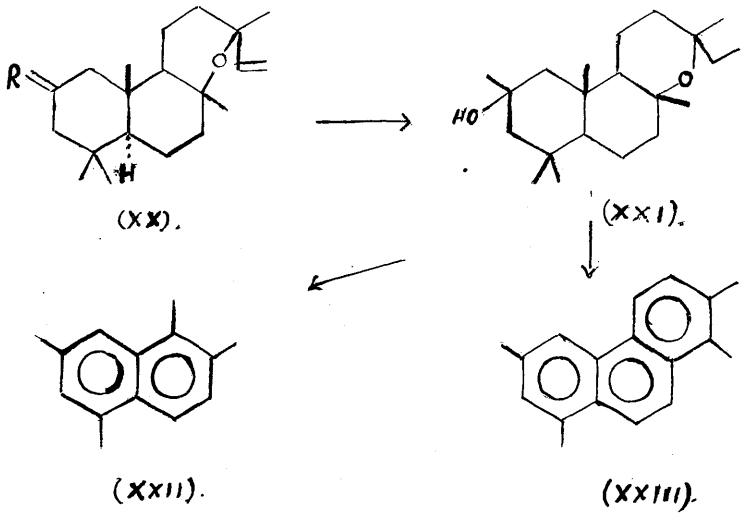
Manoöl,[8] isolated from the wood oil of the yellow pine, Dacrydium biforme, is a doubly unsaturated diterpenoid alcohol, $C_{20}H_{34}O$, (XIII).

On treatment with hydrogen chloride manoöl gave the same trichloride (XII) as sclareol (p. 5) thus demonstrating that they must contain the same carbon skeleton.

The position of the hydroxyl group in manoöl was ascertained in the following manner. Hydrogenation of manoöl furnished the saturated tetrahydromanool (XIV;X=OH) which was readily converted into the corresponding chloride (XIV;X=Cl) on treatment with hydrogen chloride. On dehydrochlorination (XIV;X=Cl) afforded a mixture of two hydrocarbons, ozonolysis of which gave a C(18) ketone (XV;R=CH_2COCH_3) and a C(16) acid (XV;R=CO_2H). These products conclusively fix the position of the hydroxyl group at position 13.

The position of the double bonds in manoöl was shown by ozonolysis which afforded a C(17) diketone (XVI). Since this compound contains no hydroxyl group it follows that one of the ethylenic linkages must be present in the allylic position to the hydroxyl group. The diketone (XVI) is readily cyclised in alkali to give the hydroxyketone (XVII) which shows that the keto-groups in the former must be in the 1:5- relationship, thus establishing the structure of manoöl as shown in (XIII).

The constitution and absolute configuration of manoöl has been confirmed by relating it to abietic acid. This was done by converting (XVII) to the hydrocarbon (XVIII) using isopropyl magnesium bromide, followed by dehydration and selective dehydrogenation. The hydrocarbon (XVIII) was identical with dehydroabietane obtained from dehydroabietic acid (XIX) by standard methods (see methyl vouacapenate, p. 42).



(XX). (XXI).

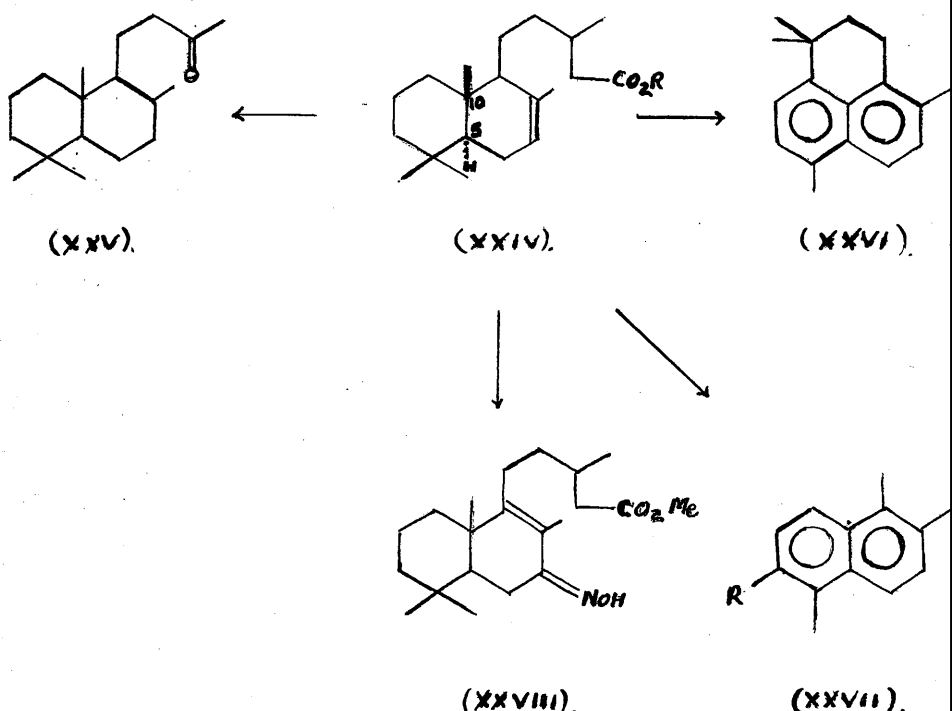
(XXII). (XXIII).

Manoyloxide[8] and ketomanoyloxide.[8] These compounds occur together in the wood oil of the silver pine (Dacrydium colensoi).

Manoyl oxide, $C_{20}H_{34}O$, (XX:R=:H_2) contains one olefinic double bond and an inert oxygen function which is present in an oxide ring. The close relationship between manoyl oxide, manoöl and sclareol follows from the observation that they all form the same trichloro-compound (XII, p. 4). Permanganate oxidation of manoyl oxide gave a saturated C(19) acid, thus proving the presence of an exocyclic methylene group and hence the structure of manoyl oxide as shown (XX;R=:H_2).

Ketomanoyl oxide, $C_{20}H_{32}O_2$ (XX;R=:O) when reduced by the Wolff-Kishner method affords manoyl oxide. The position of the keto-group was established in the following way. Treatment of (XX;R=:O) with methyl magnesium iodide followed by hydrogenation furnished (XXI). The oxide ring of (XXI) was split using hydrogen chloride to give the corresponding dichloro-compound. Dehydrochlorination followed by dehydrogenation of the latter gave a mixture of 1:3:5:6- tetramethylnaphthalene (XXII) and probably 1:3:7:8- tetramethylphenanthrene (XXIII). This clearly proves the position of the original keto-group is as shown in (XX;R=:O).

(b) **Acids**



(XXV). (XXIV). (XXVI).

(XXVIII). (XXVII).

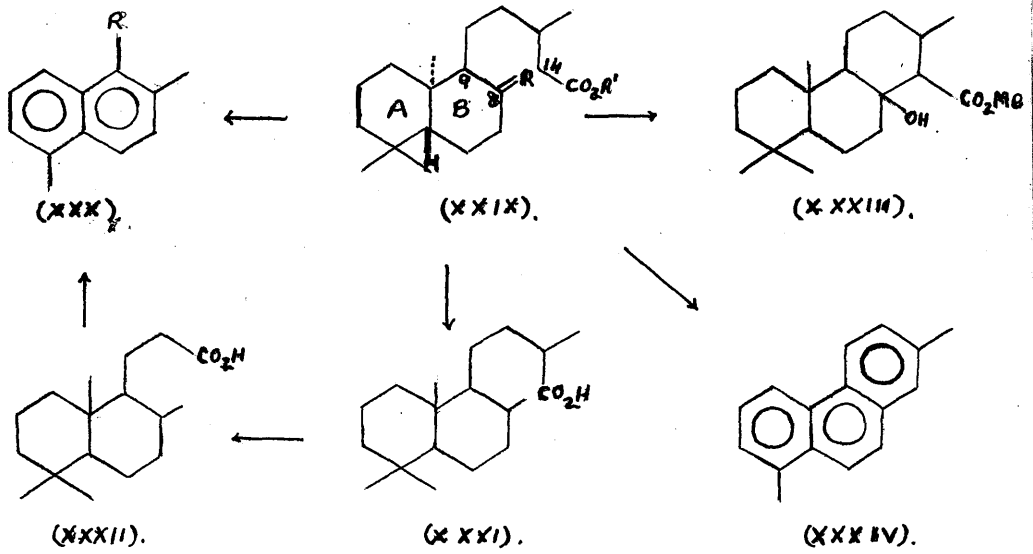
Cativic acid[9]. Cativio gum, the oleoresinous exudate of the cativa tree Prioria copaifera, Griseb., consists primarily of the mono-unsaturated bicyclic acidic diterpenoid, cativic acid, $C_{20}H_{34}O_2$ (XXIV;R=H) and the corresponding ester cativyl acetate.

The reactivity of the carboxyl group suggested that it was present in a side chain. This was confirmed and the nature of the complete carbon skeleton of cativic acid (XXIV;R=H) established by a two stage Barbier-Wieland degradation on methyl dihydrocativate. The product obtained was the methyl ketone

$C_{18}H_{32}O$ (XXV) identical with the C(18) ketone obtained from manoöl.[10]

The dehydrogenation of cativic acid and its methyl ester has produced some interesting results. Thus dehydrogenation of cativic acid (XXIV;R=H) using selenium afforded 1:1:4:7-tetramethylphenalan (XXVI) which has been recently synthesised,[11] whereas the product obtained using palladium-charcoal was 1:2:5:6-tetramethylnaphthalene (XXVII;R=Me). When methyl cativate (XXIV;R=Me) was dehydrogenated using selenium 1:2:5- trimethylnaphthalene (XXVII;R=H) was formed. These products illustrate the difference in reactivity of side chains containing carboxyl and carboxymethyl groups, and also the well-known[12] tendency of methyl migrations in palladium-charcoal dehydrogenation of compounds containing a gem- dimethyl group.

Cativic acid is very sensitive to acid and this can be attributed to the facile isomerisation of the ethylenic linkage. The position of this double bond was established in the following manner. Methyl cativate (XXIV;R=Me) obtained by methylation with diazomethane, was treated with nitrosyl chloride at low temperature to give an unstable adduct. This adduct spontaneously eliminated and isomerised yielding an α :β-unsaturated oxime (XXVIII) with a characteristic maximum in the ultraviolet. The formation of this oxime is consistent only with the presence of a trisubstituted ethylenic linkage. Since ozonolysis of cativic acid takes place without loss of carbon it follows that (XXIV;R=H) represents the constitution of cativic acid. Formulation (XXIV;R=H) also depicts the absolute configuration at positions 5 and 10 in cativic acid which follows from its correlation with manoöl.



Eperuic acid,[13] a bicyclic unsaturated acid, $C_{20}H_{34}O_2$ (XXIX;R=:CH_2 ; R'=H), is the major constituent of an oleoresin from the Wallaba tree (Eperua Spp.) of British Guiana. An interesting feature in the chemistry of eperuic acid and its transformation products is that they are almost all liquids.

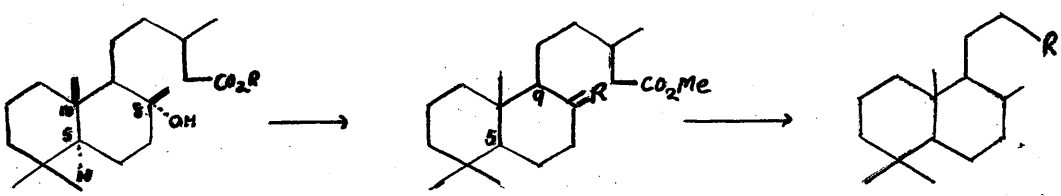
Eperuic acid contains one double bond, it is therefore bicyclic. In agreement dehydrogenation affords, as the principal product, 1:2:5- trimethylnaphthalene (XXX;R=Me). The nature of the side chain was established in the following manner. Barbier-Wieland degradation of methyl dihydroeperuate gave the nor-acid $C_{19}H_{34}O_2$ (XXXI). The product obtained when the methyl ester of (XXXI) was subjected to Barbier-Wieland degradation was a methyl ketone, $C_{18}H_{32}O$, hypoiodite oxidation of which afforded the acid $C_{17}H_{30}O_2$ (XXXII). The latter on dehydrogenation afforded 1-ethyl- 2:5- dimethylnaphthalene (XXX;R=Et), thus accounting for every carbon atom in the side chain.

Ozonolysis of methyl eperuate gave a keto-ester (XXlX;R=:O, R'=Me), together with formaldehyde and formic acid, which shows that the ethylenic linkage terminates as a methylene group. Under basic conditions this keto-ester cyclises with the formation of a tricyclic hydroxy-ester (XXXlll). It is interesting to note that dehydrogenation of the keto-ester (XXlX;R=:O, R'=Me) by analogy with its ready cyclisation in base, gave 1:7-dimethylphenanthrene (XXXlV) rather than a naphthalene derivative which, a priori, might have been expected.

On the basis of the experiments described above the exocyclic methylene group must be placed at position 8.

Owing to the remarkable similarity between the physical constants of various derivatives of eperuic and labdanolic acid (p. 14) it was tentatively suggested[14] that these two acids are antipodes, in so far as rings A and B are concerned. This led to careful study[15] of the rotary dispersion curves of keto-ester (XXlX;R=:O, R'=Me) and the corresponding derivative of labdanolic acid (XXXVl;R=:O). These curves, however, although virtually mirror images, were not completely so as is required by antipodes thus indicating some slight difference in stereochemistry, probably at position 9. In agreement with this it was shown that the corresponding Δ^{8}- unsaturated isomers of (XXlX;R=:CH_2, R'=Me) and (XXXVl;R=:CH_2) had identical infrared spectra and rotation; (but opposite in sign.)

The constitution and absolute configuration of eperuic acid is therefore expressed by (XXlX;R=:CH_2; R'=H). Recent investigations seem to indicate that cafestol (see p. 52) also belongs to this series of diterpenoids with the "wrong" absolute configuration.



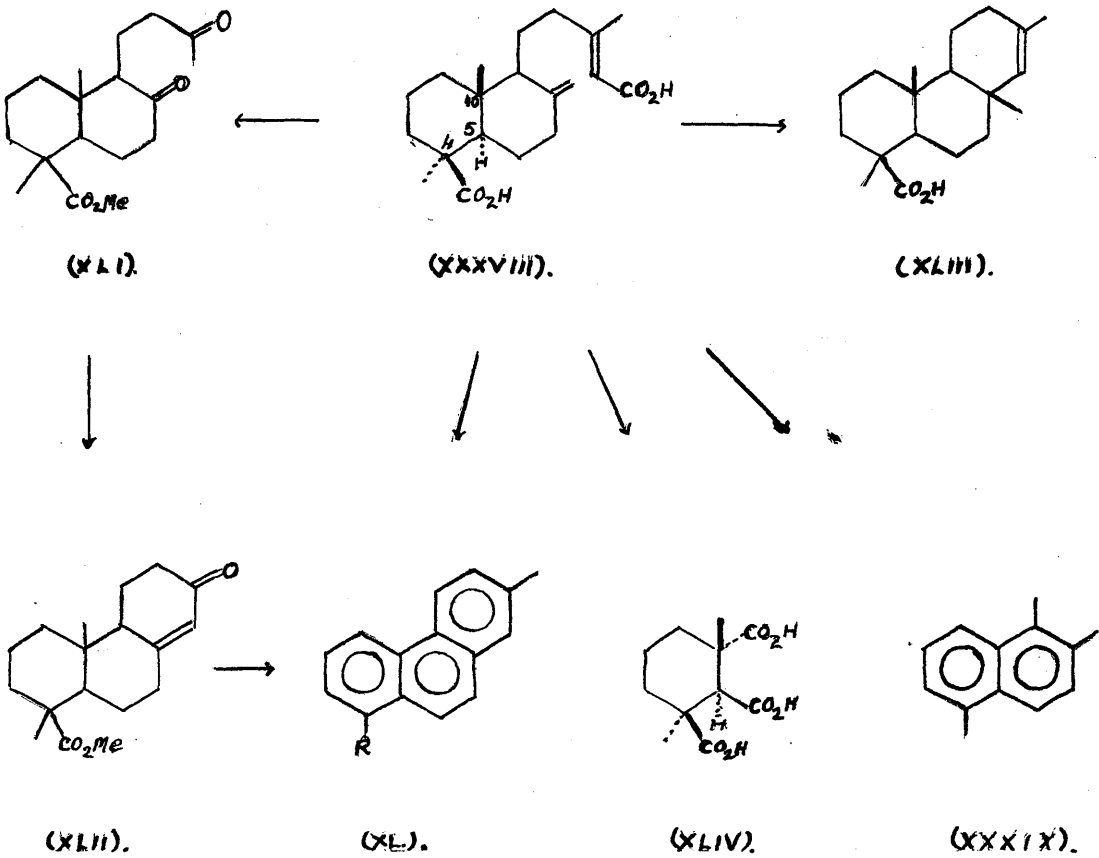
(XXXV). (XXXVI). (XXXVII).

Labdanolic Acid.[14] This bicyclic hydroxy acidic diterpenoid, isolated from gum labdanum, has been shown to have the constitution and absolute configuration formulated in (XXXV;R=H).

Methyl labdanolate (XXXV;R=Me) contains a tertiary hydroxyl group, which dehydrates to give a homogeneous product (XXXVl;R=:CH_2). The infrared spectrum of the latter suggested the presence of a vinylidene group which was confirmed by ozonolysis; the products being formaldehyde and the nor-keto ester (XXXVl;R=:O).

The environment of the carboxyl group and the nature of the entire carbon skeleton in labdanolic acid was confirmed in the following manner. Barbier-Wieland degradation of the dihydroderivative of the unsaturated ester(XXXVl;R=:CH_2) afforded the C(19) acid (XXXVll;R=CHMe.CO_2H), the methyl ester of which when subjected to the same procedure gave a C(18) methyl ketone (XXXVll;R=$COCH_3$). Hypoiodite oxidation of the latter furnished the acid $C_{17}H_{30}O_2$ (XXXVll;R=CO_2H) which was identical with the product obtained from ambrein.[16] This inter-relationship also proves that the absolute configuration in labdanolic acid at positions 5 and 10 is as shown in (XXXV;R=H).

The spectral and chemical properties of the keto-ester, obtained from methyllabdanolate by dehydration followed by ozonolysis are in complete harmony with its formulation as (XXXVl;R=:O). The hydroxyl group is therefore assigned to position 8, which also explains the ready isomerisation of the dehydration product of methyl labdanolate, depicted as (XXXVl;R=CH_2) to give an isomer containing a tetrasubstituted double bond. Finally the hydroxyl group in labdanolic acid (XXXV;R=H) must have the α configuration since dehydration to yield a pure vinylidene derivative is only possible if the hydroxyl is in the equatorial conformation.



Agathenedicarboxylic Acid[17] This doubly unsaturated bicyclic dicarboxylic acid, $C_{20}H_{30}O_4$, has been shown to have the constitution (XXXVIII).

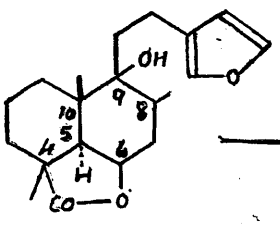
On dehydrogenation agathenedicarboxylic acid affords 1:5:6-trimethylnaphthalene (XXXIX) and pimanthrene (XL;R=Me). Since chemical evidence demonstrates that this resin acid is bicyclic the formation of the latter results presumably from the presence of an unsaturated side chain which appears partially in the former

hydrocarbon as a methyl group.

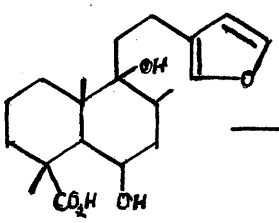
The acid contains two ethylenic linkages one of which is present in the $\alpha:\beta$- position with respect to one of the carboxyls. The ready pyrolytic elimination of one mole of carbon dioxide and the absorption spectrum demonstrated the presence of this conjugated double bond. The relative position of the two ethylenic linkages in (XXXVlll) was established by ozonolysis of the dimethyl ester. The major product was a C(18) 1:5- diketo-ester (XLl) which readily cyclised under basic conditions, affording a tricyclic $\alpha:\beta$-unsaturated ketone (XLll). The position of the carbonyl function in the latter and hence the $:CH.CO_2H$ grouping in the parent acid, was proved by reaction with methyl magnesium iodide followed by dehydrogenation which furnished pimanthrene (XL;R=Me).

The more hindered carboxyl group was shown to be attached to the 4-position as follows. Treatment of agathenedicarboxylic acid with formic acid afforded a tricyclic acid, isoagathenedicarboxylic acid, $C_{20}H_{30}O_4$. Like its progenitor the latter contained an $\alpha:\beta$-unsaturated carboxyl group which readily decarboxylated to give (XLlll). The methyl ester of (XLlll) was reduced by a modified Bouveault-Blanc reaction dehydrated and finally dehydrogenated to give 7-methyl-1-ethylphenanthrene (XL;R=Et).

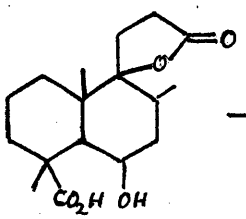
Drastic oxidation of agathenedicarboxylic acid furnished an optically active C(11) tricarboxylic acid (XLlV). Since agathenedicarboxylic acid has the normal diterpenoid configuration at positions 5 and 10 it follows that the configuration of the carboxyl group at position 4 must be different from abietic acid and as shown (XXXVlll).



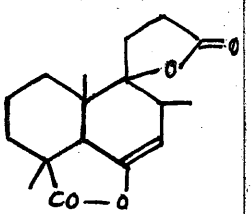
(XLV).



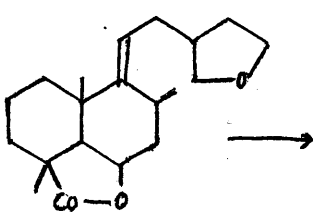
(XLVI)



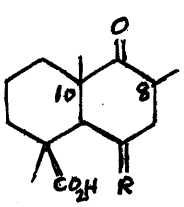
(XLVII)



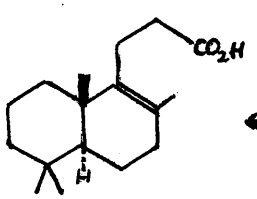
(XLVIII)



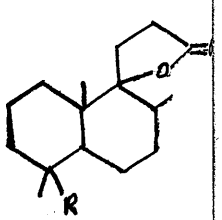
(LI).



(LII).



(L).



(XLIX).

(c) Lactones

Marrubiin,[18,19] a bitter principle of horehound (Marrubiin Vulgare L), is a tricyclic hydroxy diterpenoid lactone, $C_{20}H_{28}O_4$. In the usual way it has been shown that marrubiin (XLV) contains two double bonds and that the hydroxyl group is tertiary.

Vigorous hydrolysis of marrubiin affords the dihydroxy-acid, $C_{20}H_{30}O_5$ (XLVl), marrubic acid, in which the carboxyl group is very hindered. The principal product obtained on alkaline permanganate oxidation of marrubic acid (XLVl) is the hydroxy-lactone $C_{17}H_{26}O_5$ (XLVll). The formation of the latter, which involves the loss of three carbon atoms, three double bond equivalents and an inert oxygen function strongly suggests the oxidation of a furan ring system attached to a side chain in the α- or β-position. The simultaneous production of formic acid and the spectroscopic properties of marrubiin are in agreement with this proposal. Since, by the appropriate esterifications it is readily demonstrated that the carboxyl and hydroxyl functions present in the lactone (XLVll) are those present in marrubic acid, it follows that the carboxyl group formed by the oxidation of the furan ring must lactonise with the original tertiary hydroxyl group in marrubiin. This establishes the position of the hydroxyl group in marrubiin with respect to the furan, since infrared absorption indicates that the lactone ring must be five-membered.

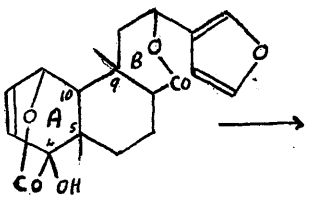
The complete carbon skeleton of marubiin and the absolute configuration at positions 5 and 10 was established by the conversion[19] of the lactone (XLVll) into a known degradation product of ambrein. Thus oxidation of (XLVll) afforded the corresponding ketone which was converted into the enol lactone (XLVlll). The major product obtained on hydrogenation of the latter was the acid

(XLlX;R=CO_2H), which was reduced, using the Rosenmund procedure, to the aldehyde (XLlX;R=CHO). Wolff Kishner reduction of the aldehyde gave a small amount of the corresponding lactone (XLlX; R=CH_3) together with the unsaturated acid (L) which was identical with the product obtained from ambreinolide[20]

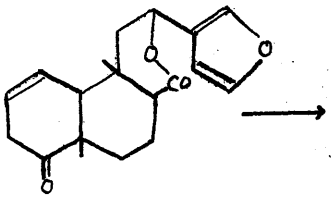
To complete the constitution of marrubiin it is only necessary to interrelate the tertiary hydroxyl group and the lactone; this has been done in the following way[18]. Tetrahydro-marrubiin obtained by hydrogenation of the furan ring in marrubiin (XLV) was dehydrated to afford the anhydro-derivative (Ll) which was smoothly ozonised to give the corresponding keto-lactone. The hydroxy- keto acid (Lll;R= H.OH) obtained by hydrolysis of the keto-lactone was stable to heat thus proving that the carboxyl group could not be attached to positions 8 or 10. Mild oxidation of the hydroxy acid (Lll;R=.H.OH) afforded the diketo-acid (Lll;R=:O) which was oxidised with selenium dioxide to give a yellow unsaturated 1:4 diketone. Treatment of the latter with zinc in acetic acid furnished the original diketone (Lll;R=:O). The secondary hydroxyl group of the lactone must therefore be placed at position 6.

Infrared absorption indicated that the lactone system is five-membered from which it follows that the carbonyl carbon atom in marrubiin must be placed at position 4 as indicated in (XLV) as position 8, the other alternative, has already been ruled out. This is in agreement with the known properties of the carboxyl group in marrubic acid.

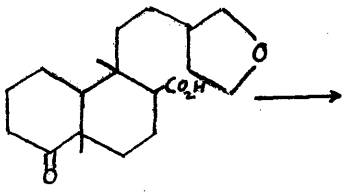
Marrubiin is therefore formulated as (XLV) in which the only uncertainties are the position of substitution of the furan ring and the configuration at positions 4,6,8 and 9. In regard to the latter Cocker[21] on the basis of molecular rotation differences and conformational agruments has attempted to deduce the complete stereochemistry of the molecule. His argument, however, involves several doubtful assumptions; these have been discussed by Rigby[18].



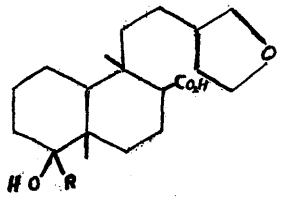
(LIII).



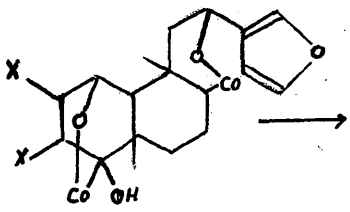
(LIV).



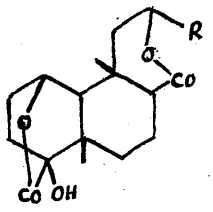
(LVII).



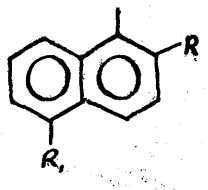
(LIX).



(LV).



(LVI).



(LVIII).

Columbin,[22] the major bitter principle of the Columbo root, (Jatrorrhiza palmata Miers.) is an unsaturated hydroxy diterpenoid lactone, $C_{20}H_{22}O_6$ (LIII).

By standard methods columbin has been shown to contain two lactones. It also possesses one tertiary hydroxyl group which is somewhat acidic. The remaining oxygen function is com - pletely inert. Hydrogenation of columbin affords an octahydro derivative. This product is a monocarboxylic acid, which must result from hydrogenolysis, thus indicating the presence of three ethylenic linkages.

A characteristic property of columbin, which disappears on hydrogenation, is the easy loss of one mole of carbon dioxide on melting with the formation of decarboxycolumbin, $C_{19}H_{22}O_4$ (LIV). The latter contains a keto group and no hydroxyl. These facts suggest the presence in columbin of a β: γ- unsaturated - α- hydroxy lactone, designated lactone (A), as shown in (LIII).

This has been confirmed and the unsaturation characterised as the group -CH=CH- in the following manner. Selective hydrogenation afforded dihydrocolumbin (LV;X=H) which did not decarboxylate. In addition treatment of columbin (LIII) with osmium tetroxide furnished columbindiol (LV;X=OH). In agreement with its formulation this compound was stable to heat, consumed two moles of lead tetra-acetate and showed furan absorption in the ultraviolet. Acetylation of the triol (LV;X=OH) under conditions which don't acetylate columbin afforded a diacetate (LV;X=OAc) thus confirming the nature of the original double bond. Spectroscopic evidence was in accord with the presence of a $\beta : \gamma$ - unsaturated ketone in decarboxycolumbin as shown (LIV).

The chemical and spectroscopic properties of columbin are in agreement with the presence of a furan ring system. This was established by ozonolysis of dihydrocolumbin (LV;X=H) which furnished a saturated C(18) keto-acid (LVl;R=$COCO_2H$) and the C(17) acid (LVl;R=CO_2H). The isolation of the former shows that the furan is β-substituted.

Hydrogenation of decarboxycolumbin (LlV) affords the octahydro-acid (LVll) thus demonstrating that the lactone which suffers hydrogenolysis is not lactone (A). The ready preparation of dihydrocolumbin (LV;X=H) shows that the hydrogenation of the lactone (A) system preceeds hydrogenolysis. It follows, therefore, that the furan ring must be the unsaturated system which is responsible for the hydrogenolysis of lactone (B). This lactone is therefore placed with its alkyl oxygen α with respect to the furan ring as shown in (Llll).

The basic skeleton of columbin and the incorporation in this of the functional groups described above follows from the following dehydrogenations on octahydrodecarboxycolumbinic acid (LVll). Wolff-Kishner reduction of the latter followed by dehydrogenation afforded 1-methyl-2-naphthoic acid (LVlll;R=CO_2H, R_1=H).

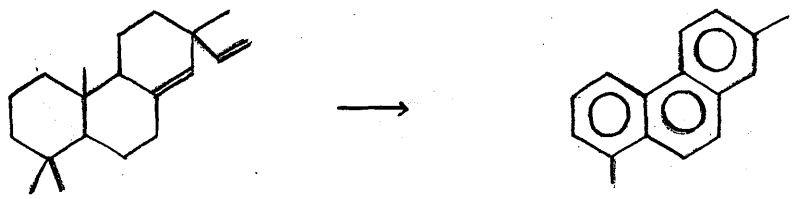
Lithium aluminium hydride reduction of (LlX;R=Me), prepared from (LVll) using one mole of methyl magnesium iodide, followed by dehydrogenation gave 1:2:5- trimethylnaphthalene (LVlll;R=Me, R_1=Me). Finally dehydrogenation of decahydrocolumbinic acid (LlX;R=H), obtained by acid catalysed hydrogenation of (LVll), furnished 1:5-dimethyl-2-naphthoic acid (LVlll;R=CO_2H, R_1=Me). From the first two experiments it is obvious that the 5 methyl group in the last experiment must have arisen from a rearrangement during dehydrog-

enation. Such migrations occurred during the dehydrogenation of other columbin derivatives. In every case, as previously found,[23] rearrangements were minimised if the carbonyl or hydroxyl functions were removed. The second dehydrogenation experiment clearly demonstrates that the carbonyl carbon atom of lactone (A) is attached to position 4 in the columbin skeleton. A further implication of these experiments is that there must be a quaternary methyl group in the adjacent position 5. The formation of the two naphthoic acids (LVlll;R=CO_2H, R_1=H) and (LVlll;R=CO_2H, R_1=Me) is important since it proves the position of lactone (B) relative to the bicyclic nucleus.

Columbin contains two C-methyl groups and therefore on the basis of the above experiments it is formulated as (Llll). The methyl group is placed at position 9 since this explains the formation of several of the previously obtained products, e.g. o-cresol, better than does the alternative constitution with the methyl group at position 10.

(C) Tricyclic Diterpenoids

(a) Rimuene and Phenols

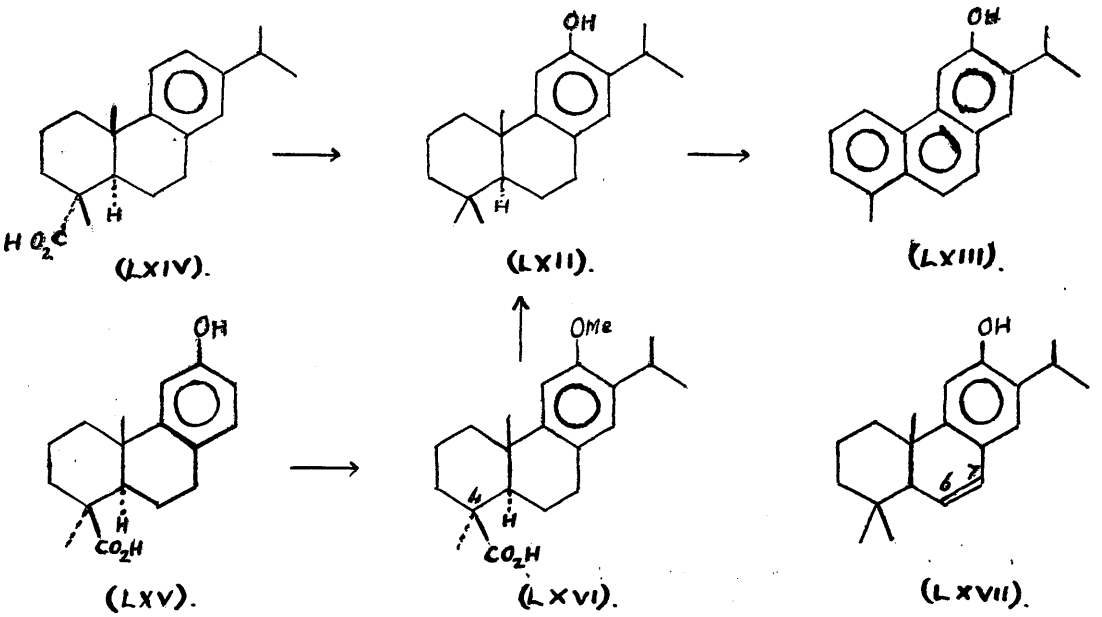


(LXI) (LX)

Rimuene or Totarene,[3,4] found in the essential oil of the Remu tree (Dacrydium cupressinum) and the essential oil of the Totara tree (Podocarus totara), is a tricyclic diterpene, $C_{20}H_{32}$. It contains two ethylenic linkages, one being present as a methylene group. On dehydrogenation rimuene affords pimanthrene (LX) whilst on treatment with formic acid it is isomerised to isophyllocladene (see p. 47.)

On the basis of this evidence Wenkert has attributed the structure (LXI) to rimuene[24].

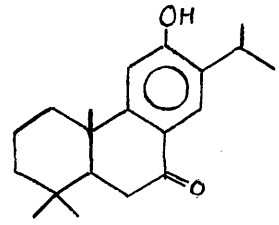
Ferruginol. This tricyclic phenolic diterpenoid, $C_{20}H_{30}O$, which comprises the major part of the resin of the Miro tree has the constitution (LXII). This structure may be deduced from the observation that dehydrogenation of ferruginol affords 6-hydroxyretene (LXIII).



The original confirmation of the constitution of ferruginol was by means of partial synthesis[25] (a) from dehydroabietic acid (LXIV) and (b) from podocarpic acid (LXV). An important observation in this work was that the compound (LXVI) from podocarpic acid was not identical with the corresponding intermediate prepared from dehydroabietic acid. It follows therefore that the carboxyl group in abietic acid is epimeric with that in podocarpic acid.

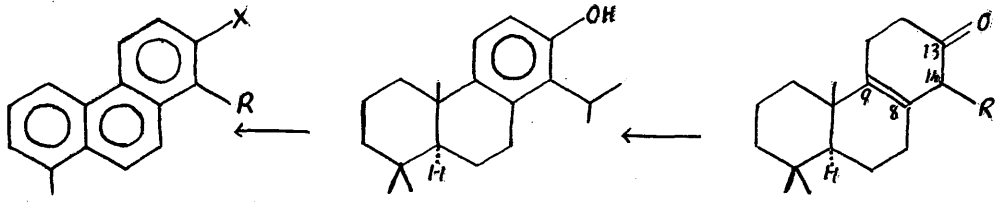
It is worthy of note that (±) ferruginol was the first naturally occuring tricyclic diterpenoid to be totally synthesised.[26]

Recently it has been shown[27] that certain samples of ferruginol are contaminated by relatively large proportions of its Δ^{6}-derivative, Δ^{6}-dehydroferruginol (LXVII).



(LXVIII).

Sugiol[3,4] (LXVIII) found in Cryotomeria japonica D. Don and also the wood of the Rimu tree (Dacrydium cupressinum) is a phenolic diterpenoid ketone, $C_{20}H_{28}O_2$. Its structure follows from the fact that sugiol methyl ether and sugiol acetate have been prepared[28] by chromic acid oxidation of the appropriate ferruginol derivatives.

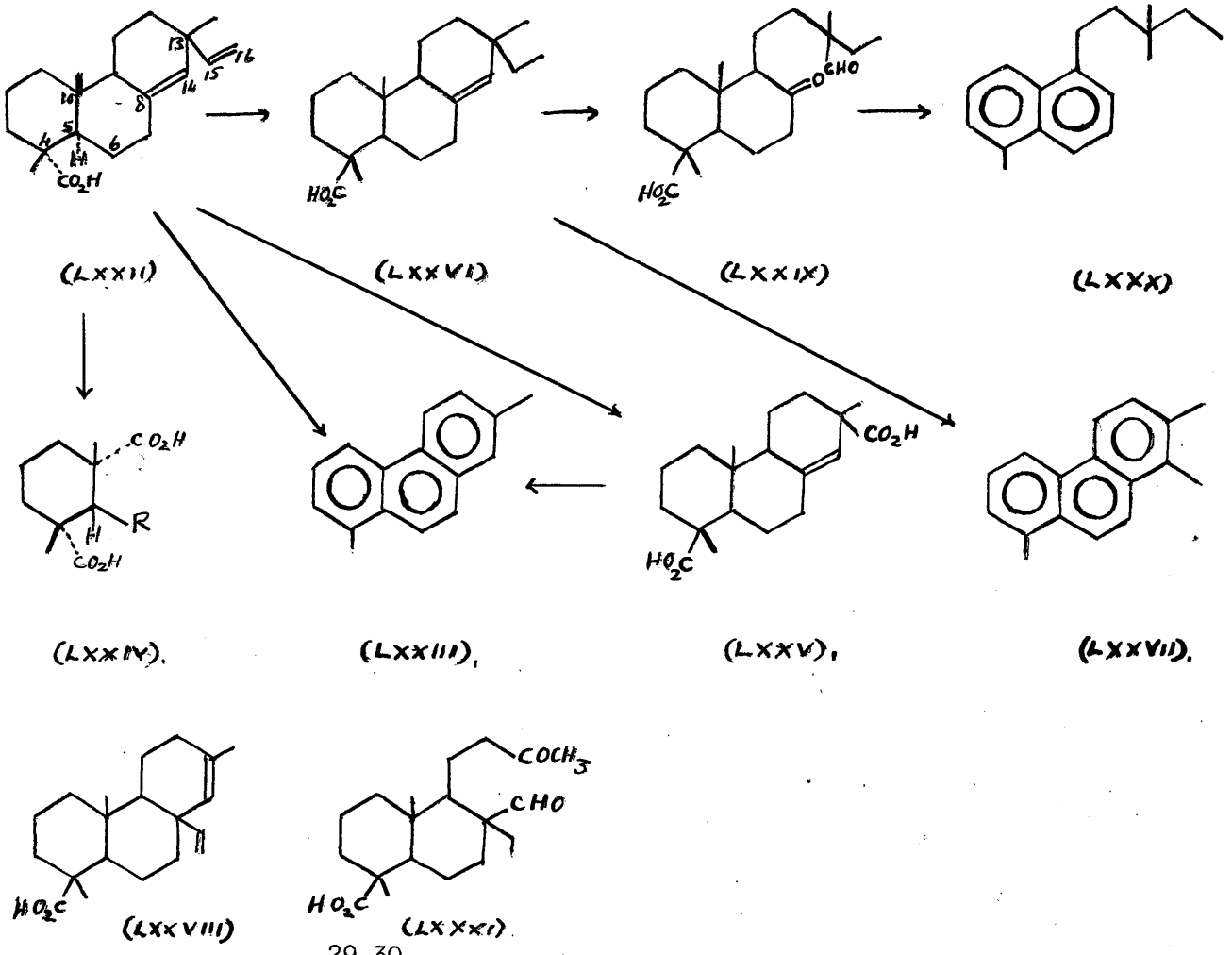


(LXX). (LXIX). (LXXI).

Totarol[3,4] The wood of the totara tree (Podocarpus totarus) contains a tricyclic diterpenoid phenol, $C_{20}H_{30}O$ (LXIX). Catalytic hydrogenation of totarol afforded a saturated hydrocarbon, totarane, $C_{20}H_{36}$, which gave, on dehydrogenation, a hydrocarbon shown to be 1-methyl-8-isopropylphenanthrene (LXX;R=Pr^i; X=H). Dehydrogenation of totarol itself furnished 7-hydroxy-1-methyl phenanthrene (LXX;R=H, X=OH). These facts indicate that (LXIX) is a possible structure for totarol. This has been confirmed recently by synthesis.[28a]

Both $\Delta^{8(9)}$ podocarpen-13-one (LXXI;R=H) and $\Delta^{8(14)}$ podocarpen-13-one when separately alkylated afford the same mixture of $\Delta^{8(9)}$-totaren-13-one (LXXI;R=Pr^i) and $\Delta^{8(14)}$-totaren-13-one. Bromination and dehydrobromination of this mixture afforded (±) totarol (LXIX).

(b) Dextropimaric acid group



Dextropimaric acid,[29,30] $C_{20}H_{30}O_2$ (LXXII) is probably present to a greater or less extent in all conifer resins, although it is not always possible to separate it from the accompanying isomeric resin acids. Unlike most of the other primary resin acids, however, dextropimaric acid is comparatively stable to heat and is not isomerised by treatment with mineral acid.

On dehydrogenation dextropimaric acid affords pimanthrene

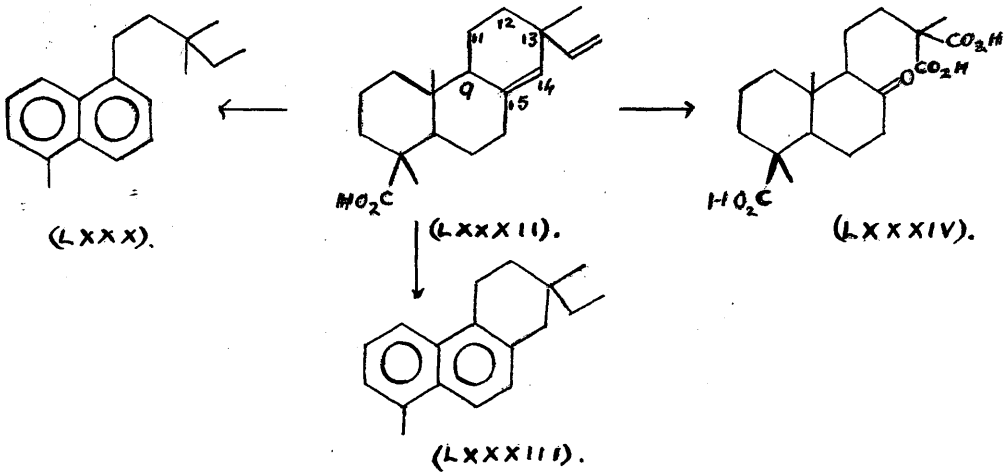
(LXXIII). Whilst on vigorous oxidation it yields the same two tricarboxylic acids (LXXIV;R=CO_2H) and (LXXIV;R=CH_2CO_2H) as are obtained in the same way from abietic acid. This proves the points of attachment of the quaternary methyl groups and the hindered carboxyl group. It also demonstrates the configuration at positions 4, 5 and 10 (Ref No.[59]).

By standard methods it can be shown that dextropimaric acid is doubly unsaturated and therefore tricarbocyclic. The two ethylenic linkages are not conjugated and differ greatly in reactivity, the more reactive being present as a tertiary vinyl grouping. Thus careful oxidation of dextropimaric acid affords a glycol which can be readily further oxidised to the nor - dicarboxylic acid (LXXV). Dehydrogenation of the latter affords pimanthrene (LXXIII).

Partial hydrogenation affords the well defined dihydro - dextropimaric acid (LXXVI) which has been used[29] in demonstration that the less reactive double bond is in the 8(14) or 13(14) position. Thus the product obtained by treatment of the oxide of dihydrodextropimaric acid with methyl magnesium iodide, afforded 1:7:8- trimethylphenanthrene (LXXVII) on dehydrogenation.

On the basis of the experiments described dextropimaric acid must be either (LXXII) or (LXXVIII). Evidence in favour of the former[30] is briefly as follows. The dihydro acid (LXXVI) was ozonised to give a keto aldehyde (negative iodoform test) (LXXIX)which was reduced by the Wolff-Kishner method and then dehydrogenated to give a C(18) disubstituted naphthalene (LXXX). The keto aldehyde (LXXXI) from (LXXVIII) would have given a positive iodoform test and would have been converted into a C(16) trisubstituted naphthalene.

iso Dextropimaric acid (A)[30] This unsaturated diterpenoid acid has been isolated along with its isomer dextropimaric acid. The original workers regard it as a C(13) epimeride of dextropimaric acid. Wenkert,[24] however, in support of his theory

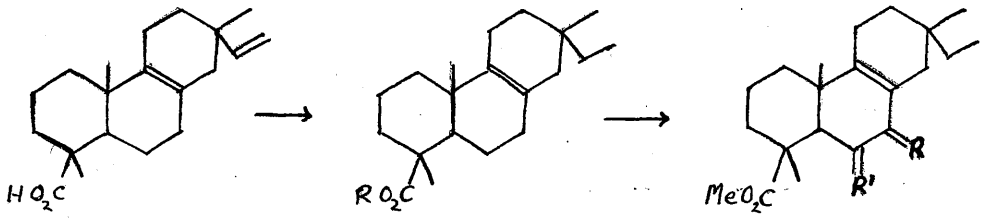


regarding the stereochemistry at C(13) of natural pimaradienes (see p.67) has suggested that the available evidence does not eliminate the possibility that the compounds are epimeric at C(9). The known facts may be summarised as follows.

When this isomer was subjected to the same reaction sequence as was applied to dextropimaric acid the same C(18) disubstituted naphthalene (LXXX) was obtained. Similarly partial dehydrogenation of both acids afforded the same tetrahydrophenanthrene (LXXXIII). Finally when dextropimaric acid and its isomer were ozonised and the products oxidized with hydrogen peroxide the same tricarboxylic acid (LXXXIV) was isolated in both cases.

To support their proposals the original workers suggested that racemisation occurred in the formation of the tetrahydrophenanthrene (LXXXIII) but not in the production of the tricarboxylic acid (LXXXIV). Wenkert's postulate may be arrived at by reversing these assumptions.

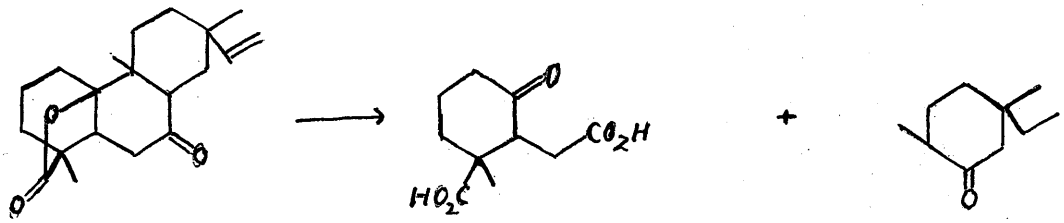
iso Dextropimaric acid (B)[31] The fruits of the Juniperus japonica contain two doubly unsaturated isomeric acids, $C_{20}H_{30}O_2$. These compounds have been shown to be isodextropimaric acid (A) (LXXXll) and the isomer (B) (LXXXV). On hydrogenation both compounds afford the same tetrahydro acid. Ozonolysis, infrared and hydrogenation studies show that (LXXXV) like (LXXXll) contains a vinylidene group which may be selectively saturated to give in each case a different dihydro acid e.g. (LXXXVl;R=H) in this case. This demonstrates that these compounds differ in the position of the less reactive double bond.



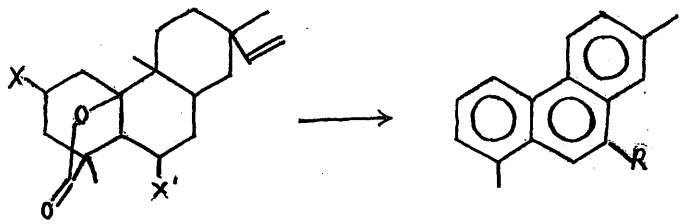
(LXXXV). (LXXXVl). (LXXXVll).

The hindered double bond has been shown[31] to be part of the system $-C=C-CH_2-CH_2-$ in the following manner. The dihydro ester (LXXXVl;R=Me) was oxidised with selenium dioxide followed by sodium dichromate to give the $\alpha:\beta$-unsaturated ketone (LXXXVll;R=:O, R'=:H_2). The latter condensed with ethyl formate under basic conditions yielding the hydroxymethylene compound (LXXXVll;R=O; R'=CHOH) with the correct spectroscopic properties.

On the basis of these experiments isodextropimaric acid (B) can only be described as in (LXXXV).



(LXXXVIII) (XCI) (XCII).



(LXXXIX) (XC).

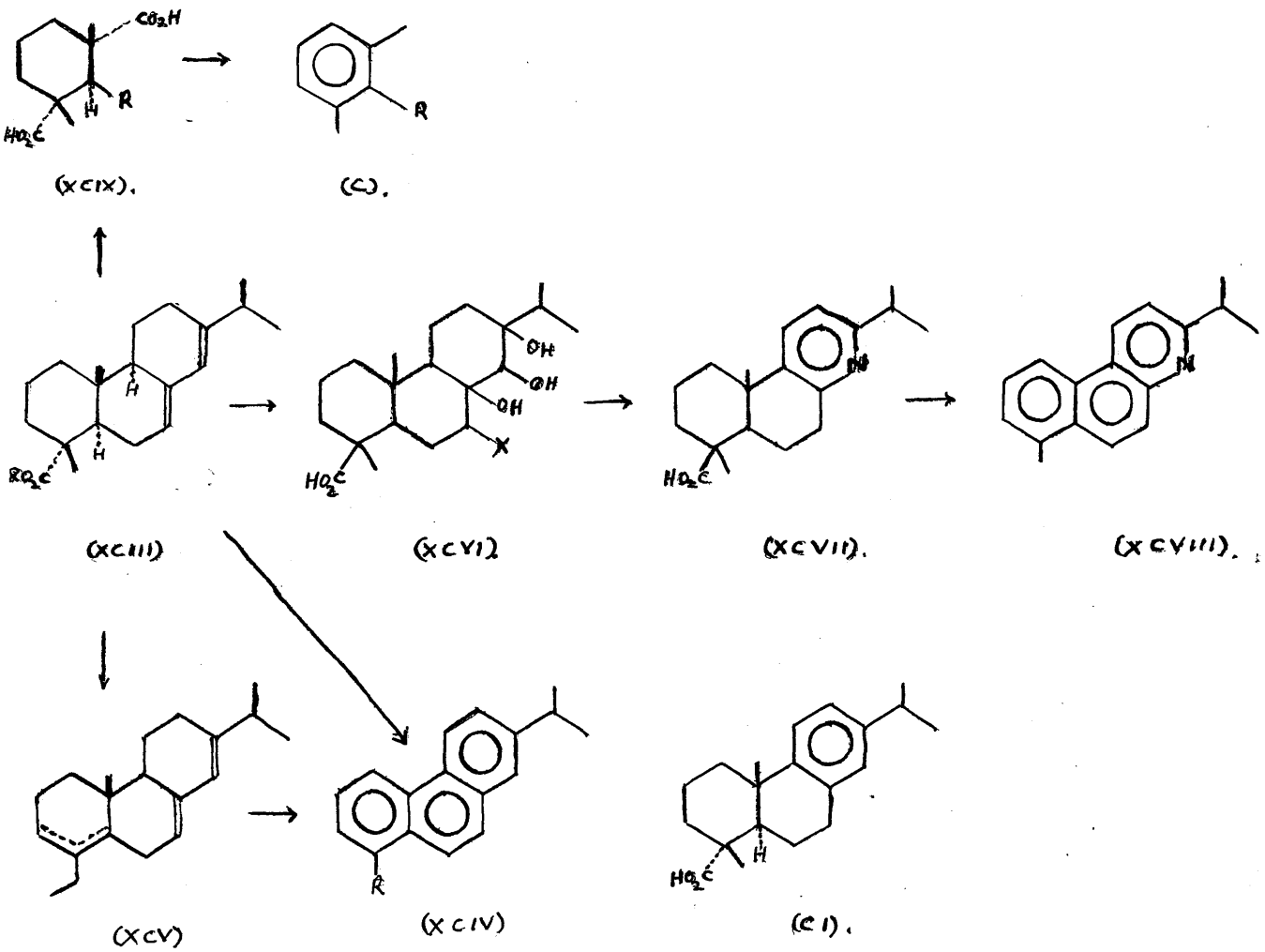
Rosenonolactone and rosonolactone[32] These interesting fungal diterpenoids are derived from the mycelium of Trichothecium Roseum Link. The former currently formulated as (LXXXVlll) is an un - saturated tricyclic keto-lactone $C_{20}H_{28}O_3$ while the latter, possibly (LXXXlX;X=OH, X'=H) or (LXXXlX;X=H, X'=OH) is an unsaturated tricyclic hydroxy-lactone $C_{20}H_{30}O_3$.

Rosenonolactone is reversibly isomerised to iso-rosenonolactone. On dehydrogenation both of these compounds furnished a mixture of 1:7-dimethyl- and 1:7- dimethyl-9-hydroxy-phenanthrene (XC;R=H and R=OH). Using standard methods rosenonolactone has been shown to contain one vinyl residue and one tertiary carboxyl group. Oxidation of dihydro-iso-rosenonolactone followed by treatment with alkali affords a cyclic keto dibasic acid, $C_{10}H_{14}O_5$ (XCl) together with a cyclic ketone $C_{10}H_{18}O$ (XCll).

On the basis of these and other facts formula (LXXXVlll) has been suggested as a possible description of rosenonolactone.

Oxidation of rosonolactone affords the corresponding ketone, dehydrorosonolactone, $C_{20}H_{28}O_3$ which is not identical with the isomeric rosenonolactone (LXXXVlll). On dehydrogenation both rosonolactone and dehydrodihydrorosonolactone afford 1:7- dimethylphenanthrene (XC;R=H). Rosonolactone contains a vinyl group. Acid treatment of dehydrorosonolactone affords an $\alpha;\beta$ - unsaturated keto acid, iso-dehydrorosonolactone. The currently suggested structures for this metabolite are (LXXXlX;X=OH, X'=H) or (LXXXlX;X=OH, X'=H).

(c) Abietic acid group



(XCIX). (C). (XCIII) (XCVI) (XCVII). (XCVIII).

(XCV) (XCIV) (CI).

Abietic acid[3,4] (XCIII;R=H) the best known of the resin acids, is prepared from colophony (rosin) by treatment with acidic reagents. It is a so-called secondary resin acid, being formed from a precursor levopimaric acid by isomerisation.

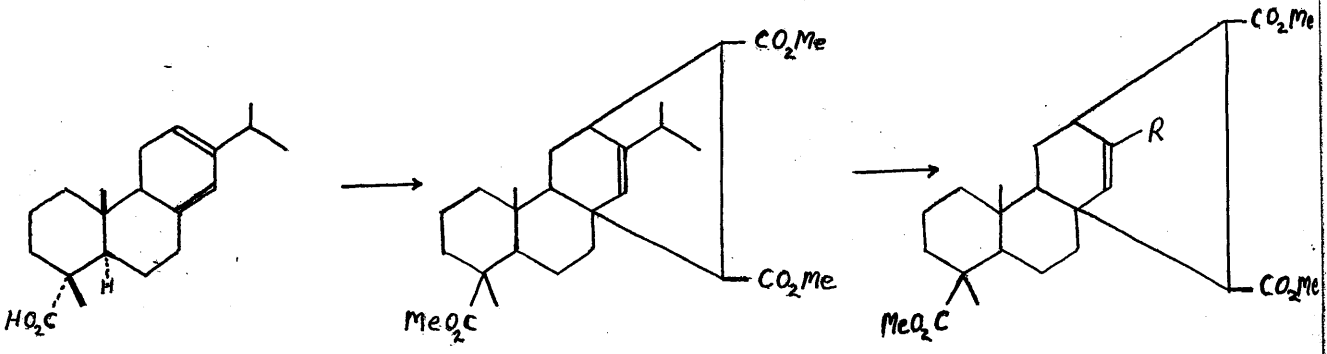
The usual spectroscopic and chemical procedures indicate that abietic acid is an unsaturated tricyclic acid, $C_{20}H_{30}O_2$ which contains two conjugated ethylenic linkages situated in different rings.

Dehydrogenation of abietic acid affords a high yield of retene (XClV;R=Me). The position of attachment of the hindered carboxyl group which is eliminated in dehydrogenation has been clearly shown in the following manner. Bouveault Blanc reduction of methyl abietate (XClll;R=Me) afforded the corresponding alcohol abietinol which was dehydrated with rearrangement to methyl abietin[33] (XCV). The structure of the latter was confirmed by dehydrogenation to yield homo-retene (XClV;R=Et) which was later synthesized by Haworth[34]

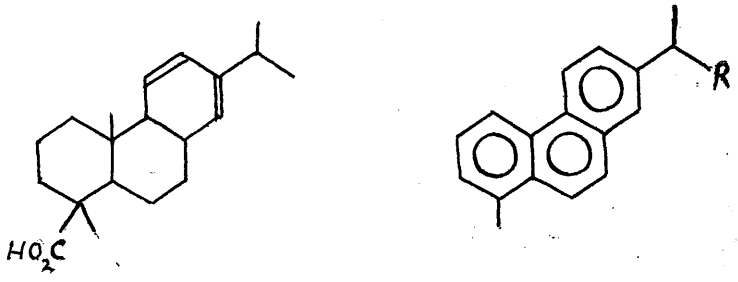
The position of the conjugated ethylenic linkages has been demonstrated[35] as follows. Mild oxidation of abietic acid affords, amongst other products, an oxidodihydroxyabietic acid, which is unstable in aqueous media rapidly hydrating to the so-called " δ "-tetrahydroxyabietic acid (XCVl;X=OH). In dilute aqueous solutions of hydrogen halides, the latter is converted into (XCVl;X=halogen) which readily reacts with two moles of lead tetraacetate to give the corresponding halogenodiketo-acids. Hydriodic acid treatment of the iododiketoacid removed the iodo group and the resulting diketo acid was cyclised with ammonia to give 8-azadehydroabietic acid (XCVll). Dehydrogenation of the latter afforded 8-azaretene (XCVlll), which was synthesised.

The position of the quaternary methyl group, the only remaining undecided feature in abietic acid follows from energetic oxidation of the latter which affords two homologous tricarboxylic acids (XClX;R=CO_2H) and (XClX;R=CH_2CO_2H). Dehydrogenation of these afforded respectively m-xylene (C;R=H) and hemimellitine (C;R=CH_3). This clearly demonstrates the 1:3 relationship of the two methyl groups in (XClX;R=CO_2H) and (XClX;R=CH_2CO_2H) from which it follows that (XClll; R=H) completely describes the constitution and absolute configuration (see p.28) of abietic acid.

Dehydroabietic acid (Cl) readily prepared from abietic acid by pyrolysis, amongst other methods[3], has recently been synthesised[36] in the ($\pm$) form.



(C II). (C IV). (C V).



(C VI) (C III).

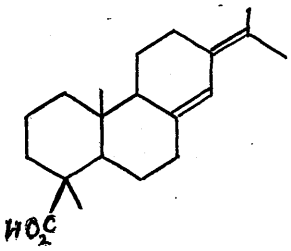
Levopimaric acid[3,4] $C_{20}H_{30}O_2$ (C11), a primary constituent of all resins from pine and fir trees, is readily isomerised by heat or acids to abietic acid. That it contains two double bonds and therefore three rings is clearly shown by hydrogenation and per-acid titration. The ultraviolet absorption and quantitative reaction of levopimaric acid with maleic anhydride at room temperature, show that it contains a homoannulardiene system. Maleic anhydride forms the same adduct with abietic acid but under much more vigorous conditions. As would therefore be expected levopimaric acid, like abietic acid, affords retene (C111;R=Me) on dehydrogenation.

The following reaction sequence[37] clearly demonstrates that the isoproyl grouping is attached directly to one of the ethylenic linkages. The trimethyl ester (C1V) of the maleic anhydride adduct, on ozonolysis afforded, amongst other products, an unsaturated keto ester (CV;R=$COCH_3$). The ultraviolet absorption of (CV;R=$COCH_3$) showed that the double bond was conjugated to the ketone. In addition the absorption spectrum of the tetramethyl ester (CV;R=CO_2Me) obtained by hypoiodite oxidation of (CV;R=$COCH_3$) confirmed the position of the double bond in the latter.

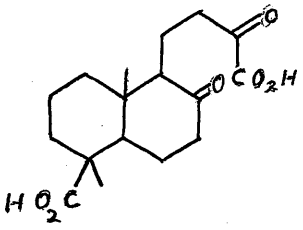
When the unsaturated keto-ester (CV;R=$COCH_3$) was treated with excess ethyl magnesium iodide and the product dehydrogenated, 1-methyl-7-sec-butylphenanthrene (C111;R=Et) was obtained. This confirms that the acetyl group in (CV;R=$COCH_3$) was derived from the isopropyl group.

On the above evidence levopimaric acid may be represented by formulae (C11) or (CV1). The former is preferable since it more readily explains the facile rearrangement to abietic acid.

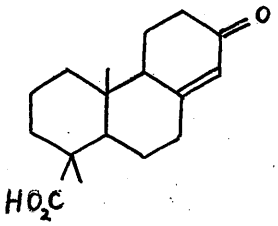
Neo-abietic acid[38] a primary constituent of the oleoresin of Pinus palustris, is conveniently prepared by heating abietic acid at 300° in an inert atmosphere for short periods. The chemical and spectroscopic properties are in agreement with neo-abietic acid $C_{20}H_{30}O_2$ (CVll) being a simple double bond isomer of abietic acid. The ethylenic linkages are in conjugation but are not present in the same ring.



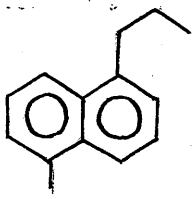
(CVII).



(CIX)



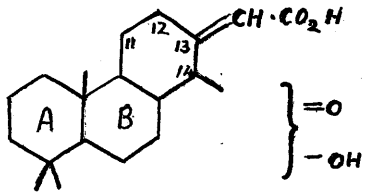
(CVIII).



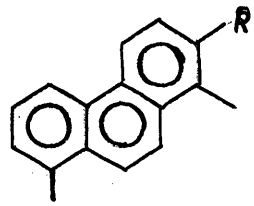
(CX).

On mild ozonolysis neo-abietic acid affords acetone and an $\alpha:\beta$-unsaturated ketone (CVlll) thus showing the presence of an isopropylidene group. Drastic ozonolysis to give (ClX) followed by dehydrogenation gave 1-methyl-5-n-propylnaphthalene (CX) rigidly confirming structure (CVll) for neo-abietic acid.

(d) Miscellaneous tricarbocyclic compounds



(CXI).



(CXII).

Cassaic acid[39] The remarkably physiologically active Erythropheum alkaloids are known to be alkamine esters of diterpenoid carboxylic esters. Thus acidic hydrolysis of cassaine yields a tricyclic hydroxy keto acid, cassaic acid, $C_{20}H_{30}O_4$ (CXl) together with dimeth aminoethanol.

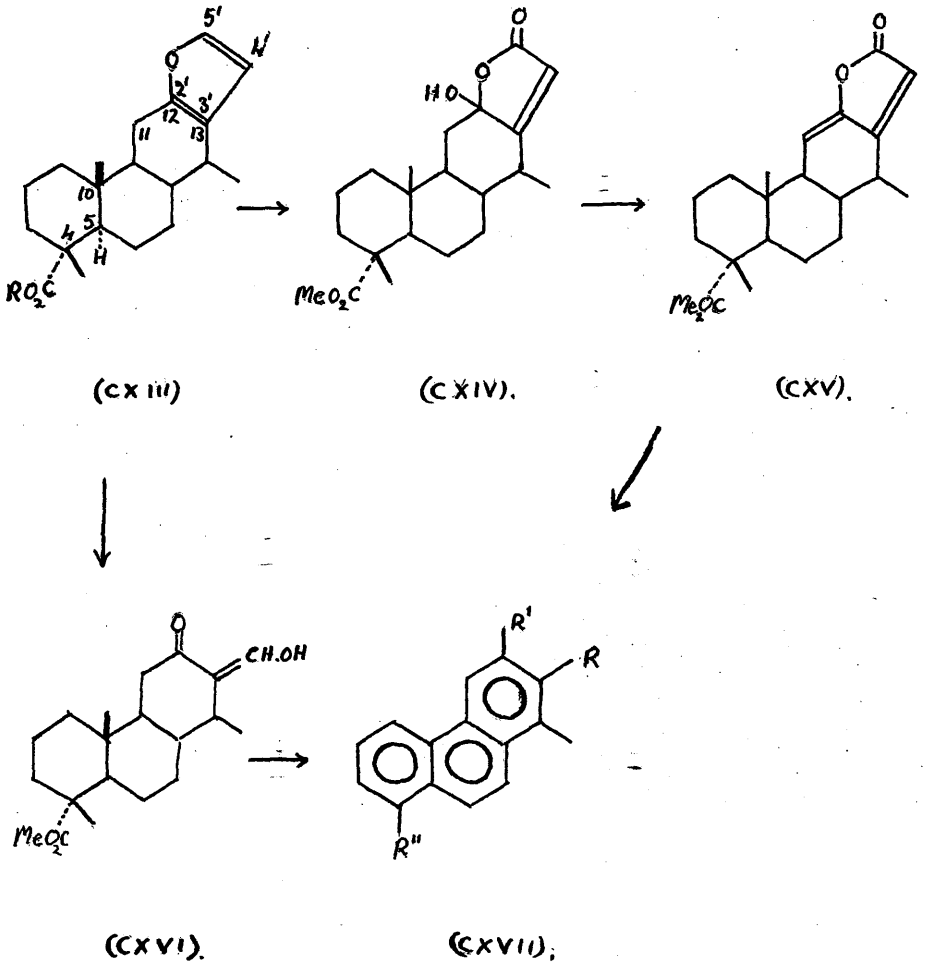
The nature of the fundamental carbon skeleton and the position of attachment of the carboxyl group to this was elucidated by

Ruzicka in the following manner. Cassaic acid was hydrogenated to dihydrocassaic acid which on oxidation afforded the diketo acid $C_{20}H_{30}O_4$. The latter was reduced and then dehydrogenated to give 1:2:8- trimethylphenanthrene (CXll;R=Me). When the reduction product cassanic acid, $C_{20}H_{34}O_2$, was methylated and treated with excess methyl magnesium iodide and then dehydrogenated, the product obtained was 2-isobutyl-1:8-dimethylphenanthrene (CXll;R=^{i}Bu). The structure of the latter was confirmed by synthesis.

The ultraviolet absorption of cassaic acid indicates that the ethylenic linkage is present in the $\alpha:\beta$-position with respect to the carboxyl group. From the formation of (CXll;R=^{i}Bu) on dehydrogenation it would therefore appear that cassaic acid is a derivative of 7-perhydrophenanthrylidene acetic acid. This explains why basic treatment partially isomerises cassaic acid to allocassaic acid, both of which afford the same dihydro-derivative. This is consistent with the known behavior of cyclohexylidene acetic acids in which exocyclic double bonds readily migrate under basic conditions to the more stable endocyclic positions.[39]

On the basis of the above facts and also by biogenetic analogy the working structure (CXl) may be attributed to cassaic acid. The presence of the methyl group in the non-isoprenoid position 14 finds analogy in the diterpenoid esters methyl vinhaticoate and methyl vouacapenate (see p.42).

The absorption spectrum of the diketo acid $C_{20}H_{30}O_4$ indicates that the hydroxyl and carbonyl functions are not present in ring C, and also that they must be present in different rings.



(CXIII) (CXIV). (CXV).

(CXVI). (CXVII).

Vinhaticoic acid[40] The heart wood of Plathymenia reticulate contains the methyl ester of an interesting unsaturated tetracyclic acidic diterpenoid, vinhaticoic acid, $C_{20}H_{28}O_3$.

Methyl vinhaticoate (CXIII;R=Me) forms a tetrahydro derivative, an unstable maleic anhydride adduct and also reacts with perphthalic acid to form a weakly acidic product $C_{21}H_{30}O_5$ (CXIV). On fusion the latter dehydrates to give the unsaturated lactone (CXV). The spectroscopic properties of (CXIV) and (CXV) are analogus with those

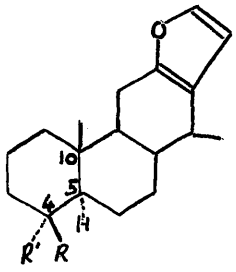
obtained by Woodward[41] when menthofuran was treated with hydrogen peroxide. These reactions suggest that the two ethylenic linkages and the unreactive oxygen function are present in a furan ring system.

The following experiments conclusively demonstrate the presence of a furan ring, unsubstituted in positions 4' and 5', which is fused in positions 2' and 3' to a perhydrophenanthrene skeleton. Partial ozonolysis of methyl vinhaticoate afforded one mole of formic acid and the hydroxymethylene ketone $C_{20}H_{30}O_4$ (CXVl). Dehydrogenation of the latter afforded 1:8-dimethylphenanthrene (CXVll;R=H, R'=H, R"=Me). Treatment of (CXVl) with excess methyl magnesium iodide, however, followed by dehydrogenation yielded 1:3:8-trimethylphenanthrene (CXVll;R=H, R'=Me, R"=Me). This clearly shows that the furan oxygen is attached to position 12. Moreover basic hydrolysis of the partial ozonolysis product $C_{20}H_{30}O_4$ (CXVl) furnished another mole of formic acid and the nor ketone $C_{19}H_{30}O_3$ thus establishing the absence of a substituent in the 4' position of the furan ring.

Conclusively proof of the attachment of the furan ring was provided by dehydrogenation of the lactone (CXV) to give 1:2:8 trimethylphenanthrene (CXVll;R=Me, R'=H, R"=Me). Thus the furan is attached to perhydrophenanthrene skeleton at positions 12 and 13.

Lithium aluminium hydride reduction of methyl vinhaticoate followed by dehydration and dehydrogenation afforded 1-ethyl-8-methylphenanthrene (CXVll;R=H, R'=H, R"=Et). This places the hindered carboxyl group at position 4[33] and enables us to formulate methyl vinhaticoate as (CXlll) although there is no positive evidence for the attachment of the methyl group at C(10). The stereochemistry at positions 4, 5 and 10 is discussed in the next section.

Vouacapenic acid and Vouacapenol[42] Derivatives of these closely related diterpenoids, methyl vouacapenate $C_{21}H_{30}O_3$ (CXVlll;R=CO_2Me, R'=Me) and vouacapenol acetate $C_{22}H_{32}O_3$(CXVlll;R=CH_2OAc, R'=Me) are isolated from the heartwood of Vouacapoua americana. The simple relationship between these compounds follows from the observation that both afford the same product on reduction with lithium aluminium hydride.



(CXVIII).

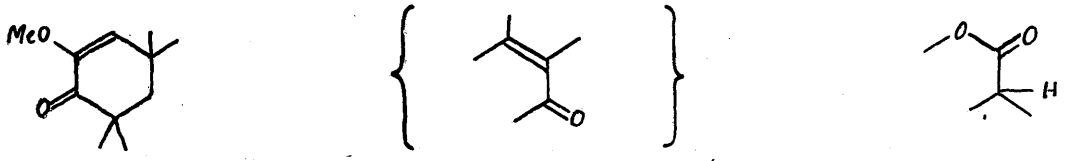
Apart from the relative reactivity of their respective carboxyl groups, the chemical transformations of vouacapenic acid (CXVlll; R=CO_2H, R'=CH_3) and vinhaticoic acid (CXVlll;R=CH_3, R'=CO_2H) are completely analogous. These compounds were inter-related by the conversion of their respective carbomethoxyl groups to methyl groups via the corresponding alcohol and aldehyde. The furanohydrocarbon (CXVlll;R, R'=CH_3) so obtained from methyl vouacapenate was identical with that similarly derived from methyl vinhaticoate.

The rate of hydrolysis of methyl vouacapenate is much smaller than that of methyl vinhaticoate. In addition comparative studies demonstrate that the rate of hydrolysis of methyl vouacapenate is very similar to methyl podocarpate whilst the hydrolysis rate of methyl vinhaticoate is very close to that of methyl abietate. Knowing the stereochemistry of the reference compounds and assuming that methyl vouacapenate and methyl vinhaticoate have trans ring junctions, the probable stereochemistry of these compounds at positions 4, 5 and 10 is respectively as shown in (CXVlll;R=CO_2Me, R'=Me) and (CXVlll;R=Me, R'=CO_2Me).

The closely related compounds quassin and neoquassin[43] which are found among the bitter constituents of Quassia wood (Quassia amara L.) appear to be diterpenoids.

Quassin, $C_{20}H_{22}O_4(OMe)_2$ is an unsaturated lactone which contains two methoxyl groups, possibly two carbonyl functions and two or three C-methyl groups. Hydrogenation and per-acid titrations seem to indicate the presence of one double bond in quassin. Under acidic conditions quassin is demethylated giving nor- and bisnor-quassin, whilst basic treatment affords an unsaturated acid isoquassinic acid. Reduction of the lactone in quassin affords the corresponding hemi-acetal, which is identical with neoquassin, $C_{20}H_{24}O_4(OMe)_2$. The latter is readily reoxidized to quassin thus indicating the close relationship of these compounds.

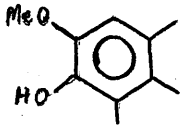
Chemical and spectroscopic evidence has been presented for the presence of the three systems shown in (CXIX) which is therefore a possible partial formula for quassin.



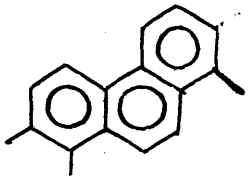
(CXIX).

Dehydrogenation of these compounds, possibly because of the high oxygen content, appears to produce extensive breakdown. Thus the only identifiable product obtained from neoquassin was (CXX) which was probably derived from the diketone enol ether system in (CXIX).

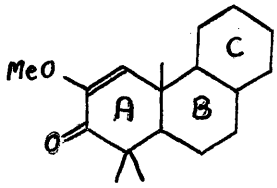
However, Clemmenson reduction followed by dehydrogenation furnished 1:2:8 trimethylphenanthrene (CXXl) thus giving the first indication that these compounds are diterpenoids.



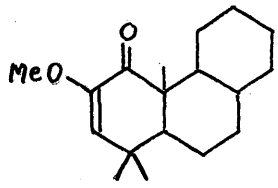
(CXX).



(CXXI).



(CXXII).



(CXXIII).

It is thus possible that quassin contains a perhydrophenanthrene skeleton in which the α- diketone enol ether system is as shown in (CXXll) or (CXXlll). The complete structure for quassin must include :

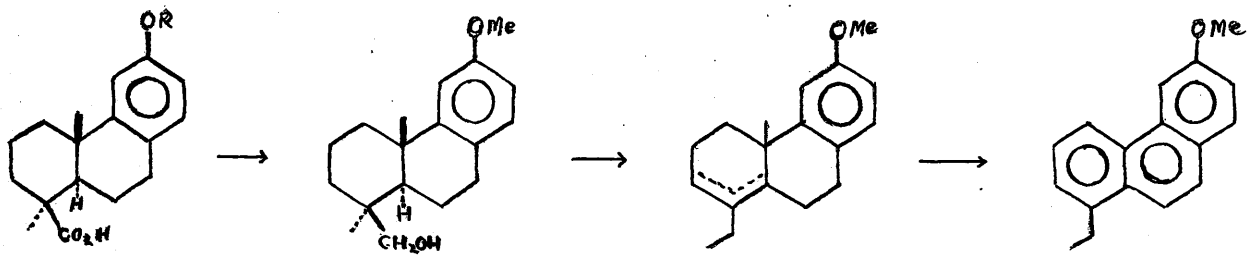
i) three further carbon atoms probably attached to ring C and associated with the lactone system.

ii) a second $\alpha:\beta$-unsaturated ketone system possibly in ring B.

iii) a second methoxyl group.

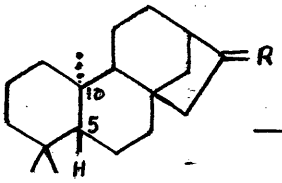
Podocarpic acid[44] Although podocarpic acid is not strictly a diterpenoid its chemistry is closely related to this group and it is convenient, therefore, to give a short account of it here.

Podocarpic acid, $C_{17}H_{22}O_3$ (CXXIV;R=H) isolated from several resins, e.g. Podocarpus cupressinum, is a tricyclic phenolic carboxylic acid. Its constitution has been elucidated in the following manner.

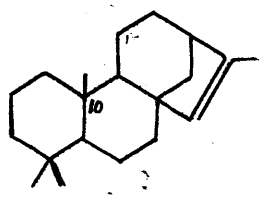


(CXXIV). (CXXV). (CXXVI). (CXXVII).

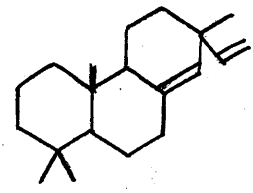
Reduction of podocarpic acid methyl ether (CXXIV;R=Me) in two stages afforded podocarpinol methyl ether (CXXV), which was dehydrated to give (CXXVI). Dehydrogenation of the latter afforded 6-methoxyl-1-ethylphenanthrene (CXXVII). The tertiary carboxyl group present in podocarpic acid has been shown to be epimeric with that in abietic acid (see p. 25). It is therefore assigned the configuration shown in (CXXIV;R=H) which has been confirmed in a recent synthesis[45] of the (±) acid.



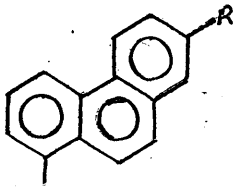
(CXXVIII).



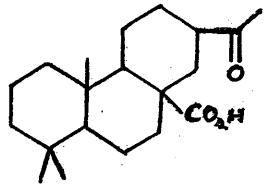
(CXXIX).



(CXXXI).



(CXXX).



(CXXXII).

(D) Tetracyclic Diterpenoids

Phyllocladene[46] (CXXVlll;R=:CH_2), $C_{20}H_{32}$ is a mono-unsaturated tetracyclic diterpene found in leaf oils, e.g. the foliage of the Norfolk Island pine. Treatment of the mother liquors from the phyllocladene extraction with acid affords a crystalline isomer iso-phyllocladene (CXXlX). Since phyllocladene and isophyllocladene give the same hydrochloride and also the same mixture of α- and β-dihydrophyllocladene on hydrogenation it would appear that they differ only in the position of the ethylenic linkage.

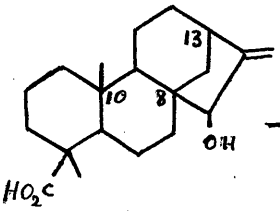
Both hydrocarbons, on dehydrogenation, afford in poor yield a mixture of 7-isopropyl-1-methylphenanthrene (CXXX;R=^{i}Pr) and 1:7-dimethylphenanthrene (CXXX;R=Me). It is worthy of note that rimuene, currently formulated as (CXXXl) (see p. 24) affords, on dehydrogenation, only one product 1:7- dimethylphenanthrene (CXXX;R=Me) and this in much better yield.

Permanganate oxidation of isophyllocladene[46] (CXXlX) furnished amongst other products a tricyclic keto-acid $C_{20}H_{32}O_3$ (CXXXll) which gave a positive iodoform reaction. This keto-acid on dehydrogenation was converted into 1-methyl-7-ethylphenanthrene (CXXX;R=Et). When, however, the methyl ester of the keto acid (CXXXll) was treated with excess methyl magnesium iodide and then dehydrogenated, 1-methyl-7-isopropylphenanthrene (CXXX;R=^{i}Pr) was produced. The isolation of these dehydrogenation products prove that the ethylenic linkage is present in the fourth carbocyclic ring which is attached to a perhydrophenanthrene ring system. Further these reactions clearly demonstrate that the carboxyl group in the keto-acid (CXXXll) must be present in a quaternary position. This observation, together with infrared evidence mentioned later completely defines the nature, size and position of attachment of the fourth ring in the phyllocladenes.

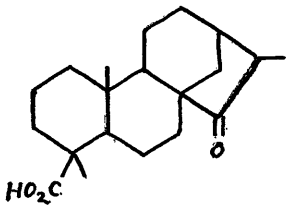
On biogenetic theory constitution (CXXIX) may be tentatively proposed for isophyllocladene. Partial substantiation of this comes from infrared measurements[47] which indicate the presence of a trisubstituted double bond, a gem- dimethyl group and also two additional methyl functions, one of which appears to be situated between two six-membered rings.

Bearing in mind the relationship between phyllocladene and isophyllocladene it is necessary to locate only the position of the double bond in phyllocladene. The infrared spectrum of the latter indicates the presence of a vinylidene group. Chemically this was proved by oxidation to the glycol, which was cleaved by periodic acid to give the nor ketone, $C_{19}H_{30}O$ (CXXVIII;R=:O). Using infrared measurements, this ketone was clearly shown to be present in a five-membered ring, thus conclusively proving the positions of attachment and the nature of the fourth ring in these tetracyclic diterpenes.

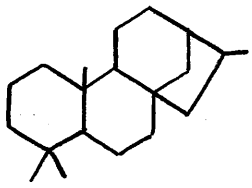
We can thus attribute constitution (CXXVIII;R=:CH_2) to phyllocladene, bearing in mind that there is, as yet, no chemical evidence for the methyl group at position 10 in either of these diterpenes. It should be noted that recent rotary dispersion studies[48] seem to indicate that the A : B ring junction in phyllocladene is different from that of the majority of the diterpenoids. The explanation[49] of this fact may be that like eperuic acid (see p. 11) and cafestol (see p. 52) the ring junction in phyllocladene is antipodal to that of the other polyterpenoids and steroids as shown in (CXXVIII;R=:CH_2).



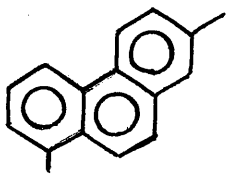
(CXXXIII).



(CXXXIV).



(CXXXVI).



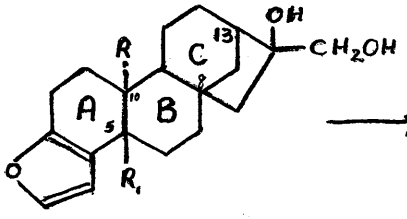
(CXXXV).

Steviol[50] The principal constituent of Stevia Rebaudiana Bertoni is stevioside, enzymatic hydrolysis of which affords a monounsaturated hydroxy- tetracyclic acid, steviol, $C_{20}H_{30}O_3$ (CXXXlll). Chemical and spectroscopic evidence indicate the presence of a vinylidene, an alcoholic and a hindered carboxyl group in steviol.

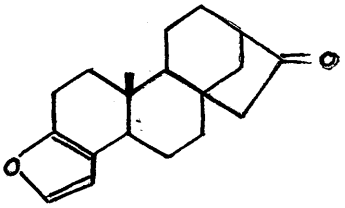
Steviol and stevioside on acidic treatment furnish isosteviol $C_{20}H_{30}O_3$ (CXXXlV) which retains the hindered carboxyl group of its precursor. On the other hand chemical and spectroscopic evidence indicate that isosteviol is a saturated ketone, the carbonyl function being attached to a five-membered ring.

On dehydrogenation isosteviol furnished 1:7- dimethylphenanthrene (CXXXV) suggesting a basic perhydrophenanthrene nucleus with a five-membered ring attached, presumably at positions 8 and 13. On this evidence, and assuming that no skelatal rearrangements occur on isomerisation, Mosettig[50] tentatively proposed structures (CXXXlll) and (CXXXlV) for steviol and isosteviol.

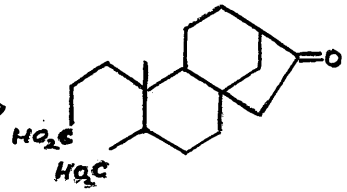
This isomerisation of a vinylidene alcohol to a saturated ketone present in a five-membered ring, finds analogy in the isomerisation of garryfoline to cauchichicine,[51] in both of which the 8:13 attachment of the cyclopentane is more rigorously justified. Complete reduction of isosteviol by standard procedures afforded a small yield of a hydrocarbon isostevane $C_{20}H_{34}$ (CXXXVl) which, however, is claimed with some reserve. Isostevane is reported to be different from α- or β-dihydro-phyllocladene. If this is the case this could either be due to differences in the carbon skeleton, e.g. the position of the quaternary methyl group placed at position 10, or more simply differences in skeletal configuration.



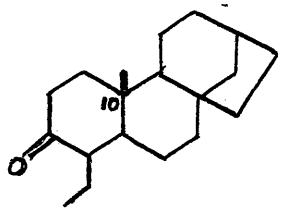
(CXXXVII).



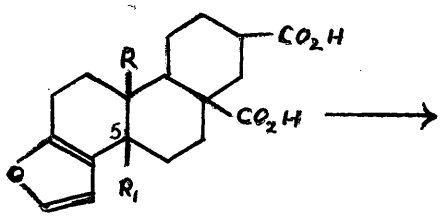
(CXXXVIII).



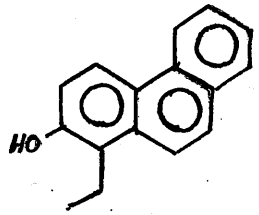
(CXXXIX).



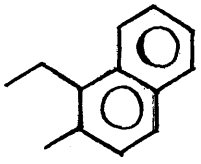
(CXLIV).



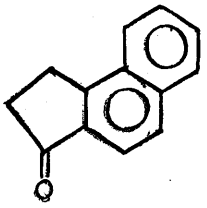
(CXL).



(CXLI).



(CXLIII)



(CXLII).

Cafestol,[52,53] $C_{20}H_{28}O_3$, the major constituent of the non-saponifiable fraction of coffee bean oil is a pentacyclic unsaturated alcohol, currently formulated[49] as (CXXXVll;R=Me, R_1=H).

Treatment of cafestol with lead tetra-acetate affords epoxynorcafestadieneone, $C_{19}H_{24}O_2$ (CXXXVlll) thus demonstrating the presence of a primary tertiary α -glycol system. The keto-group arising in this oxidation has been shown by chemical and spectroscopic methods to be present in a five-membered ring.

The ultraviolet absorption, the ease of reaction with electrophilic reagents and the formation of an unstable maleic anhydride adduct indicate the presence of a furan ring in cafestol. That the two ethylenic linkages and the inert oxygen function in cafestol are present in a furan ring fused in the 2' : 3' position to a six - membered ring is conclusively demonstrated in the following series of reactions. Ozonolysis of epoxynorcafestadieneone (CXXXVlll) afforded the saturated bisnor dicarboxylic acid $C_{17}H_{24}O_5$ (CXXXlX). The latter on pyrolysis furnished a diketone, $C_{16}H_{22}O_2$, the infrared spectrum of which showed that both ketones were present in five - membered rings.

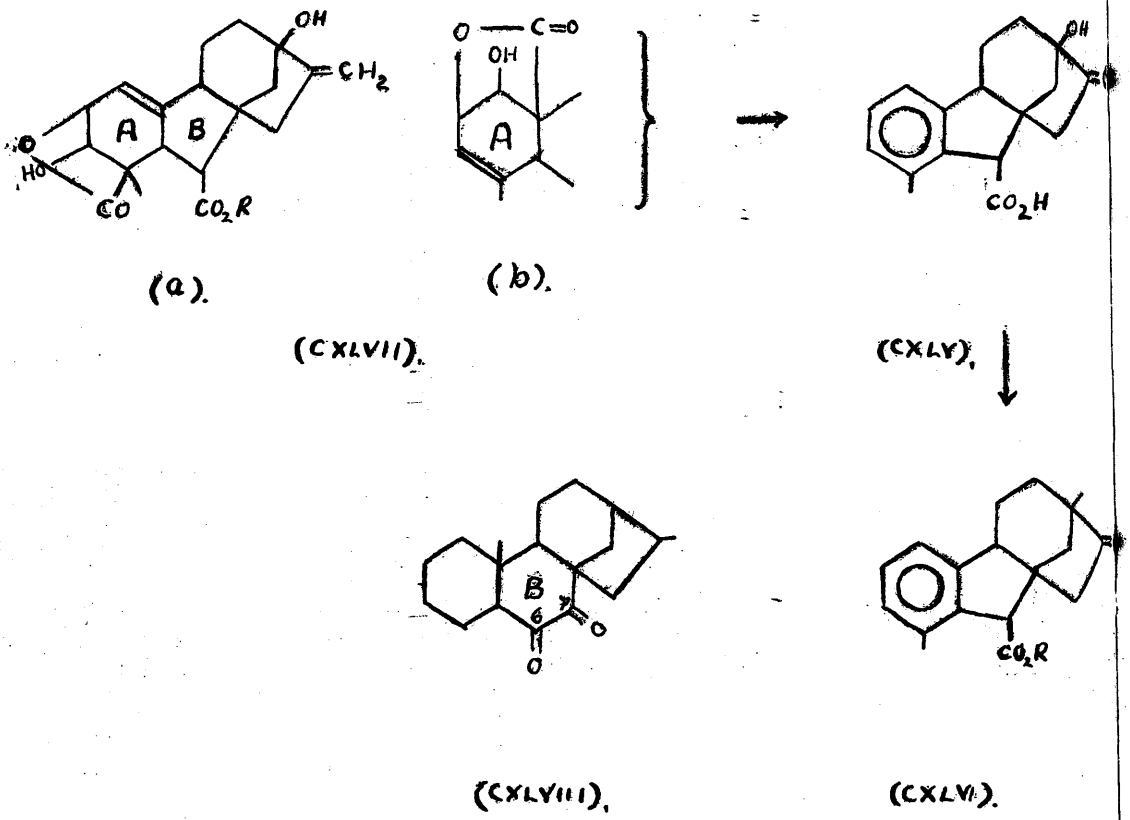
The following experiment,[53] in addition to proving the pentacyclic nature and the basic carbon skeleton of cafestol, labels the position and method of attachment of the furan ring. Dehydrogenation of the dicarboxylic acid currently depicted as (CXL;R=Me, R_1=H), obtained by hypoiodite oxidation of epoxynorcafestadieneone (CXXXVlll), afforded amongst other products 1-ethyl-2-hydroxyphenanthrene (CXL). The latter has been synthesised.[54]

Infrared measurements show that cafestol has one C-methyl group,

which, according to the next series of reactions described,[55] should apparently be placed at position 5 as depicted in (CXXXVll;R=H, R_1=Me). Ozonolysis of the dicarboxylic acid, formulated as (CXL;R=H, R_1=Me) according to the following sequence, gave a tetracarboxylic acid which was dehydrogenated to give, amongst other products, 4:5- benzindan-1-one (CXLll) and in better yield 1-ethyl-2-methylnaphthalene (CXLlll). The former must arise from a preliminary cyclisation followed by decarboxylation before dehydrogenation. Its subsequent isolation therefore demonstrates that the methyl group in cafestol must be present in an angular position from which it is eliminated during dehydrogenation. The isolation of the latter (CXLlll) would appear to establish that the angular methyl group is placed at position 5 providing, of course, that no migration or reduction of carboxyl groups has taken place in the dehydrogenation.

A recent paper[49] dealing with the absolute configuration and the position of the quaternary methyl group, however, appears to indicate that these assumptions are not valid in this case. Firstly it is shown that comparative rotatory dispersion studies lead to the conclusion that the absolute configuration of the A:B ring junction in cafestol, as in eperuic acid (see p. 11) is the mirror image of the majority of the known diterpenoids. In addition, using spectroscopic methods (see ref.114), the conformations of the products resulting from the mono-bromination and reduction of (CXLlV), 4 α -ethylcholestan-3-one (model for 10 axial methyl) and friedelin (model for 5 axial methyl) were elucidated. In every case (CXLlV) was found to behave in the same manner as 4 α -ethylcholestan-3-one and in the opposite way to friedelin. Thus in conflict with the dehydrogenation evidence it appears that the quaternary methyl group in cafestol should be placed at position 10 with the absolute configuration as shown in (CXXXVll; R=Me, R_1=H).

Finally there remains in epoxynorcafestadieneone (CXXXVlll), two carbon atoms, as yet unproved, which must serve to complete its cyclopentane system. Since the ethane bridge does not appear on dehydrogenation and since the dicarboxylic acid (CXL) contains one very hindered carboxyl group it would appear that one end of this bridge is attached to the B/C ring junction. The tetrahydro derivative of the ketone (CXXXVlll) forms a tribromo- compound and consequently the carbonyl group is not placed α-to the quaternary position at the ring junction. Such a cyclopentane system is established in phyllocladene (see p. 47) and by analogy with the latter, and in the absence of any further chemical proof, this system is tentatively attached to carbon atoms 8 and 13. Formulation (CXXXVll;R=Me, R_1=H) therefore depicts the currently accepted constitution and absolute configuration of cafestol.



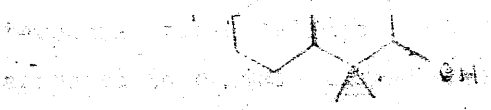
Gibberellic acid,[56] a very powerful plant growth promoting metabolite of Gibberella fujikurio, although not a diterpenoid in the normal sense, is closely related biogenetically to this series and therefore merits brief mention.

Mild acid hydrolysis of gibberellic acid, $C_{19}H_{22}O_6$, affords allogibberic acid, $C_{18}H_{20}O_3$, which is isomerised under more vigorous

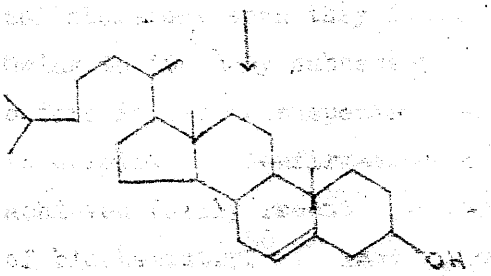
acidic conditions to gibberic acid. As a result of some excellent work, involving step-wise degradation and partial syntheses, the constitution of allogibberic acid and gibberic acid have been unambiguously demonstrated as (CXLV) and (CXLVI;R=H) respectively. From these structures it follows that the isomerisation of allogibberic acid to gibberic acid involves a Wagner-Meerwein rearrangement.

Gibberellic acid is a tetracyclic doubly unsaturated dihydroxy lactonic acid, $C_{19}H_{22}O_6$ in which, from standard reactions, it appears that one hydroxyl group is secondary and the other tertiary. The conversion of methyl gibberellate [CXLVII (a) or (b);R=Me] into methyl gibberate (CXLVI;R=Me) demonstrates that the position of the carboxyl group is the same in both compounds. Moreover since gibberellic acid [CXLVII (a) or (b);R=H] contains an exocyclic methylene group it is possible that the conversion of the latter to allogibberic acid (CXLV) merely involves aromatisation of ring A. Gibberellic acid must accommodate a five-membered lactone, a secondary hydroxyl group and a double bond, which is apparently trisubstituted (ultraviolet absorption). The methyl ester of the dicarboxylic acid obtained by opening of the lactone ring of gibberellic acid is rapidly oxidized by periodate.

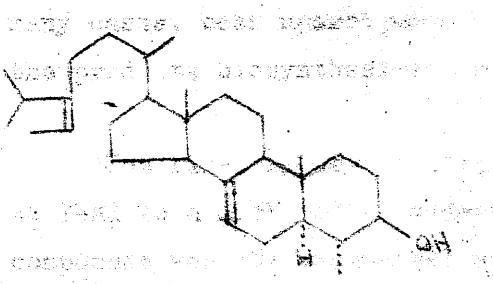
The above facts together with biogenetic considerations suggest that [CXLVII (a);R=H] or [CXLVIII (b);R=H] are the most probable structures for gibberellic acid. Apart from ring B the carbon skeleton of gibberellic acid is obviously closely related to phyllocladene (see p. 47). A possible biogenetic route to such a skeleton from the phyllocladene skeleton might involve a benzylic acid type rearrangement of a 6:7- diketo- system, e.g. (CXLVIII). Recent work[57] seems to indicate that such a system is present in xanthoperol, an artefact of the wood extract of Juniperus communis L.



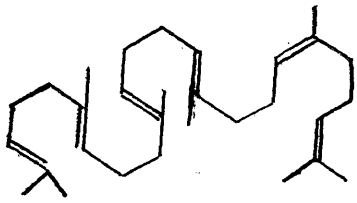
(II)



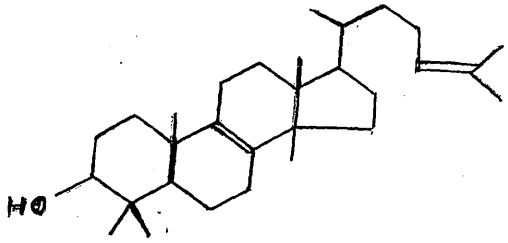
(I)



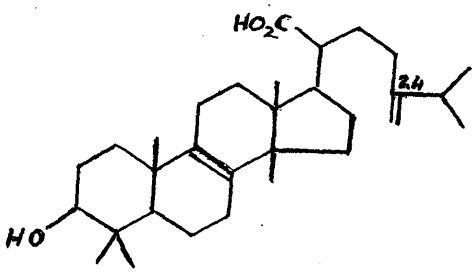
(IV)



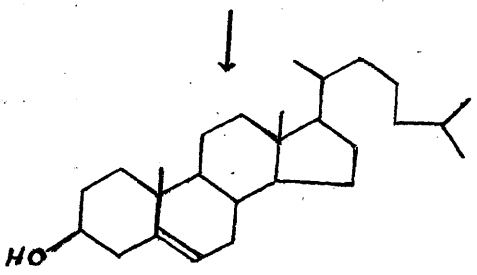
(IV).



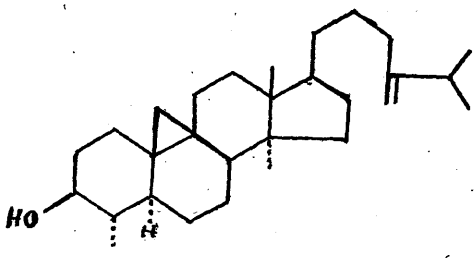
(II).



(III).



(I).



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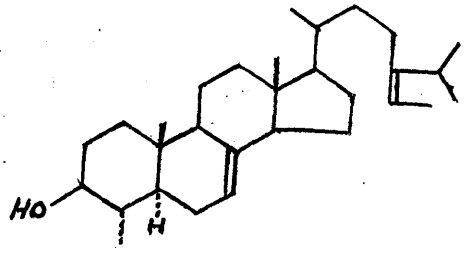
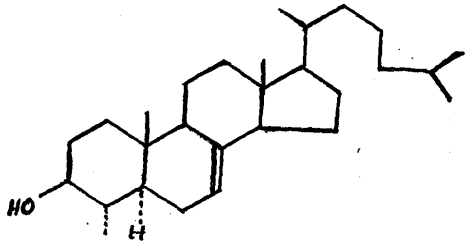


(VI).



(VII).

2. The Isoprene Rule and the Biogenesis of Diterpenoids.

While carrying out some of his pioneering work in the monoterpenoid field Wallach[58] noticed that several of these compounds appeared to contain isopentane units. In the early 1920's the importance of this observation was recognised[59] by Ruzicka and his collaborators when they formulated the empirical isoprene rule. Owing to its many subsequent successful applications it was not long before it became suspected that this hypothesis must be biogenetic in origin. Confirmation of this view however has only been achieved fairly recently by the application of the modern techniques of biochemistry.[58] Thus it has been found possible to develop enzymes which are specific for single stages in biosynthetic processes. Skillful use of such enzymes has enabled complete reaction sequences to be worked out; furthermore the arrangement of the simple precursor molecules in the biosynthetic products has, in many cases, been unambiguously established by careful degradation of the products biosynthesised from labelled precursors.[60]

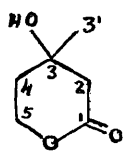
The first important discovery in the work which ultimately was to lead to a much better understanding of the biogenesis of isoprenoid compounds was the biological synthesis of cholesterol (1) from acetic acid.[61] More recently, using different enzymes, the triterpenoid lanosterol (11) and the 24- homotriterpenoid eburicoic acid (111) have been shown to originate from acetic acid.[62,63]

More recently still careful work has established three definite stages[64,65,66] in a biosynthetic route to cholesterol (1), the first step involving the transformation of acetic acid to squalene (1V). In the next stage[65] squalene (1V) is cyclised to lanosterol (11),

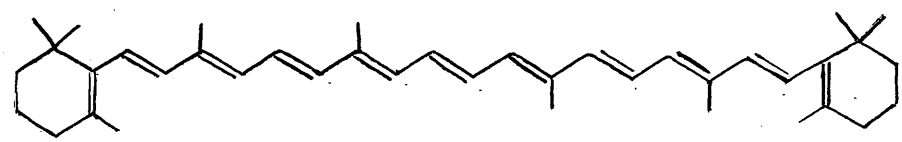
here it appears that the electrophylic moiety, which initiates the reaction is oxygen gas, presumably activated by the enzyme, and not OH^+ as postulated by Ruzicka.[59] The last step involves the conversion of lanosterol (11) to cholesterol (1) and evidence has been presented[66] to show that the three methyl groups lost in this process are oxidized to carbon dioxide.

The recent isolation of compounds containing dimethyl and monomethyl steroid nucleii, for example, cycloeucalenol[67](V) lophenol[68] (V1) and citrostadienol[69](V11) clearly indicates the probable existence of a biosynthetic route to plant sterols involving the demethylation of squalene cyclisation products.

By the early 1950 s, however, the knowledge that acetic acid was a biogenetic precursor of the steroids and triterpenoids had lost much of its earlier significance since acetic acid had by that time been shown to be similarly related to the natural fatty acids[60] and also many natural phenolic compounds.[58] It was therefore a very important advance in the biogenesis of isoprenoid compounds when Tavormina etal[70] discovered that β -hydroxy - β - methyl valerolactone (M.V.A) (V111) can be converted, in 43% yield, into cholesterol. Subsequent work has shown that M.V.A. can be successfully incorporated into β - carotene[71] (1X) and squalene[72] without prior breakdown into acetate.



(V111).

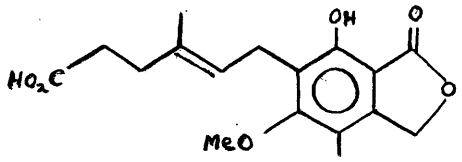


(1X).

Several investigations into the mechanism of formation of the

isoprenoid chain from M.V.A. have been carried out.[73,74] Thus it has been shown that in condensation C_1 is lost by decarboxylation and that C_2 and C_3 remain distinct. From this it would appear[73] that the chain formation involves condensation of the C_5 of one M.V.A. residue with the C_2 of another. Further it has been shown that both hydrogen atoms attached to C_5 are retained[74] during condensation, thus eliminating the possibility of a Claisen condensation. In agreement with this a recent experiment[72] has shown that a phosphate ester (probably the 5-phosphate) of one enantiomorph* of M.V.A. is an intermediate in the squalene synthesis. It has been suggested[75] that this optically active phosphate ester or a closely related derivative is a source of isoprenoid cations in the same way that methionine is known to be a source of methyl cations in natural processes.

That M.V.A. is a more immediate biological precursor of isoprenoid compounds than acetic acid has been clearly demonstrated by the biosynthesis of several compounds of mixed type.[76] Such a compound is mycophenolic acid (X) which is a phenolic compound with a terpenoid side chain. As would be expected (X) is readily synthesised from acetic acid[76] and when the reaction is carried out in the presence of labelled M.V.A. the latter only appears in the terpenoid portion of the molecule.[76]



(X).

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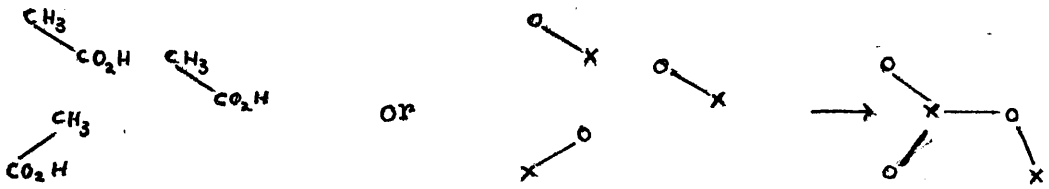
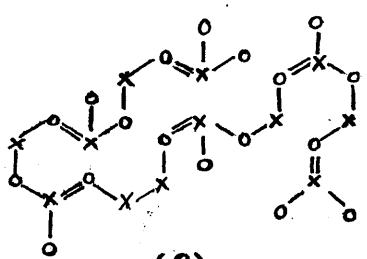
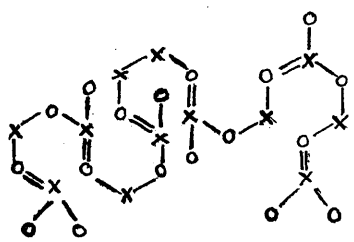
* This is particularly important since it demonstrates the very high efficiency of the conversion of one isomer of M.V.A. to cholesterol i.e. (ca 90%).

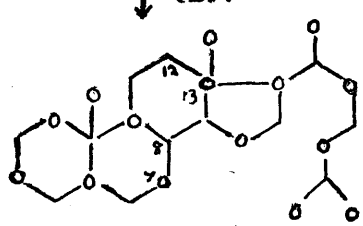
We turn now to a discussion of the theory of biogenesis of terpenoids which developed concurrently with the above advances in biochemistry. It was early recognised that three acetic acid units could condense to give an isoprenoid skeleton with the distribution of methyl and carboxyl carbon atoms as shown below.



The first important proposal in this field was that of Robinson[59] who suggested that cyclisation of squalene folded as shown in (A) might lead to cholesterol. In the early 1950's Woodward and Bloch[77] suggested the alternative scheme (B). Comparison of these schemes shows that in the cholesterol (XI) so formed the carbon atoms at positions 7, 8, 12 and 13 are derived from different acetate carbons. Subsequent work[78] on appropriately labelled cholesterol has completely elucidated the order of every acetic acid residue in biosynthetic cholesterol. The findings of these experiments are in complete agreement with the original hypothesis of Woodward and Bloch.[77]

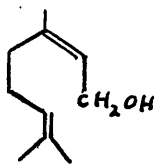

(A).


(B).

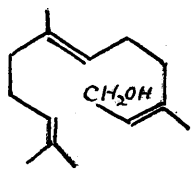


(XI)

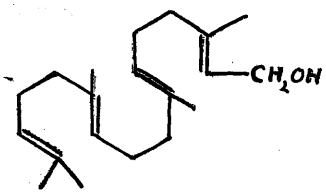
Shortly after the publication of Woodward and Bloch's hypothesis Ruzicka[59] proposed the biogenetic isoprene rule. This correlates the carbon skeletons of all the known mono-, sesque-, di- and triterpenoids which Ruzicka showed could be readily derived by accepted reaction mechanisms from the hypothesised simple acyclic precursors geraniol (XII), farnesol (XIII), geranylgeranyol (XIV) and squalene (XV) respectively.



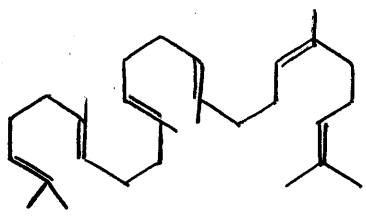
(XII).



(XIII).

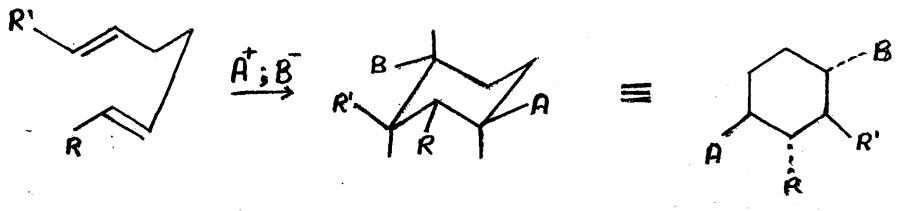


(XIV)

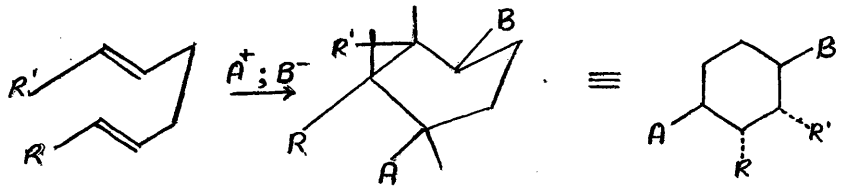


(XV).

In a later extension Ruzicka et al.[1] review the mechanisms of the many acid catalysed cyclisations and rearrangements known in terpenoid chemistry, and also point out the importance of conformation in a molecule undergoing cyclisation. The cyclisations of a simple 1:6 disubstituted hexa- 1:5- diene illustrates the latter point as shown below.



(XVI). (XVII).



(XVIII). (XIX).

When the diene reacts in the chain conformation (XVI) the product (XVII) is formed whereas reaction in the boat conformation (XVIII) affords (XIX).

These postulates are utilised to develop a scheme in which the complete skeleton and configuration of every known triterpenoid is derived from squalene. This hypothesis is based upon the following assumptions:

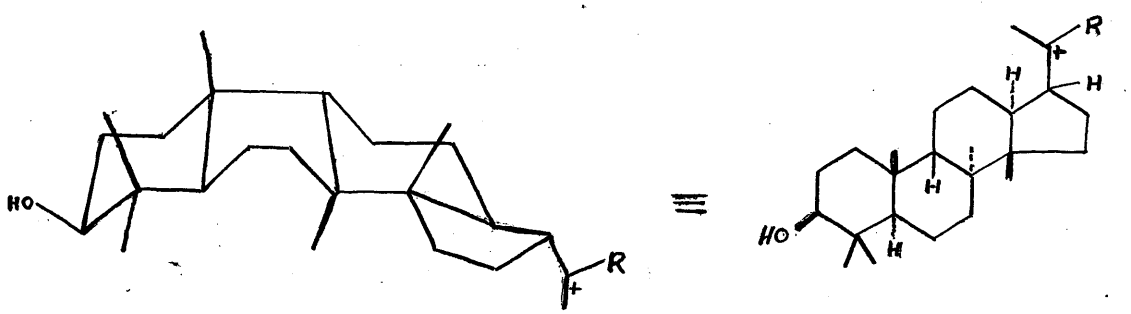
1. The squalene molecule reacts with the four middle double bonds in the trans configuration.
2. When cyclisation takes place the squalene molecule is already folded in either of two conformations (see later)

3. Cyclisation occurs in the usual trans addition manner.

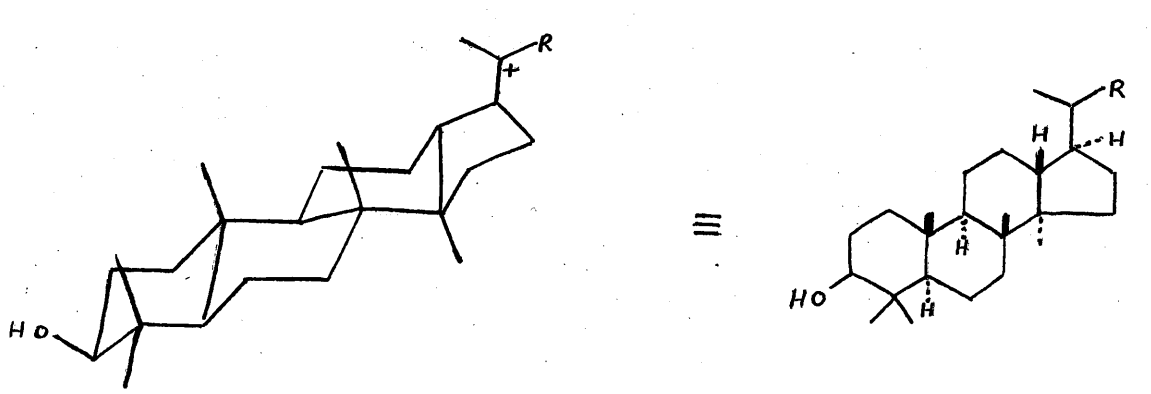
4. Wagner - Meerwein rearrangements and 1:2 eliminations only take place if the stereochemistry is correct.

5. Carbonium ions are best represented as carbonium ion double bond complexes.

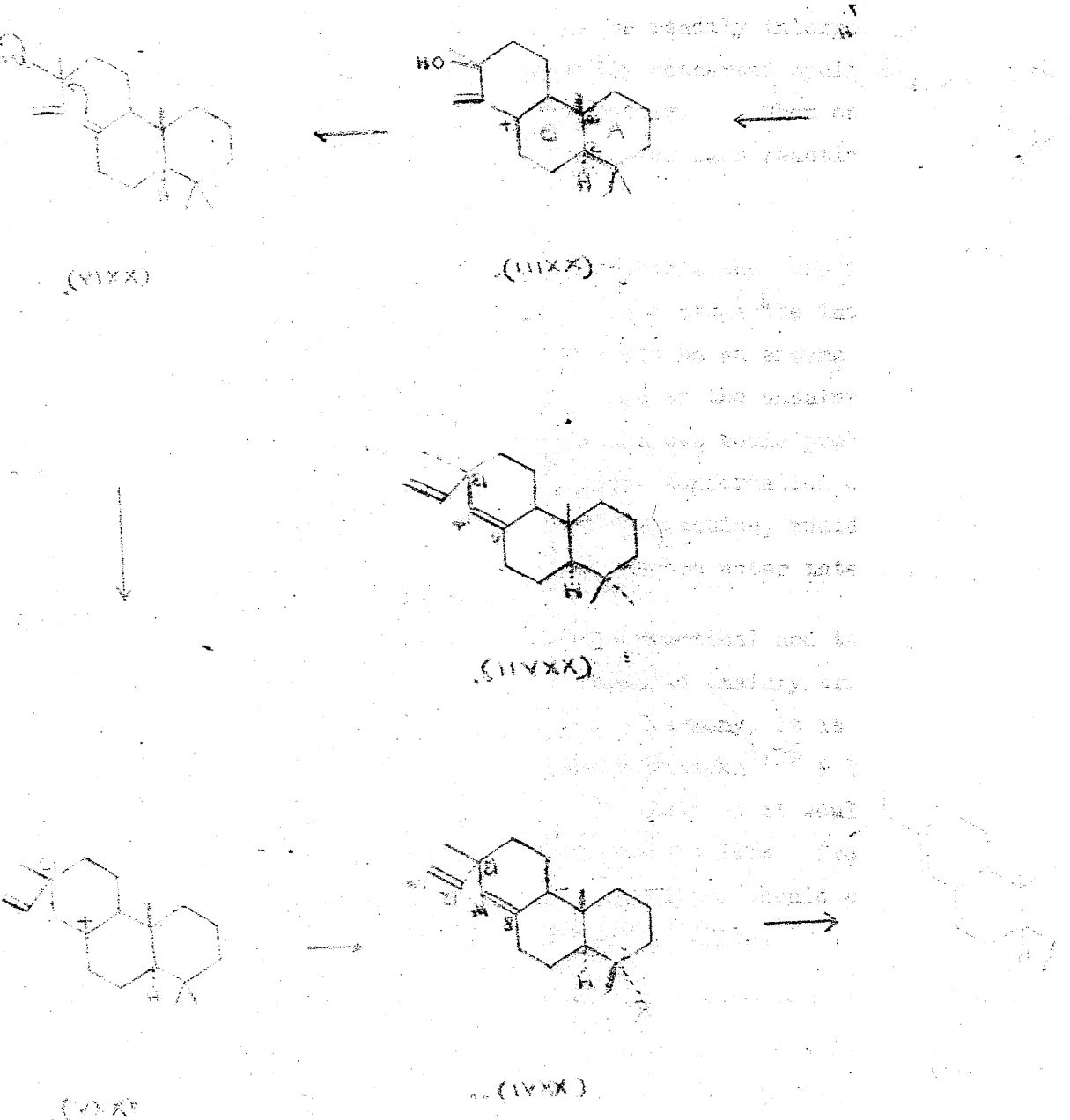
In this way concerted cyclisation of squalene in two different conformations compare (XVlll) and (XVl) affords the two tetracyclic carbonium ions (XX) and (XXl). The former can in theory be transformed into lanosterol and any of the steroids while the latter (XXl) can be similarly shown to be a precursor of the pentacyclic triterpenoids and the tetracyclic euphol group.

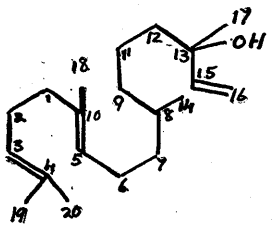


(xx).

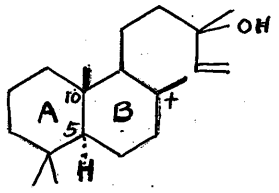


(xxl).

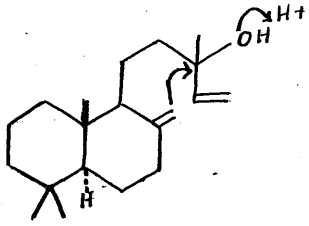




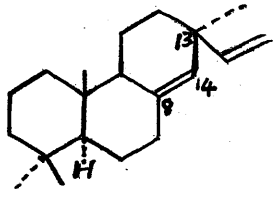
(XXII).



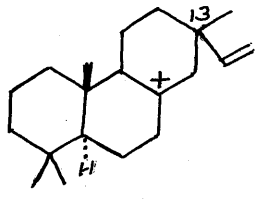
(XXIII).



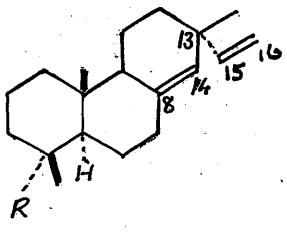
(XXIV).



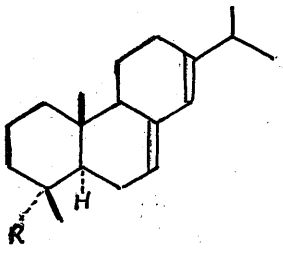
(XXVII).



(XXV).



(XXVI).



(XXVIII).

Within recent years many attempts[79,80] have been made to achieve, under laboratory conditions, the fully concerted cyclisations which the above biogenetic theory requires. The results of these experiments can be readily interpreted,[80] and they clearly demonstrate that fully concerted cyclisation is very difficult to achieve in the laboratory. When one considers the strict conformational requirements for such reactions these results are not unexpected.

In nature, however, it is probable that the formation of such asymmetric compounds requires at some stage the intervention of some asymmetric agent. This could be an enzyme in which specific groups interact with the π eletrons of the unsaturated hydrocarbon; furthermore, reactions involving enzymes would probably take place in aqueous solution where the folded conformation of the hydrocarbon, so necessary for fully concerted cyclisation, would be favoured owing to the smaller area of hydrocarbon water interface.

Having briefly discussed the practical and theoretical developments in the theory of terpenoid (mainly triterpenoid) biogenesis and shown these to be in harmony, it is now proposed to discuss in more detail the ideas of Ruzicka[1,59] & Wenkert[81] on the biogenesis of diterpenoids. In addition it would appear that the stereochemical implications, which follow from Ruzicka's second paper[1] on the squalene cyclisation, should also be applicable to the cyclisation of geranyllinalool (XXll).

On this basis it is possible to derive the carbon skeletons and make tentative proposals regarding the stereochemistry, for example the high probability of a trans A : B ring fusion, of all diterpenoids.

The precursor in this scheme,[59] geranyllinalool (XXll) has not as yet been found in nature. Protonation of the $\Delta^{3:4}$ linkage initiates a concerted cyclisation to give trans- decalin carbonium ion (XXlll),* the conversion of which into manool (XXlV) sclareol (p.5) and the other isoprenoid bicyclic diterpenoids (p.p.5-23) is acceptable.

Acid catalysed cyclisation of manool (XXlV) as shown would afford the intermediate (XXV) which could readily lose a proton to yield the pimaradiene (XXVl;R=CH_3).

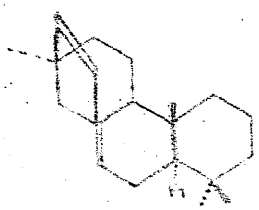
The potentialities and implications of the pimaradiene (XXVl; R=Me or CO_2H) as a biogenetic precursor for the tri- and tetracyclic diterpenoids have been thoroughly analysed by Wenkert.[81] The possible rearrangements of (XXVl) or (XXVll) depend on the stereochemistry of the substituents at position 13. This follows from stereo- electronic considerations which indicate that only a pimaradiene, with a quasi- axial methyl and a quasi- equatorial vinyl function, e.g. (XXVl) can undergo smooth concerted rearrangements to the abietadiene (XXVlll;R=Me). The relationship of this to the abietic acid group of tricyclic diterpenoids (p.p. 33-37) is obvious. The facility of this transformation leads the author to conclude that only the pimaradienes (and diterpenoids derived from them) which are epimeric at C(13), such as (XXVll) will be found in nature. He also adds that the reaction of compounds such as (XXVll) would probably proceed through the protonation of $\Delta^{8:14}$ instead of $\Delta^{15:16}$

The chemistry of the known pimaradienes, rimuene and dextropimaric acid appear to corroborate this view. Further work

---

* Since (XXlll) is the precursor of all bi-, tri- and tetracyclic diterpenoids in this scheme, the trans A : B ring junction (See Ref[59]) in these compounds follows automatically providing no further changes take place at positions 5 and 10.

(1xxx)



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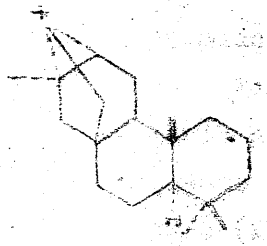
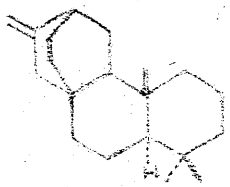
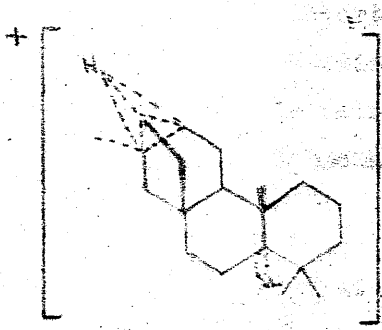


(4 xxx)

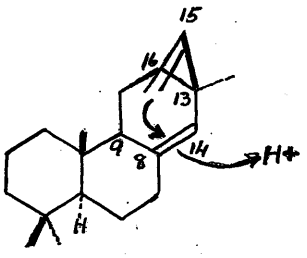
III:



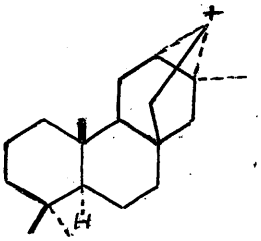
(11xxx)



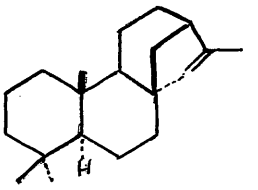
(8 xxx)



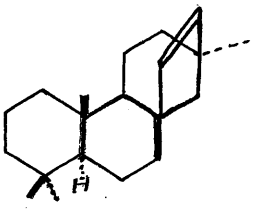
(XXVII).



(XXX A).

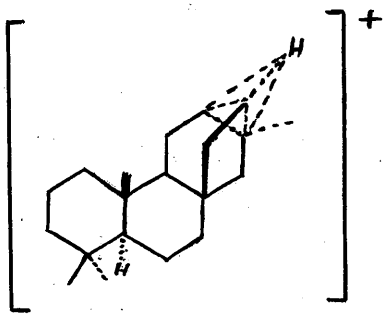


(XXXI).

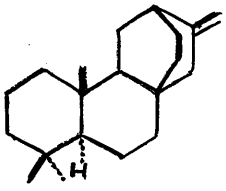


(XXIX).

III



(XXX B).



(XXXII).

is needed however to establish the relationship between the three known dextropimaric acids (see p.p.27-30).

Rimuene is known to isomerise to isophyllocladene (XXXl) * and is formulated as (XXVll) with the vinyl group in the quasi-axial position. On this formulation the isomerisation to isophyllocladene can be readily visualised via the non-classical carbonium ion (XXX A). This indicates a possible biogenetic route to the tetracyclic diterpenoids (p.p. 47-56) and the closely related family of alkaloids.[82] Wenkert suggests structure (XXlX) for the isomeric tetracyclic hydrocarbon mirene which is also converted into isophyllocladene (XXXl) on treatment with acid probably via the same intermediate (XXX A).

Reactions involving intermediateslike (XXX A) are often accompanied by 1:3 hydride shifts, which may be interpreted by formulating the appropriate nortricyclonium ion transition state.[83] In this case (XXX A) may be written as (XXX B) which could break down to yield several structures, one being (XXXll). This carbon skeleton (XXXll) appears to be important in the other family of diterpenoid alkaloids related to atisine.[84] Podocarprene,[85] a tetracyclic hydrocarbon isomeric with phyllocladene, which is isomerised by acid to yield yet another hydrocarbon, may well belong to this class of compounds.

It would therefore appear that through this scheme all tetracyclic diterpenoids regardless of the nature of their fourth ring can be related biogenetically to a pimaradiene with a quasi-axial vinyl substituent (XXVll).

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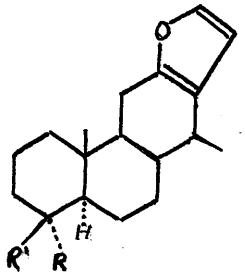
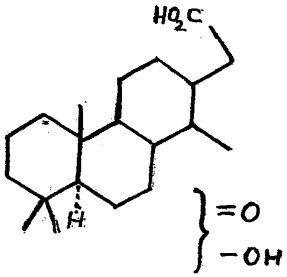
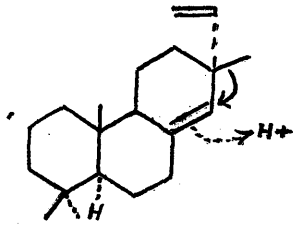
* The absolute configuration depicted in (XXXI) for isophyllocladene has recently been questioned.



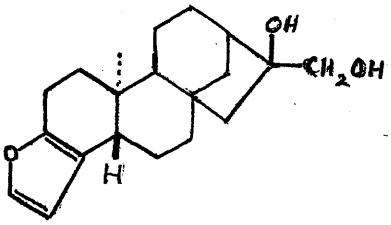
(xxxiii).



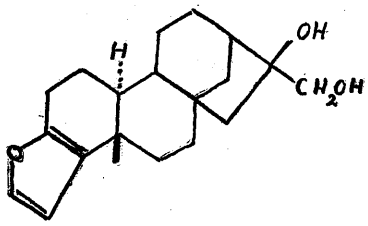
(xxxiv).



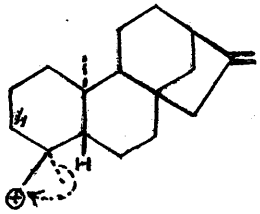
(xxxvii).



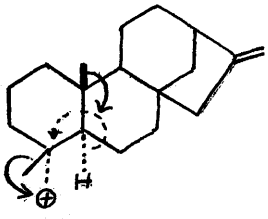
(xxxv).



(xxxvi).



(xxxviii).



(xxxix).

In the scheme described all the non-isoprenoid diterpenoids are covered apart from columbin (see later p.126) vinhaticoic acid (XXXlll;R=CO_2H, R'=Me) vouacapenic acid (XXXlll;R=Me, R'=CO_2H), cassaic acid (XXXlV) and cafestol (XXXV) or possibly (XXXVl).

The carbon frame work of (XXXlll) and (XXXlV) is probably derived from the pimaradiene (XXXVll) by methyl migration as shown. It is interesting to postulate at this stage that the carboxymethyl side chain in cassaic acid (XXXlV) may be the biogenetic precursor of the furan ring in (XXXlll; R=CO_2H, R'=Me) and (XXXlll;R=Me, R'=CO_2H).

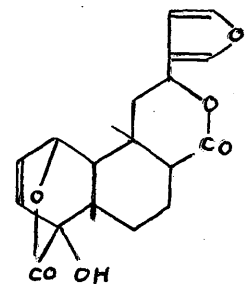
The carbon skeleton and known absolute configuration of the two most probable structures from cafestol (XXXV) and (XXXVl) (see p.52) can be derived from the enantiomorphic phyllocladenes (XXXVlll) and (XXXlX) as shown. In the case of (XXXVlll) the removal of a proton from position 4 prevents the concerted rearrangement shown in (XXXlX). Current chemical evidence (see p.53) favours formulation (XXXV) for cafestol, and when one considers the subsequent formation of the furan ring this is probably more feasible biogenetically. It is interesting to note that, apart from cassaic acid, these migrations take place prior to or during the formation of the aromatic furan ring system.

3. The Structure of Chasmanthin, Jateorin and Palmarin

(a) Discussion

The root of Jateorhiza palmata Mier's (Colombo root) which is indigenous to East Africa contains a number of interesting organic compounds. These fall into two distinct classes; one contains three closely related alkaloids[86,87] of established structure and the other a number of neutral bitter principles. This investigation is concerned with the latter group.

Although the Colombo root bitter principles were first investigated in an impure form as early as 1830,[88] no significant chemical work was carried out before 1935. The correct molecular formula for chasmanthin $C_{20}H_{22}O_7$ was first proposed by Feist,[95] whereas the two remaining bitter principles columbin, $C_{20}H_{22}O_6$, and palmarin, $C_{20}H_{22}O_7$, were first correctly formulated by Wessely.[89,90] During the period 1935 to 1938 these compounds, particularly columbin, were extensively investigated by the schools of Wessely[89-94] and Feist.[95-100] Although this work furnished much information, very few conclusions were reached and no definite proposals concerning the functional groups or structure were made for columbin, chasmanthin or palmarin.



I

In 1955 Barton and Elad[101] re-analysed the above work and on the basis of additional experiments proposed the constitution (1) for columbin (see p. 21).

The object of the investigation now to be described was to elucidate the structures of the remaining bitter principles chasmanthin and palmarin. As already mentioned the only previous significant contributions to this problem are those of Wessely[90,94] and Feist[95,96,98,99] and their respective collaborators.

The first experiments to be undertaken were essentially repetitions of some of the more important observations of Wessely and Feist. This exercise had two objectives, in the first place it was hoped to rationalise many of the apparently confusing results recorded, and secondly to gather spectral data for the various compounds described.

The isolation of the crude mixture of bitter principles in approximately 0.2% yield from the Colombo root was conveniently carried out using ether extraction. Fractional crystallisation of this crude extract effected a preliminary separation of the two major components columbin and chasmanthin. The extent of the separation was best judged by optical rotation. Further purification of the chasmanthin rich material using Wessely's[94] crystallisation procedure afforded a relatively pure sample of 'chasmanthin' m.p. 230°, $[\alpha]_D \pm 0°$.

Feist[95] has claimed that heat treatment of columbin affords, in addition to decarboxycolumbin, two isomeric compounds $C_{20}H_{22}O_7$ of m.p. 265° and 212°, which he considered to be polymorphic forms of chasmanthin. This clearly arose because his columbin contained some chasmanthin. Consequently this experiment was repeated using chasmanthin- rich fractions and it was found to be a very efficient means of isolating 'chasmanthin'. The great difference in the relative solubility of 'chasmanthin' and decarboxycolumbin permits their ready separation. By repeated crystallisation of

this 'chasmanthin' there was obtained, in small yield, an obviously pure compound $C_{20}H_{22}O_7$, $[\alpha]_D$ + 12°, m.p. 256°, the properties of which were identical with those described for palmarin by Wessely.[90] The bulk of the undecarboxylated material, m.p. 230-240°, $[\alpha]_D$ ± 0° appeared to be identical with Wessely's pure 'chasmanthin'[94] m.p. 236-246°. Like Wessely,[94] however, we could find no trace of Feist's polymorphic form of chasmanthin m.p. 212°.[95]

It seemed to us ab origine that Wessely's rather confusing results,[94] which will be discussed subsequently, could be most simply rationalised if the 'chasmanthin' used by him contained, in addition to traces of palmarin, similar proportions of two isomeric compounds. Wessely himself, with his extensive previous experience of columbin[89], was fully aware of the characteristic tendency to mixed crystal formation in this series and consequently directed much effort[94] to the purification of his 'chasmanthin'. He therefore felt[94] despite its m.p. range, that his eventual product was a homogenous compound. It was hoped however, by the use of chromatography to achieve the purification which it was assumed had eluded Wessely. Unfortunately this proved not to be possible.

By adopting different isolation procedures (see experimental) it was hoped to obtain samples of 'chasmanthin' with differing proportions of its components. Three such samples were prepared and fractionated either by chromatography or a six-stage triangulation procedure. Although in these three experiments minor impurities, (namely, columbin, decarboxyisocolumbin and palmarin) were separated, the 'chasmanthin' was not resolved. It should be noted however that the appearance of the various 'chasmanthin' fractions under a microscope and at the m.p. seemed to indicate that these were not completely homogeneous.

Having thus isolated the two naturally occurring materials palmarin and 'chasmanthin' and found their properties to be identical with those attributed to them in the literature, it was convenient to prepare their o-acetyl and o-methyl derivatives. These compounds were readily formed using boiling acetic anhydride, and dimethyl sulphate in alkali respectively. Like Wessely[90,94] it was found that the products from palmarin were pure, whereas those from 'chasmanthin' appeared to be mixtures.

The solution to this problem of proving the non-homogeneity of 'chasmanthin' presented itself when another of Wessely's experiments was reinvestigated. Thus Wessely claimed[94] that under the mild alkaline conditions which bring about the columbin- isocolumbin change,[89] 'chasmanthin' is isomerised to give a resolvable mixture of two isomeric compounds, chasmanthin A m.p. 258°, $[\alpha]_D$ + 16° to + 20° and chasmanthin B m.p. 170°, $[\alpha]_D$ + 25° to + 30°. When this experiment was repeated and the reaction mixture fractionated using chromatography or crystallisation there was obtained two pure products having m.p. 256°, $[\alpha]_D$ + 12°, and m.p. 166°, $[\alpha]_D$ + 30° respectively. Whereas Wessely claimed[94] that he could separate his chasmanthin A from palmarin and thus demonstrate their non-identity, we found that our higher melting isomerisation product to be identical in every respect (m.p., mixed m.p., rotation and infrared spectra) with the palmarin which had been previously obtained from the root (p. 74).

The spectral and chemical properties of these two isomerisation products are very similar and indicate that they are most probably stereoisomers. Assuming this, there are two possible ways in which these products could arise. In the first place if the starting material contains two compounds, each of which contains one

optically active centre which under alkaline conditions is almost quantitatively inverted. The second possibility is that the starting material is essentially pure but contains two epimerisable centres, one of which is almost quantitatively inverted, whilst the other is merely racemised under equilibrating conditions. It should be noted that the presence of one centre which is almost quantitatively inverted is necessary since no trace of starting material 'chasmanthin' could be found. The latter possibility is rigorously excluded by the stability of o- methylchasmanthin A (methylpalmarin) and o- methylchasmanthin B (methyl*iso*jateorin) under much more vigorous alkaline conditions.

It would therefore appear that the 'chasmanthin' of Wessely[94] and Feist[88,95] is a mixture of two isomeric bitter principles which will be called chasmanthin and jateorin. On isomerisation chasmanthin furnishes palmarin whereas jateorin affords *iso*jateorin. Consequently all Wessely's derivatives[94] of chasmanthin A are derivatives of palmarin and those of chasmanthin B are derived from *iso*jateorin.

This isomerisation of 'chasmanthin' under alkaline conditions has a number of important consequences. Firstly it suggests that the relationship between the two naturally occurring compounds chasmanthin and palmarin is most probably analogous to that between columbin and *iso*columbin.[89,101] Secondly it establishes the existence of the new bitter principle jateorin. Lastly, from the practical point of view it provided reasonable amounts of pure palmarin on which the degradational work to be described was carried out. Moreover the first two conclusions make it possible to rationalise the otherwise confusing results of Wessely, which will now be briefly discussed.

The specific optical rotations of the starting materials and

TABLE I

Ref.	Compound	$[\alpha]_D$	$[\alpha]_D$ O-acetate
24	Oleandrin A (crude helleborin)	+ 15° to + 20°	+ 44.5°
24	Oleandrin (oleandrin A, Jacobs)	± 0°	+ 44.5° to 46.1°
24	Oleandrin B (crude isohelleborin)	+ 25° to + 30°	45.5° to 46.1°
	isohelleborin	+ 30°	+ 52°

Ref.	Compound	$[\alpha]_D$	$[\alpha]_D$ o-methyl ether
90	Palmarin	+ 12°	+ 39.5°
94	Chasmanthin A (crude palmarin)	+ 16° to + 20°	+ 44.5°
94	'Chasmanthin' (chasmanthin & Jateorin)	± 0°	+ 44.5° to 49.5°
94	Chasmanthin B (crude iso jateorin)	+ 25° to + 30°	45.5° to 48.5°
	iso Jateorin	+ 30°	+ 58°

TABLE 1

Ref.	Compound	$[\alpha]_D$	$[\alpha]_D$ O-acetate
90	Palmarin	+ 12°	+ 13°
	iso Jateorin	+ 30°	

TABLE 11

products obtained by Wessely[90,94] when he treated palmarin, chasmanthin A (crude palmarin), 'chasmanthin' (mixture of chasmanthin and jateorin) and chasmanthin B (crude isojateorin) with dimethyl sulphate and alkali to give mono o- methyl ethers are shown on Table 1. There is also included in this table the corresponding data for a similar experiment which we carried out on isojateorin. Bearing in mind that the 'chasmanthin' must also be isomerised under the methylation conditions, the results are in complete accord with the compositions assigned to each material in brackets. Methylation of the 'chasmanthin' used in this work furnished a product $[\alpha]_D$ + 50° as would be expected since it has been shown to contain similar proportions of chasmanthin and jateorin. Finally it should be noted that both palmarin and isojateorin show essentially the same change (+ 28°) in specific rotation on methylation.

It has been demonstrated by Wessely[94] that 'chasmanthin' on treatment with boiling acetic anhydride containing sodium acetate affords acetylchasmanthin 11, and that when the sodium acetate is omitted from this reaction another distinct product acetylchasmanthin 1 is produced. Examination of Table 11 reveals that the specific rotation change, which takes place when the hydroxyl group of palmarin is acetylated, is + 1°. Since palmarin and isojateorin show a very similar rotation change on o- methylation it would be reasonable to assume that both compounds show similar rotational changes on o- acetylation. Thus acetylisojateorin might be expected to have a rotation of about + 31°, which is very similar to the value (+ 30°) reported for acetylchasmanthin 11. It is therefore concluded that the acetylchasmanthin 11 of Wessely is acetylisojateorin. Furthermore it has been shown that hydrolysis of acetylchasmanthin 1 affords a product which, from its reported constants, appears to be essentially pure isojateorin. Since acetylchasmanthin 1 cannot be

Isolauteorin (CH_3)			1763	1743(KBr)
Hexahydrocolumbamin (M)	3700 - 2390		1760	1720
Tetrahydrocolumbamin (M)	2390		1764	1720
Isocolumbamin (M)			1770 (1755)	1725
(CH_3)			1713	1720
(Ket)			1715	1747
Methylcolumbamin (M)		3160(m)	1770	1735
Columbamin (M)	3435	3140(m)	1778	1710

Compound	HO- cm^{-1}	Furan cm^{-1}	Carbonyl cm^{-1}	cm^{-1}	Furan cm^{-1}
Chasmanthin (N)	3480	3130(w)	1760	1710	1504(w)
Palmarin (N)	3453	3140(w)	1778	1710	1507(w)
methylpalmarin (N)		3160(w)	1770	1735	1505(w)
(KCl)			1772	1727	1505(w)
($CHCl_3$)			1773	1728	1504(w)
Acetylpalmarin (N)			1770 (1755)	1723	1505(w)
Tetrahydropalmarin (N)	3390		1764	1730	
Hexahydropalmarin (N)	3700 - 2390		1760	1720	
isoJateorin ($CHCl_3$)			1763	1743(sh*)	1504(w)
(N)	3510	3150(w)	1765	1745($\frac{2}{3}$)	1509(w)
Tetrahydroisojateorin	3440		1760	unresolved	

T A B L E 111

sh* = Shoulder
N = nujol mull

acetyl*iso*jateorin it must be acetyljateorin. Indeed, there is available independent evidence,[94] namely, hydrogenation of acetylchasmanthin 1 followed by hydrolysis, interpretation of which in the light of conclusions subsequently to be drawn clearly establishes that this compound belongs to the normal series.

The isolation of essentially pure derivatives of jateorin by Wessely[94] in his acetylations of 'chasmanthin', which has been shown to be a mixture of jateorin and chasmanthin, is probably due to the relative insolubilities of the acetyljateorins. Thus it was found that acetylation of chasmanthin in the presence of sodium acetate furnished a product with the specific rotation $[\alpha]_D$ + 19° which is the value expected for a mixture of palmarin and *iso*jateorin acetates. On further crystallisation a product $[\alpha]_D$ + 26° was obtained thus demonstrating the relative insolubility of acetyl*iso*jateorin $[\alpha]_D$ + 30°.

We now turn to a discussion of the functional groups in the molecule of palmarin $C_{20}H_{22}O_7$. Of the seven oxygen atoms in palmarin six are contained in functions which are very similar to those found in columbin.[89,101]

Thus, in spite of the conflicting evidence of the earlier workers,[90,94,95,100] it has been clearly demonstrated that both palmarin and methylpalmarin consume two moles of alkali, the first much more readily than the second. In agreement the infrared spectrum (chloroform) of methylpalmarin shows bonds at 1773 and 1728 cm^{-1} (lactone carbonyls) which disappear on treatment with base with the simultaneous appearance of a single peak at 1573 cm^{-1} (carboxylate anion).

Like columbin, palmarin contains a tertiary acidic hydroxyl

group which will not acetylate under normal conditions but which does form a mono-acetate and mono-methyl ether under the conditions previously described (see p. 75). The reduction of palmarin with lithium aluminium hydride to a pentol which is cleaved by sodium metaperiodate to a nor triol-one (see p.104) has two important consequences. Firstly it confirms the presence of two lactones and secondly it indicates that the acidic hydroxyl group in palmarin must be attached in the α-position to one of the lactone carbonyl functions.

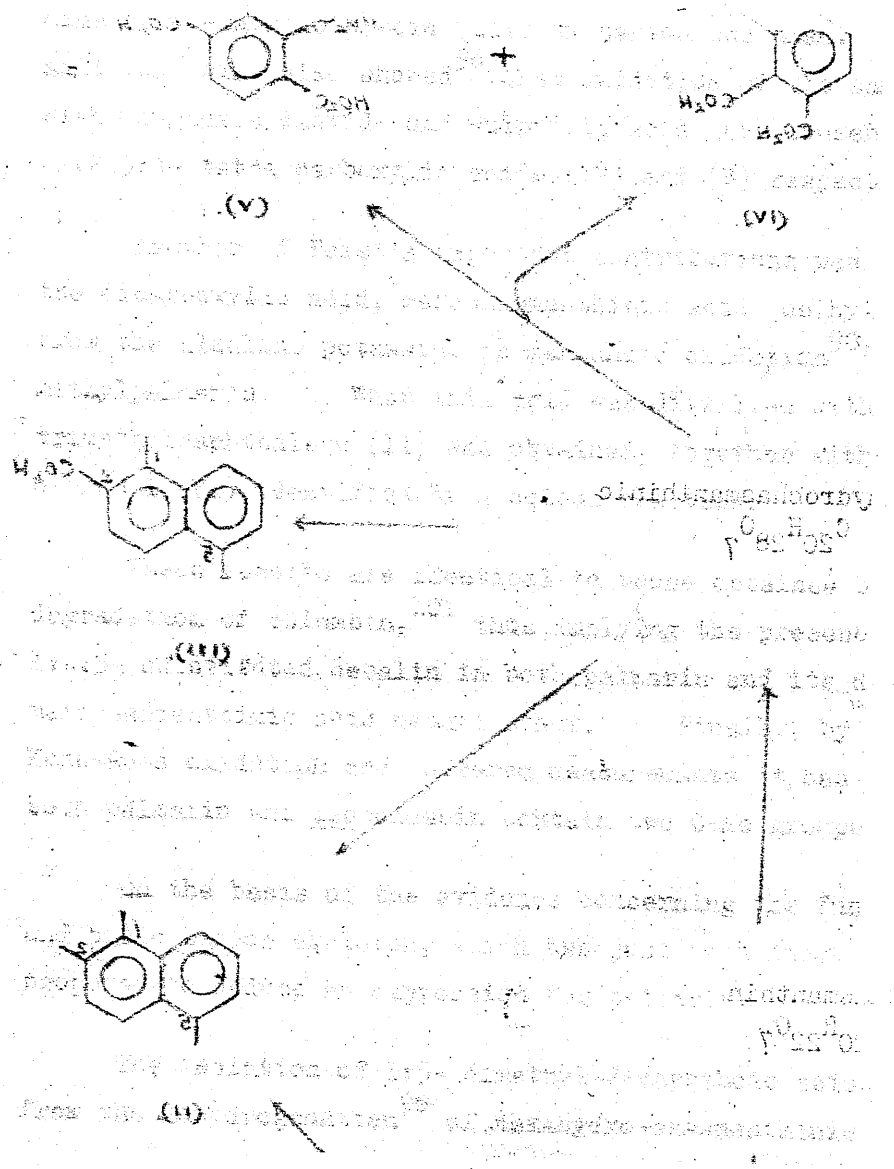
Again the presence of a mono α - or β - substituted furan ring system and its position relative to one of the lactonesfollows from familiar arguments.[101,102] Thus oxidation of methylpalmarin, $C_{21}H_{24}O_7$ affords, amongst other products, the crystalline methyl ester of the tris-nor acid $C_{18}H_{22}O_8$ (p. 94). Furthermore, using ozonolysis, the corresponding crystalline acid $C_{17}H_{20}O_8$ of the palmarin series has also been obtained (see p.110). Spectroscopically (see Table 111) a furan ring system is also indicated. Thus, like columbin[101] and marrubiin,[102] palmarin and all its derivatives, which have not undergone hydrogenation or oxidation, show bands at 1505 and 3110-3160 cm^{-1} (furan) in the infrared and end absorption at 210 mμ (ε = 5500) in the ultraviolet. By hydrogenation of palmarin over palladium charcoal in acetic acid Wessely[90] obtained the hexahydro acid $C_{20}H_{28}O_7$. Repetition of this work furnished in addition to the latter a neutral tetrahydro- compound $C_{20}H_{26}O_7$ thus confirming the presence, in palmarin, of two ethylenic linkages which must be present in the furan ring. The formation of Wessely's hexahydro-palmarinic acid must involve hydrogenolysis of the lactone ring, analagous to the hydrogenation of decarboxycolumbin (see p. 21).

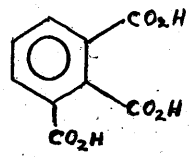
It is convenient at this stage to designate the lactone involved in hydrogenolysis as lactone (B) and the other as lactone (A). It

follows from its easy hydrogenolysis that the alkyl oxygen atom of lactone (B) (which, because of its reversible opening, cannot be vinylic) must be attached allylically with respect to some unsaturated system, most probably the furan ring. This situation exists in columbin[101] and is conclusively established in palmarin by experiments which will be described later (see p.111). A comparison of the carbonyl absorption in the infrared spectra (nujol) of tetrahydro-palmarin (1764, 1730 cm^{-1}) and hexahydro-palmarinic acid [1760, 1720 (broad) cm^{-1}] seems to indicate that the lactone which does not hydrogenolyse, that is lactone (A), is probably a γ-lactone whereas the other, lactone (B), is probably δ. The carbonyl absorption of the various derivatives of chasmanthin, palmarin and isojateorin shown in Table III (p. 79) clearly demonstrates the presence of two different types of lactones.

Before leaving this discussion of the functional groups of palmarin the nature of the remaining oxygen function must be briefly considered. A more detailed examination will be made later. This function is clearly not hydroxylic since, firstly, palmarin and methylpalmarin show respectively 1 and 0 active hydrogen atoms by the Zerewitinoff method,[90] and secondly from the absence of hydroxyl absorption (see Table III) in the infrared spectrum of acetylpalmarin, methylpalmarin and the methyl ester of the tris-nor acid from permanganate oxidation of methylpalmarin. A ketonic function is also excluded, inter al by the absence of selective absorption in the 280-290 m.μ region, and by the nature of many of the compounds which will subsequently be described. It follows therefore that the remaining oxygen atom of palmarin is present as an inert ether function.

Feist's drastic degradations[96,98,99] must now be briefly discussed since it is from these experiments that the carbon skeleton of palmarin is deduced.





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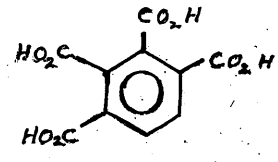
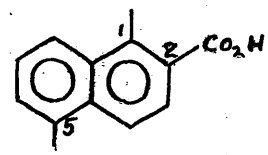


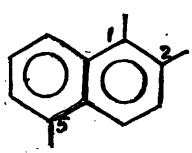
(V).

Hexahydrochasmanthinic acid $C_{20}H_{28}O_7$



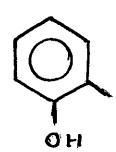
(III).

Chasmanthin $C_{20}H_{22}O_7$



(II).

Merochasmanthinic acid Methyl ether $C_{16}H_{20}O_8$ (via Methylpalmarin)



(VI).

Thus, dehydrogenation[96] using selenium of the hexahydro acid, obtained from chasmanthin, furnished 1:2:5-trimethylnaphthalene (11) and the so called 'lactone' $C_{15}H_{16}O_2$, subsequently shown to be 1:5-dimethyl-2-naphthoic acid (111) by Barton and Elad.[101] In addition Feist also showed[96] that oxidation of the same hexahydro acid with manganese dioxide and sulphuric acid gave benzene -1:2:3- tri and -1:2:3:4- tetra carboxylic acids (1V) and (V) respectively.

Another of Feist's important contributions was his isolation of the dicarboxylic acid, merochasmanthinic acid methyl ether $C_{16}H_{20}O_8$, from the alkaline potassium permanganate oxidation[98,100] of methylpalmarin. When this acid was distilled with zinc 1:2:5-trimethylnaphthalene (11) was obtained, together with a trace of o-cresol (V1) identified by a colour reaction.

These results are identical to those obtained by similar degradation of columbin,[101] thus implying the presence of a similar 1:2:5- substituted decalin in both palmarin and its derivative merochasmanthinic acid methyl ether. Finally, by careful Kuhn-Roth oxidation and infrared measurements it has been shown that both palmarin and <u>iso</u>columbin contain two C-Me groups.

On the basis of the evidence concerning the functional groups and basic carbon skeleton, which has just been discussed, it is now proposed to deduce an expression for palmarin.

The isolation of 1:5- dimethyl-2-naphthoic acid (V11;R=CO_2H) from the dehydrogenation[96] of hexahydro-chasmanthinic acid clearly demonstrates that the carbonyl group of lactone (B) is attached to position 8* in the basic decalin skeleton of palmarin (viz V111).

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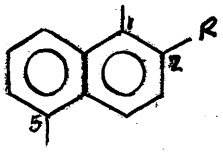
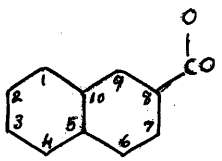
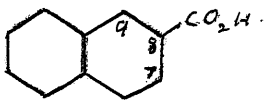
* For convenience in the later parts of the discussion the numbering system used for the basic decalin skeleton is as shown in (V111). This bears no relation to the system used in naphthalene dehydrogenation products (viz. V11).



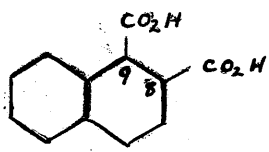
(VII).



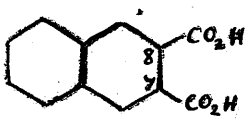
(VIII).



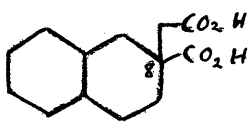
(IX).



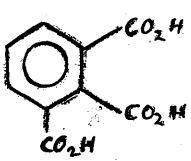
(X).



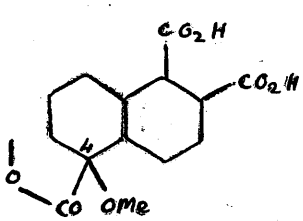
(XI).



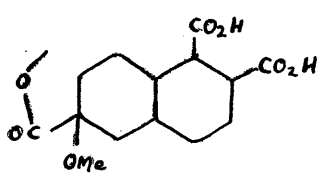
(XII).



(XIII).



(XIV).



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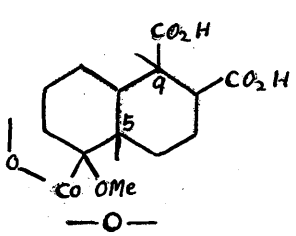


(XVI).

Now under the alkaline conditions, present during potassium permanganate oxidation of methylpalmarin $C_{21}H_{24}O_7$ to give the dicarboxylic acid merochasmanthinic acid methyl ether $C_{16}H_{20}O_8$, it has been readily shown that the lactone (B) is open. Since the carboxylate group derived from lactone (B) must be directly attached to the decalin nucleus as shown in (1X) it would be expected to be stable under the oxidation conditions employed. Consequently the lactone (B) carbonyl in methylpalmarin is presumably the precursor of one of the carboxyl groups in the dicarboxylic acid $C_{16}H_{20}O_8$. This is indirectly confirmed by the zinc dust distillation of the dicarboxylic acid to give 1:2:5 trimethylnaphthalene (V11;R=Me) which shows that the basic decalin skeleton of palmarin has not been destroyed. It is unlikely that the basic decalin nucleus would have remained had the carboxyl group at 8 been oxidized. The partial expression (1X) may therefore be used to describe the dicarboxylic acid merochasmanthinic acid methyl ether ('mero acid') $C_{16}H_{20}O_8$.

When the 'mero acid' is heated <u>in vacuo</u> it sublimes to form an anhydride with infrared absorption (nujol) 1850, 1773 (succinic anhydride and γ- lactone) and 924 cm^{-1}. Furthermore when the dicarboxylic acid is treated with refluxing acetic anhydride or acetylchloride at 100°, another anhydride, with infrared (nujol) bands at 1860, 1785 (succinic anhydride) 1762 (γ-lactone) and 891 cm^{-1}, is formed. The latter anhydride on being heated under nitrogen is slowly converted to the former which must be the thermodynamically more stable, that is the <u>cis</u> anhydride.

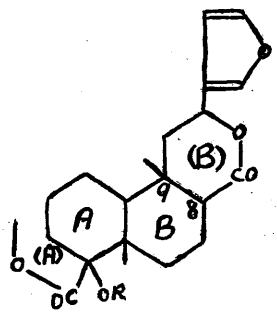
Since the 'mero acid' must be a substituted succinic acid it follows that the other carboxyl group must be placed at positions 9 or 7, that is (X) or (X1). The only other alternative structure

(XII) can be readily dismissed since, amongst other things, it would not be expected to equilibrate under thermal conditions. Later work (p.118) also requires that there is a hydrogen atom at position 8. To differentiate between (X) and (XI) as possible partial formulae for the 'mero acid' it is necessary to consider once more the zinc dust dehydrogenation of the 'mero acid'. In such a reaction carboxyl groups would be expected to be reduced to methyl groups and hence the formation of 1:2:5- trimethylnaphthalene (VII;R=Me) very strongly favours (X) as a partial expression for the 'mero acid'.

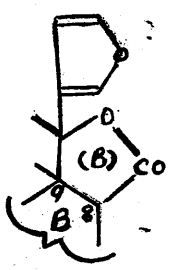
The 'mero acid' is also known to possess, in addition to two carboxyl groups, one inert oxygen atom which must be the cyclic ether of palmarin, one methoxyl group which is derived from the acidic hydroxyl group of palmarin, and finally a lactone which is the lactone (A) system of palmarin. The infrared spectra of various palmarin derivatives (see p. 79) indicate that lactone (A) is γ and this is also indicated by the infrared spectrum of the dimethyl ester of the 'mero acid' which shows bonds at 1775 (γ - lactone) and 1730, 1718 (carbomethoxyl carbonyls) cm^{-1}. From its acidic properties the hydroxyl group in palmarin must be placed α- to a lactone carbonyl which must be the lactone (A) carbonyl (see p.114).

Consideration of the drastic degradation products from the 'mero acid' and hexahydrochasmanthinic acid*, in particular 1:2:5-trimethylnaphthalene (VII;R=Me) by zinc dust distillation of the former and benzene -1:2:3- tricarboxylic acid (XIII) by oxidation of the latter, clearly favours position 4 for the attachment of the

* The lactone (A) system is attached to the basic decalin skeleton and hence information concerning this lactone can be taken from the degradation of any compound containing this basic decalin skeleton.



(XVII).



(XVIII).

methoxyl and carbonyl of the lactone (A) system as shown in (XlV). The alternative possibility (XV) would not be expected to give the above degradation products.

To complete the expression for the 'mero acid' $C_{16}H_{20}O_8$ it only remains to place the unknown oxygen function and two carbon atoms. The latter must be present as methyl groups, one of which must be placed at position 5 to fit in with the degradational evidence. The other methyl group is placed at 9 mainly by analogy with columbin (see p. 21) although position 10 is in no way rigorously excluded chemically. It should be noted however that the data given[100] on the rate of alkali consumption by the dimethyl ester of the 'mero acid' suggests preferential uptake of one mole of alkali which cannot be due to the lactone (A) system. Since it is known that the carboxyl group in the 'mero acid' attached to position 8 is secondary it might be inferred from the above that the other carboxyl group at position 9 is tertiary as would be expected if a methyl group were placed at position 9. From the above conclusion we may write the partial formula (XVl) to define the 'mero acid'.

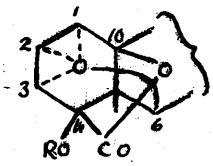
In the formation of the 'mero acid' $C_{16}H_{20}O_8$ from methylpalmarin $C_{21}H_{24}O_7$, five carbon atoms and one oxygen atom are lost. Since it has been previously established that the lactone (B) carbonyl must be attached to position 8, and that the alkyl oxygen of this lactone must be attached to a carbon atom α- to the furan ring, and finally that four of the carbon atoms and one oxygen atom must be present in a mono-substituted furan ring system, palmarin can only be formulated as (XVll;R=H) or (XVlll). Of these the latter is rejected on the basis of later experiments (see p. 113 and p. 122) which clearly demonstrate that a hydrogen atom is attached to the carbon atom α- to the furan ring. Moreover formulation (XVll;R=H) for palmarin is consistent

with infrared data (see Table 111, p. 79) which indicates that lactone (B) is δ . Finally the above conclusions lead to a structure for palmarin containing two C-methyl groups which is in complete accord with the experimental findings.

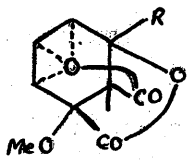
Is should be noted that the only points of difference from columbin (see p. 21) in expression (XV11) for palmarin are the γ--lactone (A) system, the absence of the ethylenic linkage in ring A, the presence of an additional as yet unplaced oxygen function, and of course any stereochemical differences not implicated in the two dimensional representations.

At this stage therefore, apart from confirmation of the above conclusions, it only remains to establish the positions of three carbon-oxygen bonds. These are the points of attachment of the ethereal oxygen function and the alkyl oxygen termination of the γ - lactone system.

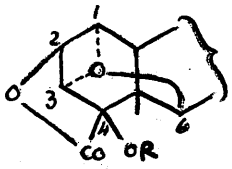
The simplest solution to the first problem, namely that the olefinic double bond in columbin is merely epoxidized in palmarin came early under suspicion and was proved by later events to be untenable. Thus hexahydropalmaric acid was recovered unchanged after standing overnight at room temperature in constant boiling hydriodic acid. Similarly, when the same compound in acetic acid was shaken with a platinum catalyst, no hydrogen was consumed and unchanged starting material recovered. The survival of an ethylene or propylene oxide under these conditions seems unlikely.[103] The stability of the anhydride of the 'mero acid' to boiling acetic anhydride for three hours and acetyl chloride at 100° under pressure certainly suggests that the ether function is very stable[104] and is most likely to be present as a 1:4 or 1:5 bridge system. The



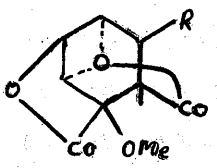
(XVIII).



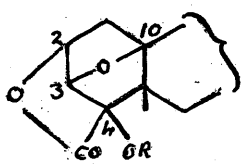
(XXII).



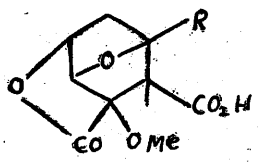
(XIX).



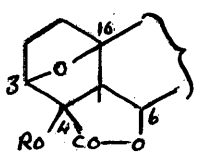
(XXIII).



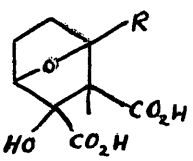
(XX).



(XXIV).



(XXI).

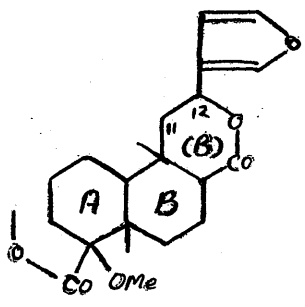


(XXV).

various possible positions for such an ether bridge are strictly limited. Thus in the first place it must be present in the A:B ring system of palmarin since it remains completely intact when methylpalmarin is oxidized to the 'mero acid'. Further the existence of two C-methyl groups in palmarin clearly shows that the ether bridge can only be attached to the carbon atoms of the A:B ring system. Lastly of the various possible free positions in the A:B ring system evidence to be presented on page118 clearly eliminates position 7 as a possibility.

Using the above limitations concerning the attachment of the ether bridge in conjunction with the limited possibilities for a γ - lactone system attached to position 4 it is possible to deduce that only seven possible structures for palmarin are feasible. Thus when the γ -lactone is attached to positions 4 and 10, ether bridges spanning positions 1 and 6, 2 and 6 or 3 and 6 (see XVlll;R=H) must be considered. Alternatively 1:6, 3:6 (see XlX,R=H) or 3:10 ether bridges (see XX;R=H) would be possible if the γ - lactone were attached to positions 2 and 4. Finally with the γ - lactone spanning positions 4 and 6, a 3:10 ether bridge (see XXl;R=H) is the only possibility.

Of the many possible ways of tackling the rather formidable task of deciding which of the structures represented by (XVlll;R=H) (XlX;R=H) (XX;R=H) and (XXl;R=H) describes palmarin one scheme in view of its apparent simplicity and flexibility was particularly attractive. The key step in this scheme was the oxidation of ring B in methyl - palmarin with potassium permanganate under alkaline conditions which were not strong enough to open the γ - lactone (A). This was apparently quite feasible since it was known that very strongly alkaline conditions were required to hydrolyse this lactone. It



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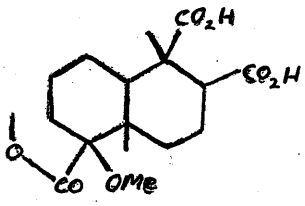
(XXVI).



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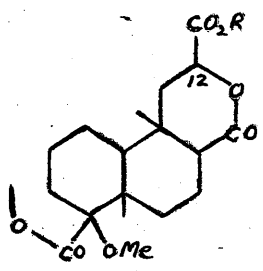
(XXVII).



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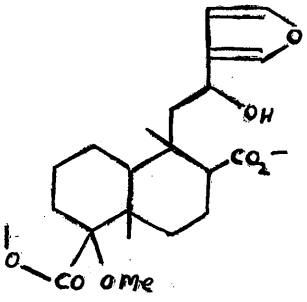
(XXVIII).



—o—

(XXIX).

was hoped in this way, depending on which of the expressions (XVIII;R=Me) (XIX;R=Me) (XX;R=Me) and (XXI;R=Me) represented methylpalmarin, to get a product having a structure described by (XXII) (XXIII) (XXIV) or (XXV) in which R would presumably be carboxyl. In all cases apart from the two products represented by (XXIII) it should be possible to establish the structure of the oxidation product, and hence palmarin, by careful examination of its infrared spectrum together with the number of moles of sodium periodate consumed after reduction with lithium aluminium hydride.

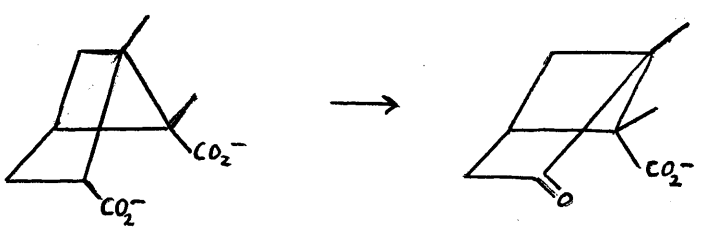
Now the alkaline potassium permanganate oxidation of methylpalmarin (XXVI) had already been studied by Feist,[98,100] his only crystalline product being a 10% yield of the 'mero acid' $C_{16}H_{20}O_8$ (XXVII). It was decided therefore to reinvestigate the remainder of Feist's oxidation product. Thus by methylation and chromatography there was obtained two crystalline methyl esters $C_{18}H_{24}O_8$, and $C_{19}H_{24}O_8$, the former being the dimethyl ester of the 'mero acid' (XXVII) and the latter being the methyl ester of the trisnor acid (XXVIII;R=H).

Since our project requires a much more drastic breakdown of the molecule it would be expected that a preliminary oxidation of the basic A:B ring system of methylpalmarin would facilitate this degradation. This could possibly be achieved by oxidation of the enolisable hydrogen at position 8 (see p.118) using selenium dioxide in boiling acetic acid. A two stage oxidation using selenium dioxide and then alkaline potassium permanganate was therefore carried out several times but despite careful working up, involving methylation and chromatography there were obtained only two crystalline products. These were the dimethyl ester of the 'mero acid' (XXVII) and a compound analysing for $C_{19}H_{24}O_8$, the infrared

spectrum and optical rotation of which were different from those of the isomer (XXVlll;R=Me). It is probable that the two compounds $C_{19}H_{24}O_8$ are isomeric at position 12.

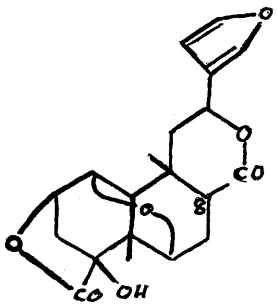
It follows from the nature of the above reaction products that, under Feist's oxidation conditions lactone (B) is only partially open. It was found possible, under carefully controlled conditions, to open lactone (B) without affecting lactone (A), thus affording an alkaline solution of (XXlX). By varying the conditions of potassium permanganate addition to this solution of (XXlX) an optimum yield of 50% 'mero acid' was obtained. A careful investigation of the mother liquors of this reaction revealed two or three crystalline products with very interesting spectral properties. The yields in which these interesting compounds were obtained were too small for their use in structural arguments, consequently further work on them was abandoned.

At this stage it should be noted that the stability of the 'mero acid' to oxidizing agents is not due to its insolubility in the oxidizing medium. Further attempts to achieve a more drastic degradation of methylpalmarin (XXVl) using strongly alkaline solutions and reversing the order of addition afforded as the only crystalline product the 'mero acid' in yields of 3% and 25% respectively.

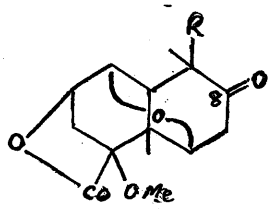


(XXX). (XXXl).

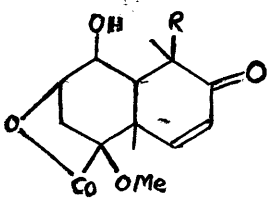
The selective degradation of a secondary carboxylate anion, such as is present in (XXlX) has recently been demonstrated by Buchi et al[105]



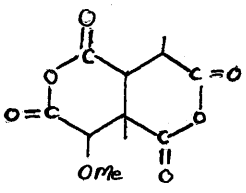
(XXX).



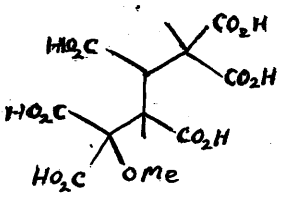
(XXXI).



(XXXII).



(XXXIV).



(XXXIII).

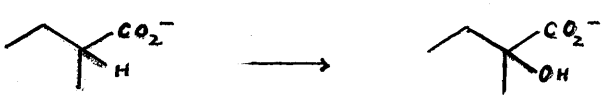
who converted (XXX) to (XXXl). Application of these conditions to the mono-salt (XXlX) furnished a complex reaction product, careful working up of which afforded two crystalline acids. The major product was the 'mero acid' and the infrared spectrum (CCl_4) of the other water soluble acid showed a bond at 1715 cm^{-1} (carboxyl) but no hydroxyl absorption. When the unknown acid was sublimed it was converted into another crystalline product with no hydroxyl absorption and carbonyl absorption (nujol) at 1800 and 1750 cm^{-1} (glutaric anhydride).

In the light of the current probable structure of palmarin (see p.114) it is possible to propose tentatively that the above acid has structure (XXXlll) and its sublimation product is (XXXlV). Thus this acid, since it is clearly not the tris-nor acid $C_{18}H_{22}O_8$ or the mero acid $C_{16}H_{20}O_8$ must surely have been formed by degradation of at least part of the A:B ring system. The relative stability of the 'mero acid' under these conditions surely indicates that if any oxidation of the A:B ring system of methylpalmarin (XXX) were to occur this should take place at position 8 to give the key intermediate of type (XXXl;R=?). In the formation of the latter it is probable that the keto group at position 8 is formed before the side chain degradation is complete since the 'mero acid' appears to be more stable under the reaction conditions. The most probable next stage involves β - elimination under alkaline conditions to give the hydroxy - α : β -unsaturated ketone (XXXll;R=?) in which the γ - lactone should be much easier to open. Hydrolysis and complete oxidation of (XXXll;R=?) should give a product (XXXlll), the chemical and spectroscopic properties of which should be those found for our acid. Moreover on sublimation it should lose 2 moles of carbon dioxide and 2 moles of water to give the di-glutaric anhydride (XXXlV).

In the light of the above reasoning drastic alkaline oxidation

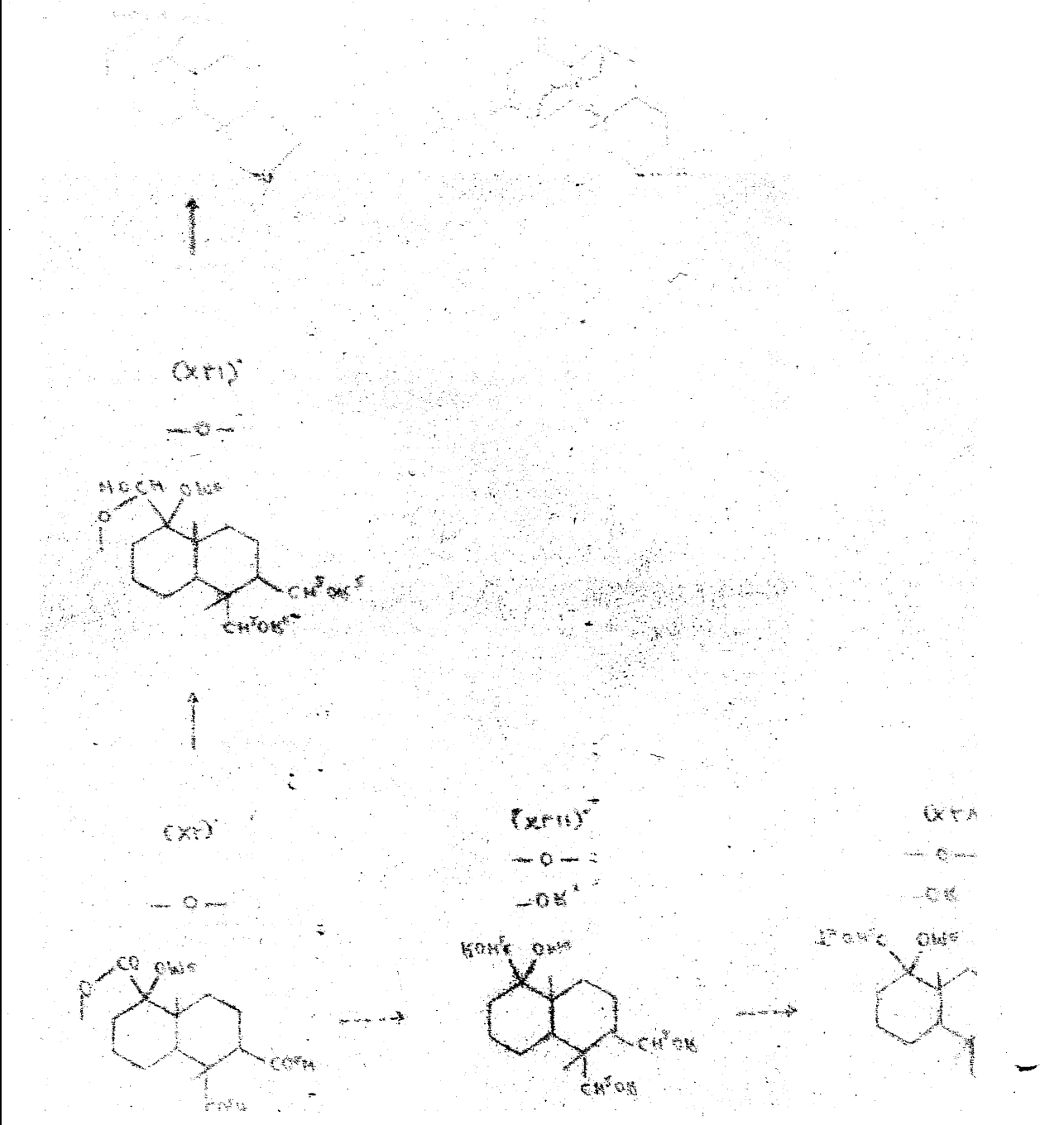
reactions were abandoned and the above experiment repeated in the presence of a large excess of magnesium sulphate solution to act as a neutral buffer. Neutral conditions were achieved but careful working up furnished, as the only crystallisable product, a 10% yield of the 'mero acid'.

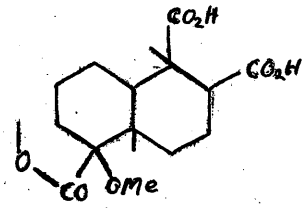
Having failed to get a workable yield of interesting material by oxidation of methylpalmarin several attempts were made to obtain a suitable product from the less complex 'mero acid'. The methods tried were: (a) strongly alkaline permanganate at 40° after Kenyon and Symons[106] who oxidized (XXXV) to (XXXVI) in 80% yield, (b) alkaline potassium permanganate at 100° after Buchi et al,[105] (c) aqueous potassium permanganate containing one equivalent of sulphuric acid added at 100° as it was consumed, and (d) boiling with concentrated nitric acid for $2\frac{1}{2}$ hours. In each case the only crystallisable product which could be obtained was the 'mero acid' the yield varying between 20 and 50%.



(XXXV) (XXXVI).

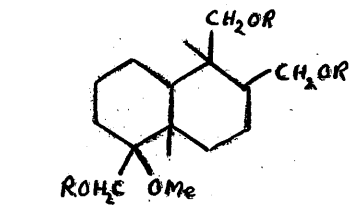
At this stage it was decided that perhaps after all the final decision on the structure of palmarin might be more easily reached using the more selective and easily controlled reactions of modern organic chemistry.





— O —

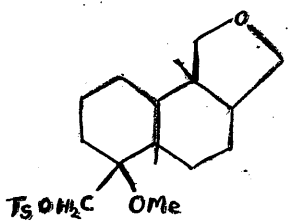
(XL).



$-OR_1$

— O —

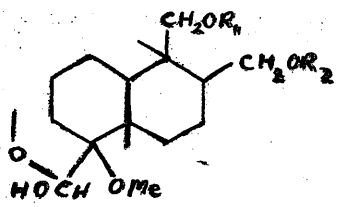
(XLII).



-OR

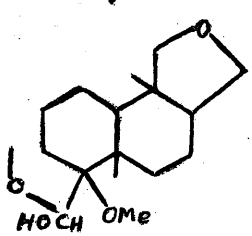
— O —

(XLV).



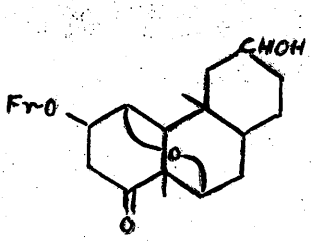
— O —

(XLI).

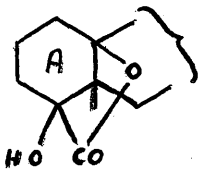


— O —

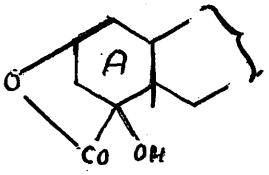
(XLIII).



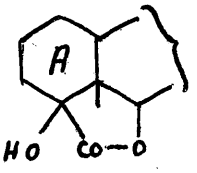
(XLIV).



(XXXVII).



(XXXVIII).



(XXXIX).

Formulations (XXXVII) (XXXVIII) and (XXXIX) represent the three possible positions for the γ - lactone system in palmarin. Examination of these show that it should be possible to confirm or eliminate (XXXVII) relatively easily since only this structure would give a tertiary hydroxyl group after suitable cleavage of the lactone. The 'mero acid' (XL) since its only hydroxyl group is protected by methylation would appear an ideal compound for this purpose. Consequently (XL) was reduced with lithium aluminium hydride in boiling ether to give a crude product, which showed no infrared absorption in the region 1800 to 1650 cm^{-1}, thus indicating complete reduction of the carbonyl and carboxyl functions. The product was carefully worked up and shown to be a mixture from which the only well defined product obtainable was the hemi-acetal $C_{16}H_{26}O_6$ (XLI;R_1=H, R_2=H). The structure of this compound follows from its analysis and subsequent reactions. Such semi-reduction of a lactone under these conditions is not without precedent,[107] and in this case is most probably due to steric hindrance.

Bearing in mind the spectroscopic proof of complete reduction and

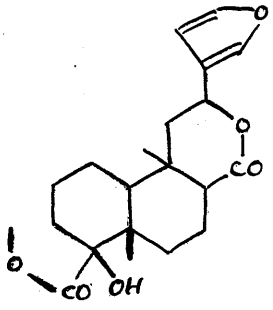
the small yield of the hemi-acetal (XL1;R_1=H, R_2=H) it would not be unreasonable to assume that the crude reduction product contains a significant proportion of the desired tetrol (XL11;R=H, R_1=H). It was therefore decided to carry on and attempt to characterise the crude reduction product in the hope that a suitable crystalline derivative of (XL11;R=H, R_1=H) might be obtained.

Acetylation in the presence of pyridine at room temperature or in the presence of sodium acetate at 140° failed to produce products which could be crystallised. Tosylation, however, resulted in two different compounds depending upon the reaction conditions. By adding an excess of p-toluene sulphonyl chloride in one portion (slow portion-wise addition does not improve the yield) to a well-stirred solution of the crude reduction product in pyridine, a complex mixture was obtained. Careful chromatography however furnished only one well-defined product, the tetrahydrofurano-hemi-acetal $C_{16}H_{24}O_5$. This compound showed no tosylate absorption in the ultraviolet or infrared[108] regions and must be formulated as (XL111). The conversion of 1:4- dihydroxy compounds into tetrahydrofurano- derivatives under tosylation conditions is well known[109,110] and most probably involves the intermediate formation of the monotosylate (XL1;R_1=H, R_2=Ts) or (XL1;R_1=Ts, R_2=H). When the crude reaction product containing (XL1;R_1=H, R_2 =H) and perhaps (XL11;R=H, R_1=H) was added gradually to a well-stirred large excess of p-toluene sulphonyl chloride in pyridine the only crystalline product which could be obtained was a ditosylate $C_{30}H_{38}O_{10}S_2$ (XL1;R_1=Ts, R_2=Ts). In agreement with its formulation the latter showed bands at 3480 cm^{-1}(-OH) and 1368 and 1180 cm^{-1} (tosylate)[108] in the infrared (CCl_4 solution), and its ultraviolet spectrum in ethanal showed a maximum at 226 m.μ. (ε 27,000) clearly indicative of two tosylate residues.

When (XL1;R_1=Ts, R_2=Ts) was treated with excess chromium trioxide in acetic acid 0.98 atom equivalents of 'oxygen' were consumed and the γ - lactone system regenerated. The infrared spectrum (CCl_4) of the product showed bonds at 1777 cm^{-1} (γ -lactone) 1368 and 1180 cm^{-1} (tosylate)[108] but nothing in the hydroxyl region. The consumption of one atom equivalent of 'oxygen', the disappearance of a hydroxyl group and the appearance of a γ -lactone carbonyl can only be explained if the original hydroxyl function was present in a hemi-acetal system. It is worthy of note at this stage that the ditosylate hemi-acetal (XL1;R_1=Ts, R_2=Ts) could not be acetylated under standard conditions, in complete contrast to the hemi-acetal (XL1V) (see p.159). This is surely further evidence of the steric compression existing in the environment of the γ -lactone carbonyl.

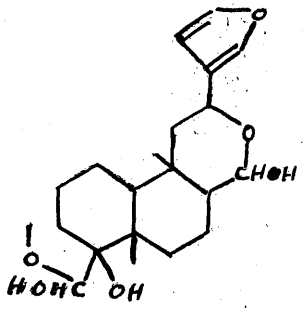
The yields of (XL111) and (XL1;R_1=Ts, R_2=Ts) prepared from the crude reaction mixture containing (XL1;R_1=H, R_2=H) and perhaps (XL11;R=H, R_1=H) appear to represent a reasonable percentage conversion of the hemi-acetal (XL1;R_1=H, R_2=H) present. It would be quite rational to assume therefore that the tetrol (XL11;R=H, R_1=H) if present should be converted into the tetrahydrofurano- compound (XLV;R=H or Ts) or the appropriate fully tosylated compound (XL11; R=Ts, R_1=H or Ts) depending upon the conditions used and whether the alkyl oxygen of the γ -lactone system was secondary or tertiary. No trace, however, of crystalline compounds of such structure could be obtained. It thus appears that, if complete reduction of the γ -lactone is possible, the product and its derivatives do not crystallise as readily as the corresponding derivatives of the semi-reduced γ -lactone.

There was however an obvious method of circumventing the problem



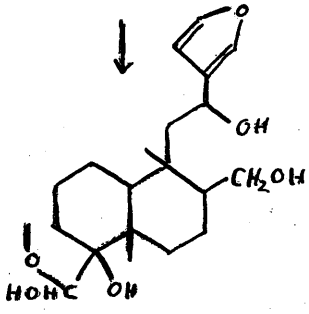
—O—

(XLVI)



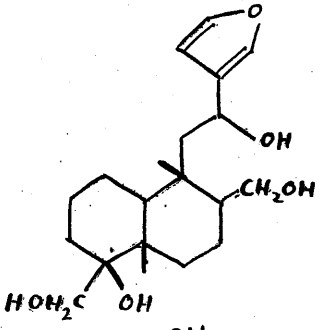
—O—

(XLVIII).



—O—

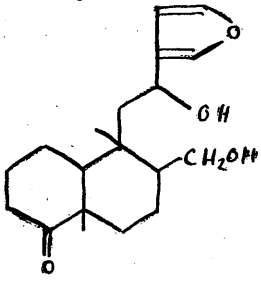
(XLVII).



—OH

—O—

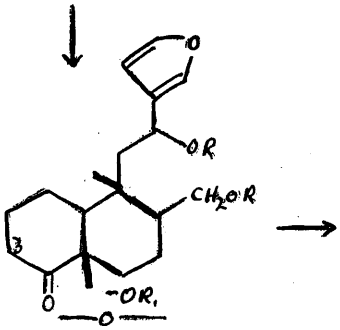
(XLIX).



—O.CHO

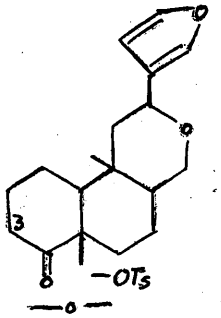
—O—

(LI)



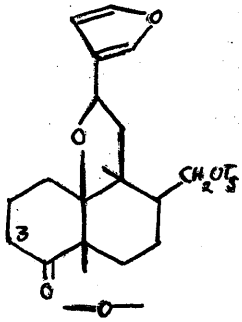
—O—

(L)



—O—

(LII)



—O—

(LIII).

of incomplete reduction of the γ-lactone and obtaining a useful product. Thus the α-hydroxy-γ-lactone system of palmarin clearly presents a potential carbonyl which should act as a useful probe for the exploration of ring A. On reduction with excess lithium aluminium hydride in boiling tetrahydrofuran palmarin (XLVI) was converted into a mixture of products, presumably (XLVII) (XLVIII) and the completely reduced system (XLIX) which showed no carbonyl absorption in the infrared. The total reduction product was carefully acetylated and chromatographed but no crystalline products could be obtained. When another sample of the crude reduction product in methanol was treated with excess aqueous sodium metaperiodate one mole of the latter was consumed and a non crystalline ketonic product formed. As with its precursor attempts to form a crystalline derivative of the crude oxidation mixture, by acetylation followed by chromatography, were not successful. Tosylation, on the other hand, did produce a crystalline derivative which is more conveniently discussed later.

When the crude oxidation product was subjected to a very mild basic hydrolysis there was obtained, in 45% overall yield from palmarin, the crystalline triol-one $C_{19}H_{26}O_6$ (L; R=H, R_1=H). Distillation of the acidified hydrolysis solution afforded, in 20% yield, a volatile acid which was shown to be formic acid by infrared analysis of its sodium salt, indicating a relatively large proportion of (LI) in the oxidation mixture. In agreement with its formulation the infrared spectrum (nujol) of the triol-one (L;R=H, R_1=H) showed bands at 3380 cm^{-1} (hydroxyl) 3110 and 1505 $cm.^{-1}$ (furan) and 1710 cm^{-1} (cyclohexanone). A quantitative analysis showed the presence of one carbonyl function.

It is convenient at this stage to mention an interesting reaction

involving the triol-one (L;R=H, R_1=H). When this compound is heated at 100° in 5% aqueous sodium hydroxide there develops immediately a strong violet colour which remains on cooling. A similar reaction takes place, although more slowly, in 0.5% aqueous alkali. A series of these reactions were carried out for different times at 100° and the ultraviolet spectra of the product measured in alkaline and acidic ethanol (for all the results see Experimental p.156). The results show that a peak at 290 m.μ. in alkali, which shifts to 270 m.μ. in acidic ethanol, develops very rapidly reaching a maximum (ε =15,600) after about 2½ minutes and then slowly falls off. The ratio of the absorption intensity in alkaline solution to that in acidic solution is about 1·4. These values are very similar to those characteristic of the chromophore of a substituted enolisable β-diketone. Thus dimedone was shown to exhibit a maximum in alkaline solution at 282 m.μ. (ε =28,300) and at 255 m.μ. (ε =13,500) in acidic solution. Obviously the triol-one (L;R=H, R_1=H) undergoes some rearrangement to give some system analagous to an enolisable β-diketone. The exact nature of this rearrangement is somewhat difficult to discern.

In theory triol-one (L;R=H, R_1=H) should have been an ideal compound to demonstrate whether the potential hydroxyl group of the γ-lactone was secondary or tertiary. In practice this compound was found to be labile under acetylation conditions and all attempts to prepare a crystalline acetate were unsuccessful.

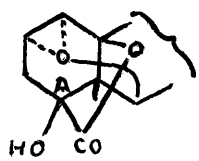
Tosylation in pyridine solution was an obvious alternative and although a wide variety of conditions were employed in no case could the desired di- or tri- tosylate (L; R=Ts, R_1=Ts or H) be obtained. In every case, however, there was obtained, in yields ranging from 5 to 40%, a monotosylate $C_{26}H_{30}O_7S$ which showed bands in the infrared

(nujol) at 1705 $cm.^{-1}$ (cyclohexanone) 1595, 1360, 1180 $cm.^{-1}$ (tosylate)[108] 1505 $cm.^{-1}$ (furan), and no absorption in the hydroxyl region. Quantitative measurements in the infrared and ultraviolet regions demonstrated the presence of one carbonyl function and one tosylate residue [λ max. 223 m.μ. (ε 14,800 ethanol)] respectively. The formation of this monotosylate clearly involves cyclisation of a hydroxy ditosylate, in which the hydroxyl group and one of the tosylate residues must be in a 1:4- or 1:5-relationship.[109,110] In view of the specific requirements of this reaction it is not surprising that the yield of monotosylate varied with the reaction conditions. On the basis of the above facts the monotosylate may be described in two ways (Lll) or (Llll). It should be possible to distinguish these two possibilities by showing whether the tosylate residue is primary or secondary. Unfortunately experimental results at this stage were not conclusive.

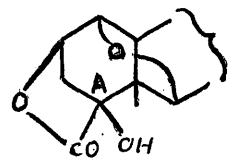
It is worthy of note that the tosylate obtained from the crude sodium metaperiodate oxidation mixture, mentioned previously on p.104, was identical in every respect (m.p., mixed m.p. and infrared spectrum) with the above compound (Lll or Llll). Moreover the yield obtained was exactly that expected from the potential quantity of crystalline triol-one (L;R=H, R_1=H) known to be present in the crude periodate oxidation mixture. It follows from this that the formate group in (L;R=H, R_1=Fr) and the hydroxyl function in (L;R=H, R_1=H) react analagously under the tosylation conditions employed.

If it had been possible to obtain a crystalline poly-acetate or -tosylate from (L;R=H, R_1=H) or establish the nature of the tosylate residue in the monotosylate (Lll or Llll) then it will be apparent from the discussion which follows that our problem would have been essentially solved. Unfortunately these experiments could not be

carried out successfully, nevertheless another very significant observation was made. Thus it was conclusively shown that the tetrahydro derivatives of both (L;R=H, R_1=H) and the tosylate (Lll or Llll) consumed two moles of bromine under standard conditions, clearly demonstrating the presence of two enolisable hydrogen atoms at position 3 α- to their carbonyl functions. If we now consider the various possible structures for palmarin as represented on page 91 it becomes apparent that the ether bridge can only be 1:6 or 2:6. Furthermore it follows from this that partial structure (XXl) in which the γ-lactone spans positions 4 and 6 must be excluded. On the basis of these conclusions the only possible structures for palmarin are represented in (LlV) and (LV). By examination of these partial formulations it becomes apparent that the structure of palmarin would be essentially solved if the secondary or tertiary nature of the alkyl oxygen of the γ-lactone could be established.

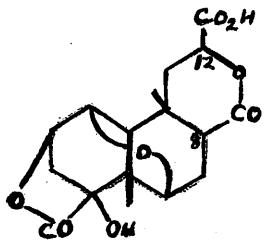


(LIV).

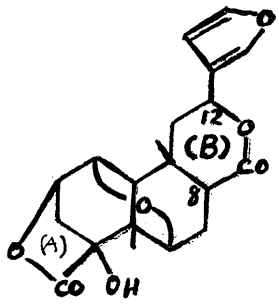


(LV).

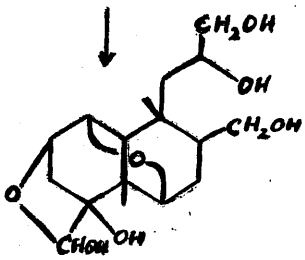
The ambiguities, which arose when it was attempted to establish the nature of the alkyl oxygen of the γ-lactone system by acetylation and tosylation of the triol-one (L;R=H, R_1=H), doubtlessly arose from the presence of three reactive hydroxyl groups in the molecule. Consequently in planning the next series of experiments



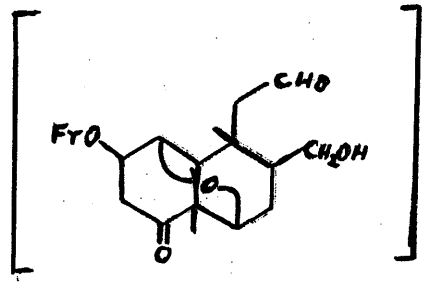
(LVII).



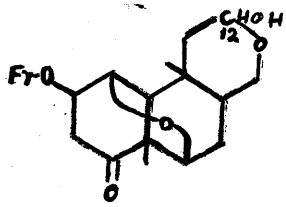
(LVI).



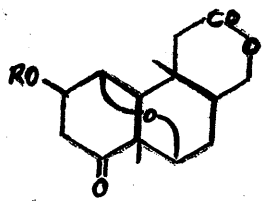
(LVIII).



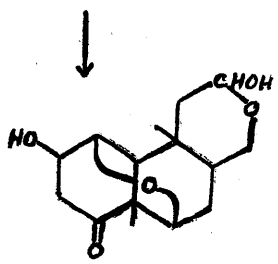
(LXIIA)



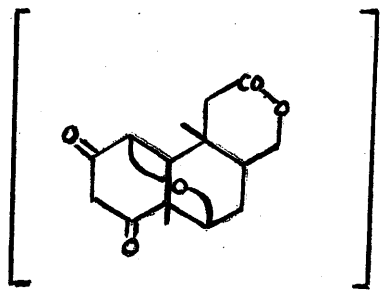
(LXII).



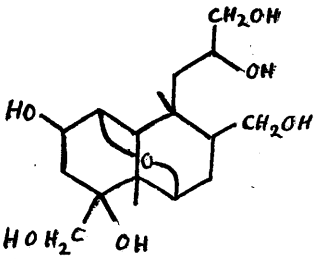
(LXIII).



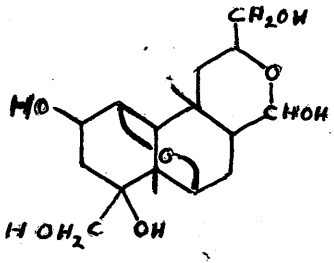
(LXIV).



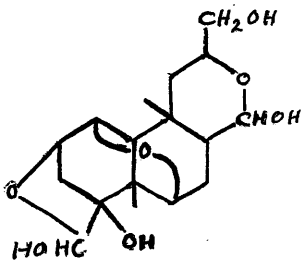
(LXV).


CH2OH
OH
HO
CH2OH
O
HOH2C
OH

(LIX).


CH2OH
O
CHOH
HO
O
HOH2C
OH

(LX).


CH2OH
O
CHOH
O
O
HOHC
OH

(LXI).

particular care was taken to ensure that, under all forseeable conditions, each stage should provide meaningful and unambiguous information. This scheme, as devised, was intended to establish finally (a) the presence, environment and size of the two lactone systems (b) the relationship of one of the latter to the α - or β -substituted furan ring system and (c) the positions of attachment of the oxide bridge. In fact most of the above aims were accomplished and where they were not it was only through lack of sufficient materials. For convenience and clarity the correct positions of the ether bridge and the γ-lactone, as deduduced in the following series of experiments, will be used in all subsequent formulations.

Ozonolysis of palmarin (LV1) in ethyl acetate at 0° furnished in good yield the crystalline tris-nor acid $C_{17}H_{20}O_8$ (LV11) which was characterised as its methyl ester. When this acid was slowly added to an excess of a boiling solution of lithium aluminium hydride in tetrahydrofuran, there was obtained a yield of the crystalline water soluble tetrol hemi-acetal $C_{17}H_{28}O_7$ (LV111).* In agreement with its formulation, the infrared spectrum (nujol) of (LV111) showed strong absorption at 3340 $cm.^{-1}$ (hydroxyl) but nothing in the region 1800 to 1650 cm^{-1}.

The hemi-acetal (LV111) reacted smoothly with aqueous sodium metaperiodate, 1.98 moles being consumed, to give a crystalline product $C_{16}H_{22}O_6$. This product, from its subsequent reaction with chromium trioxide, is best formulated as the keto formate hemi-acetal (LX11), which presumably arises by subsequent cyclisation of the

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* Analytical data does not completely eliminate structures (LIX) (LX) and (LX1) for the hemi-acetal. These alternatives are readily dismissed however by the hemi-acetals subsequent reaction with two moles of sodium metaperiodate with the formation of a nor-compound which contains a formate residue.

aldehydro keto formate (LX11A). In accordance with structure (LX11) the keto formate hemi-acetal showed bands in the infrared (CCl_4) at 3622 $cm.^{-1}$ (hydroxyl), 1725 $cm.^{-1}$ (cyclohexanone) and 1733 $cm.^{-1}$ (formate).

As has already been discussed (see p.81) the consumption of one mole of sodium metaperiodate by the lithium aluminium hydride reduction product of palmarin conclusively establishes the presence of an α-hydroxy-lactone in palmarin. In the same way the consumption of two moles of sodium metaperiodate by the lithium aluminium hydride reduction product (LV111) from the tris-nor acid (LV11) clearly demonstrates the presence of two potential α-glycol systems in the latter. Since the only difference between palmarin $C_{20}H_{22}O_7$ (LV1) and the tris-nor acid $C_{17}H_{20}O_8$ (LV11) is that the furan ring in the former is replaced by a carboxyl group in the latter, it follows that the difference in the sodium periodate consumption of their reduction products must be due to a potential hydroxyl group α- to the carboxyl group in the tris-nor acid. This potential hydroxyl group α- to the carboxyl group in the tris-nor acid and hence α- to the furan in palmarin must be the alkyl oxygen bond of lactone (B). This completely confirms our previous conclusions (see p.82), regarding this function, which were based on its ready hydrogenolysis.

When the formate hemi-acetal (LX11) was treated with excess chromium trioxide in acetic acid, 1.2 atom equivalents of 'oxygen' were consumed and the neutral formate lactone (LX111;R=Fr) was formed. The infrared spectrum (CCl_4) of the latter showed peaks at 1727 $cm.^{-1}$ (cyclohexanone) 1734 $cm.^{-1}$ (formate) and 1745 $cm.^{-1}$ (δ-lactone) and no hydroxyl absorption. The amount of oxidant consumed and the spectral changes can only be explained by the

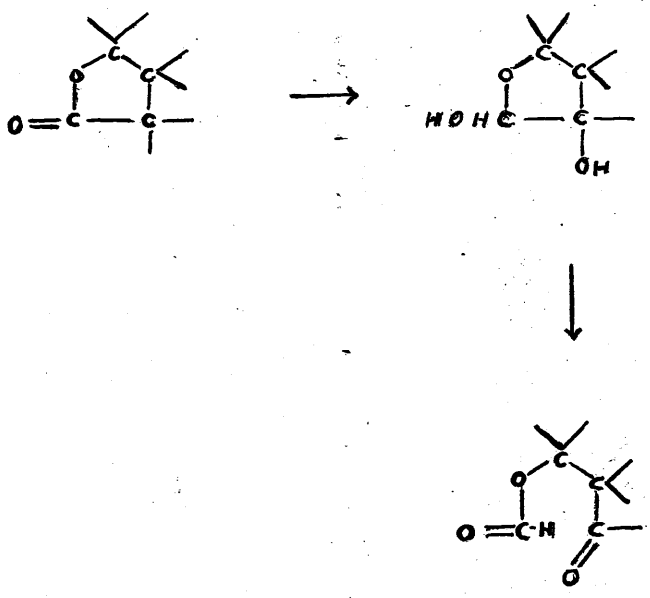


Figure 1.

conversion of a six-membered hemi-acetal system into the corresponding δ-lactone. Moreover it follows from the conversion of (LXll) into (LXlll;R=Fr) that there must be a hydrogen atom attached to C_{12} in the formate hemi-acetal (LXll) and consequently the same situation must also exist in palmarin (LVl). This fact forms an important part of an argument (see p. 89) which conclusively establishes, in agreement with the infrared data, that lactone (B) must be six-membered.

When the formate lactone (LXlll;R=Fr) is treated with mild base, hydrolysis occurs and the keto hydroxy lactone (LXlll;R=H) is formed. This shows bands in the infrared (CCl_4) at 3633 $cm.^{-1}$ (hydroxyl), 1727 $cm.^{-1}$ (cyclohexanone) and 1749 $cm.^{-1}$ (δ-lactone). In agreement with its formulation (LXlll;R=H) was readily acetylated under mild conditions to give the corresponding acetate (LXlll;R=Ac) which showed bands in the infrared (CCl_4) at 1730 $cm.^{-1}$ (cyclohexanone) and 1757 $cm.^{-1}$ (δ-lactone and acetate).

The importance of the formate residue, which first appears in the product (LXll) obtained by the sodium periodate oxidation of the α-hydroxy hemi-acetal, must now be considered. Figure 1 shows the semi-reduction of an α-hydroxy lactone followed by sodium periodate oxidation of the derived hemi-acetal. From this diagram it is obvious that the formate labels the alkyl oxygen atom of the γ-lactone system. Now the presence of a formate residue in (LXll) is conclusively demonstrated by analysis and infrared. Moreover there can be no doubt that the formate residue present in (LXll) and (LXlll;R=Fr) must have been derived from the α-hydroxy-γ-lactone system present in palmarin as depicted in Figure 1. Consequently this formate residue labels the elusive alkyl-oxygen of the γ-lactone in palmarin. That this formate is secondary

and not tertiary has been proved in two ways. Firstly, as has been described above, the parent hydroxy compound (LXIII;R=H) can be smoothly acetylated under mild conditions. Secondly whereas the formate hemi-acetal (LXII) consumed 1.2 equivalents of chromium trioxide, 2.5 equivalents of chromium trioxide were consumed by corresponding hydroxy-hemi-acetal (LXIV) thus indicating that the hydroxyl group arising from hydrolysis of the formate was indeed secondary. It follows from this fact that the alkyl-oxygen in the γ-lactone in palmarin must be secondary and hence, as reasoned on page 107 this establishes the structure of palmarin as (LVI).

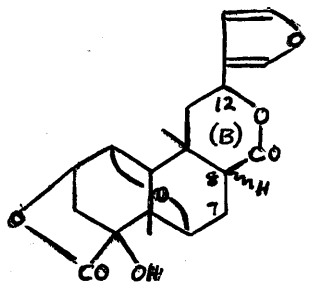
It should be noted if the product (LXV) could be isolated and shown to be an enolisable β-diketone then this would be a very elegant chemical proof for the size of the γ-lactone system and also for the position of the ether bridge. Unfortunately this reaction could only be carried out on a very small scale and hence the crude product could only be examined spectroscopically. The ultraviolet spectrum of the crude product in dilute ethanolic sodium hydroxide slowly developed a peak at 280 m.μ. ($\varepsilon \sim 2700$) which changed to 267 m.μ. ($\varepsilon \sim 2700$) on prolonged standing. On acidification the intensity of both of these maxima fell by at least 25% but owing to background interferences no definite peak could be observed. Although these results cannot be taken as conclusive such results in as far as they go would not be unexpected for a β-diketone system such as (LXV).

Before closing this section it must be pointed out how the results of the above degradation sequence clearly establish, as has been previously assumed, that the acidic hydroxyl group in palmarin (LVI) must be attached α-to the γ-lactone system (A). The four possible structures for the crystalline reduction product $C_{17}H_{28}O_7$,

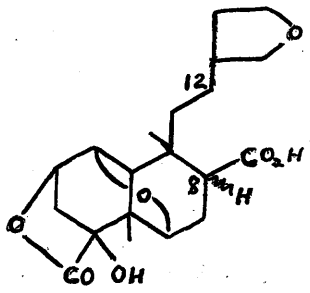
(IXXI).

(IXXI).

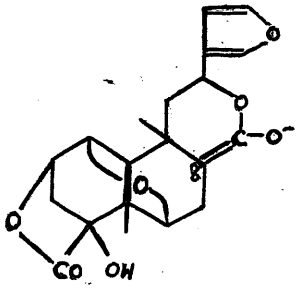
(IXXII).



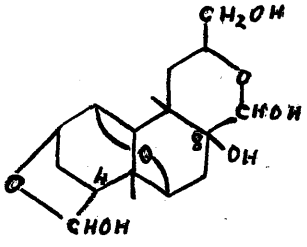
(LXX).



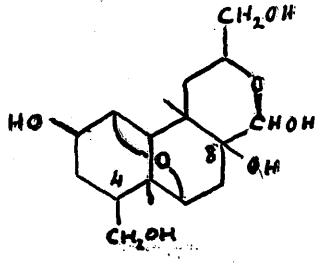
(LXXI).



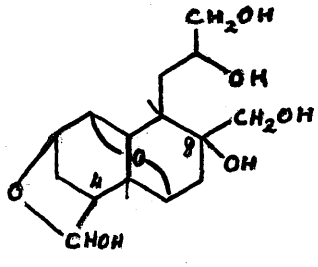
(LXXII).



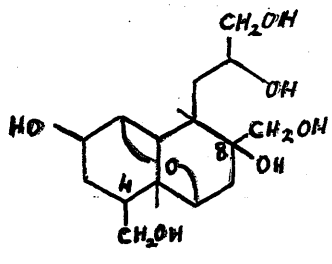
(LXVI).



(LXVII).



(LXVIII).



(LXIX).

from the tris-nor acid in which it has been assumed that the acidic hydroxyl group in the latter and hence palmarin is at position 8 are shown in (LXVl) (LXVll) (LXVlll) and LXlX). The product $C_{17}H_{28}O_7$ obtained in practice consumed two moles of sodium metaperiodate and gave a compound $C_{16}H_{22}O_6$ containing a secondary formate ester. None of the alternative formulations (LXVl) (LXVll) (LXVlll) and (LXlX) would have these properties thus clearly demonstrating that the acidic hydroxyl group in palmarin (LVl) is correctly placed at position 4.

Having proposed a definite structure for palmarin, its relationship with chasmanthin, jateorin and isojateorin must be discussed. Firstly the nature of the chasmanthin - palmarin isomerisation will be considered.

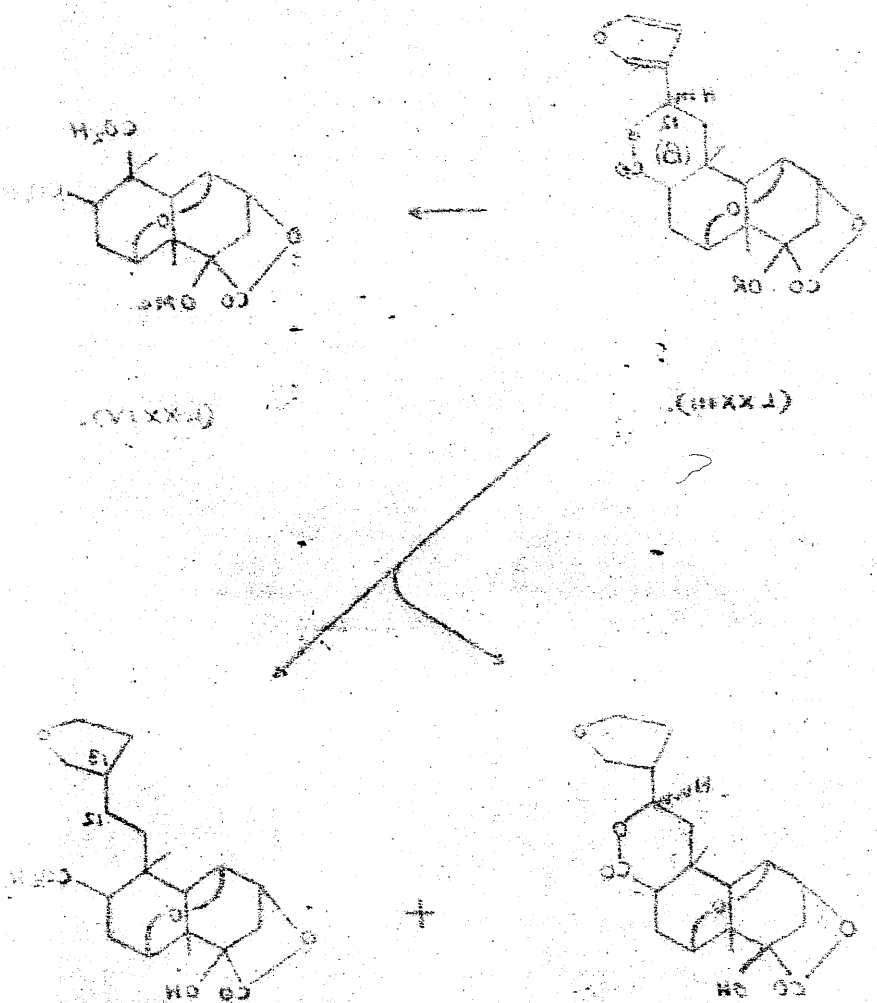
Chasmanthin (LXX) is converted into palmarin (LXX) by treatment with 2% aqueous ethanolic sodium hydroxide for five minutes at 95°. A priori there are two possible epimerisable centres; these are at carbon atoms 8 and 12. It should be noted that equilibration at position 12 could only take place if lactone (B) were open and must involve an oxidation - reduction mechanism.[111] Although the possibility of the latter happening is rather remote under such mild conditions it must be considered.

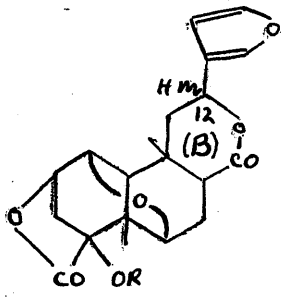
On hydrogenation palmarin (LXX) and chasmanthin (LXX) form different hexahydro-acids, both of which may be represented as (LXXl). The formation of these acids involves firstly hydrogenolysis of the $C_{(12)}$ oxygen bond followed by hydrogenation of the furan ring. In this process therefore the asymmetry at $C_{(12)}$ is lost since, in addition to the C-H bond already proved present (p.113) another such bond is formed. From the non identity of the hexahydro-acids (LXXl) it follows that chasmanthin and palmarin must also differ at some other position most probably position 8. It should be noted that this

argument does not exclude the possibility of the two compounds differing also at position 12 although it is highly unlikely that they should differ at two positions.

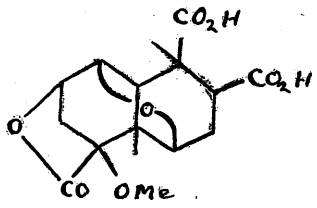
That chasmanthin and palmarin do indeed differ in stereochemistry at position 8 is strongly suggested by the difference in reactivity to base of chasmanthin (LXX) and hexahydrochasmanthinic acid (LXX1). Thus although the former is readily isomerised by base the latter is completely stable[94] under much stronger alkaline conditions. Now if we compare chasmanthin (LXX) and hexahydrochasmanthinic acid (LXX1) it is obvious that, apart from position 12 which has been previously eliminated, the only position in which each would be expected to react differently to base is position 8. This difference in reactivity is readily explained since it is to be expected[112] that a hydrogen atom α- to the carbonyl group of a lactone (lactone and ester carbonyl functions are completely analogous) would enolise much more easily under alkaline conditions than the hydrogen atom α- to the corresponding carboxylate anion. Consequently the ready isomerisation of chasmanthin can be explained by formation of the enolate (LXX11) which is then protonated to give the thermodynamically more stable keto form of (LXX) which must be palmarin. This isomerisation must take place when lactone (B) is closed otherwise position 8 in chasmanthin becomes essentially identical with position 8 in hexahydrochasmanthinic acid which is completely stable[94] under much more drastic alkaline conditions.

Previously it was stated (see p.92.) that it was highly unlikely that the oxide bridge in palmarin could be attached to position 7. This follows from the stability of the oxide bridge during the chasmanthin-palmarin change (i.e. conditions during which it has been concluded the hydrogen atom at position 8 must be enolised). Thus

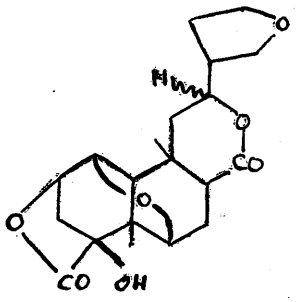




(LXXIII).

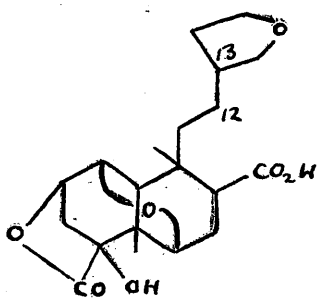


(LXXIV).



(LXXVI).

+



(LXXV).

TABLE IV

Series	Starting Material $[\alpha]_D$	Hexahydro acid $[\alpha]_D$	Hexahydro acid methyl ester $[\alpha]_D$
J*	Chasmanthin B +25°		+ 45°
P+	Chasmanthin A +18°	+ 27°	
P	Palmarin +12°	+ 29°	+ 42°
J	'Methyl-chasmanthin +47°	+ 56°	
P	Methyl-palmarin +39°	+ 58°	

TABLE V

Series	Starting Material $[\alpha]_D$	Hexahydro acid $[\alpha]_D$	Hydrolysis product from Hexahydro acid $[\alpha]_D$	Methyl ester from hydrolysis product $[\alpha]_D$
J	Acetylchasmanthin 11 + 29°	+ 39°		+ 46°
P	Acetylpalmarin +12°	+ 37°	+ 46°	+ 42°

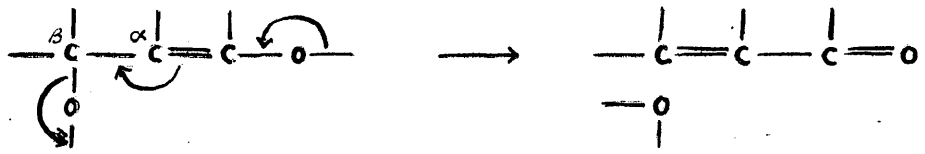
TABLE VI

Series	Starting Material $[\alpha]_D$	Total acidic hydrogenation product $[\alpha]_D$	Recrystallised Hexahydro acid $[\alpha]_D$	Crude neutral hydrogenation product $[\alpha]_D$	Recrystallised tetrahydro compound $[\alpha]_D$
J	isojateorin +29°	+ 36.5°	+ 41°	+ 37°	+ 39°
J	Palmarin +12°	+ 35°	+ 41°	+ 8°	+ 16°

*J = isojateorin series

+P = palmarin series

it is well known[113] that electronegative functions (e.g. the oxygen function here) in the β - position to an enolisable hydrogen atom, undergo spontaneous elimination under conditions which bring about enolisation as shown :



That the difference between palmarin and isojateorin is one of stereochemistry at one asymmetric centre was established in the following way. isoJateorin (LXXlll;R=H) was converted with dimethyl suphate and alkali to a methyl ether $C_{21}H_{24}O_7$ (LXXlll;R=Me) isomeric with methylpalmarin, which on oxidation with alkaline potassium permanganate gave merochasmanthinic acid methyl ether (LXXlV). This acid was identical with that obtained by a similar oxidation of methylpalmarin (LXXlll;R=Me). The identity was confirmed by a careful comparison of the methyl esters.

At this stage we turn now to reconsider some of the earlier work of Wessely, much of which could be rationalised by assuming his 'chasmanthin' to be a mixture of chasmanthin and jateorin. On this basis Wessely's complicated hydrogenation results[90,94] have been reanalysed and are shown on Table lV and V. It would appear from these results that, after catalytic hydrogenation over palladium in acetic acid, derivatives of palmarin and isojateorin become identical.

To confirm this observation palmarin (LXXlll;R=H) and isojateorin (LXXlll;R=H) were carefully hydrogenated under identical conditions. The results are shown in Table Vl. Firstly it should be noted that like Wessely there was obtained in both cases a high yield of the same hexahydro acid $C_{20}H_{28}O_7$ (LXXV). Infrared spectra, optical

rotation and mixed melting point were used to confirm this. Further careful examination of each hydrogenation product revealed in both cases, in addition to the major product, a crystalline neutral tetrahydro compound, $C_{20}H_{26}O_7$ (LXXVI). These compounds are different and were not obtained by Wessely.

The above observations are only to be expected if the difference between palmarin and isojateorin resides in the stereochemistry at carbon 12. As discussed previously (p.117) the above differences must disappear* on hydrogenolytic cleavage of lactone (B). Since during hydrogenation a new asymmetric centre is created at position 13 it might be expected that hydrogenation of palmarin and isojateorin would give different proportions of the $C_{(13)}$ isomerides. This was in fact not observed and can be readily explained since hydrogenolysis must take place before reduction of the furan ring. After this initial reaction palmarin and isojateorin become the same with the result that identical hexahydro acids (LXXV) are formed. The difference in stereochemistry of the starting material however does show itself in the ratio of acidic to neutral products. Thus tetrahydroisojateorin (LXXVI) is formed in about twice the yield of tetrahydropalmarin (LXXVI) thus indicating that the stereochemical environment of the bond about to be hydrogenolysed is more favourable for this reaction in the case of palmarin.

Finally in view of the above relationship of palmarin and isojateorin it must be concluded that jateorin and isojateorin are related in the same that chasmanthin and palmarin are related.

Before concluding this discussion of the chemistry of chasmanthin,

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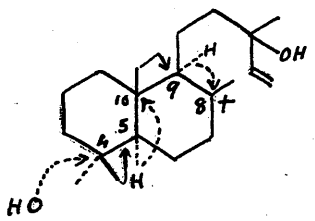
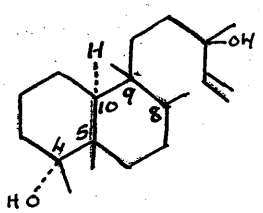
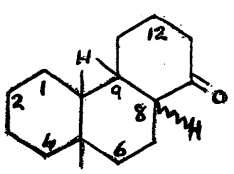
* The fact that palmarin and isojateorin form identical hexahydro acids is the second piece of evidence that there must be a hydrogen attached to $C_{(12)}$ in palmarin (p.89.)



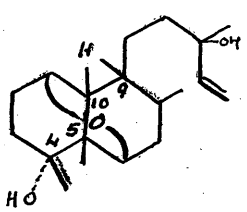
(LXXVII).



(LXXVIII).



(LXXX).

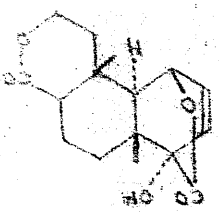


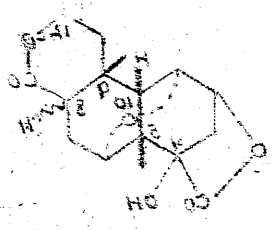
(LXXIX).

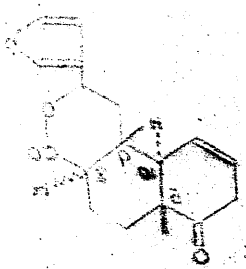
palmarin and jateorin, some consideration must be given to what, if any, are the stereochemical implications in the evidence so far presented. The first and only definite conclusion which can be drawn on this subject is that the A:B ring system must be cis fused. This follows since geometrical factors exclude the existence of a 1:6 oxide bridge in a trans fused decalin system.

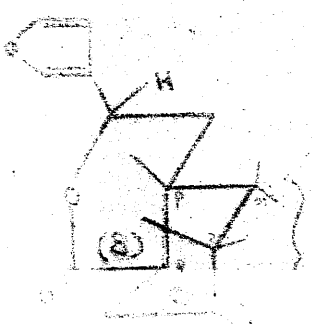
Any further proposals require certain speculative assumptions, which deal in particular with the possible biogenesis of this group of compounds. Thus a feasible biogenetic scheme in which the carbon skeleton of the normal biogenetic precursor of bicyclic diterpenoids (see p. 67) (LXXVll) may be transformed into the nonisoprenoid carbon skeleton with a hydroxyl group at position 4,i.e. (LXXVlll), as found in this group has been proposed[101] and is shown opposite. In order that this change might proceed in a fully concerted manner it should take place before the creation of the 1:6 ether bridge, the formation of which must involve inversion of configuration at position 5 or 10. Since inversion of configuration at a tertiary centre is easier than at a quaternary position it is probable that in the formation of the ether bridge inversion at position 10 takes place. If the above hypothetical scheme were in fact true then its geometrical requirements are such that the configuration at positions 4, 5 9 and 10 must be as shown in (LXXlX). If the molecules under consideration were as simple as the keto- perhydrophenanthrene* depicted in (LXXX) then the decision as to which configuration at position 8 would give the more stable molecule would be relatively simple. Thus of the two possible isomers represented by (LXXX) that in which the hydrogen atom at position 8 is trans to the methyl group at 9 would be considered to be the more thermodynamically stable.

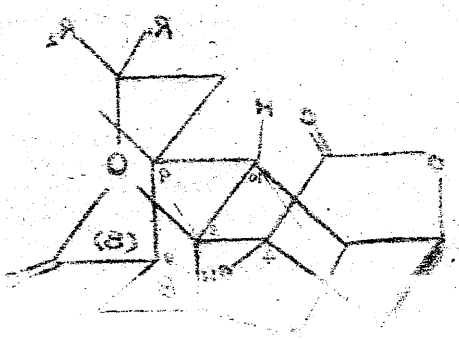
* Since the conformational requirements of a δ-lactone system are essentially analogous to those of a cyclohexanone[114] then (LXXX) may be considered the simplest possible analogue for this system.

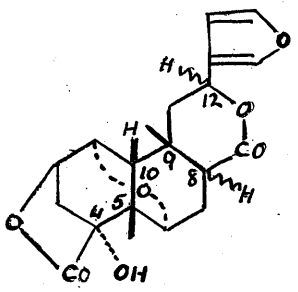




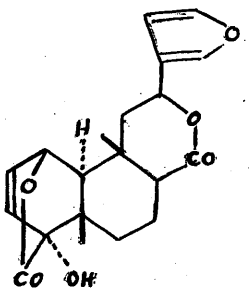




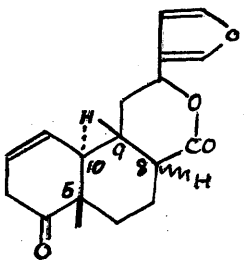




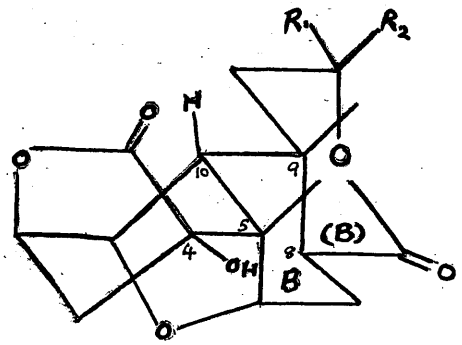
(LXXXI).



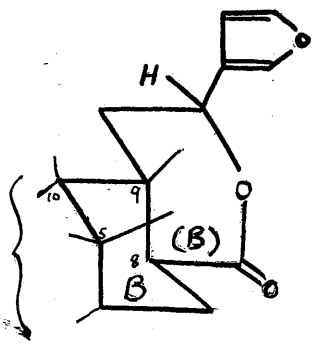
(LXXXII).



(LXXXIII)



(LXXXIV).



(LXXXV).

The extension of this conclusion to the configuration at position 8 of chasmanthin and palmarin does not follow automatically, since the modification of (LXXX) to include the hydroxy- γ-lactone system, ether bridge and furanyl side chain could very easily completely reverse the deductions based on the simple molecule (LXXX)

In spite of this however there is some evidence, again based on biogenetic assumptions, that the hydrogen atom at position 8 in the more stable isomers palmarin (LXXXl) isojateorin (LXXXl) and isocolumbin (LXXXll) is indeed trans to the methyl group at position 9. This depends on the assumption that the first stage in biogenetic scheme already mentioned applies to columbin, the stereochemistry of which, at positions 4, 5, 9 and 10, must be as defined in (LXXXll). Conclusive evidence that the columbin-isocolumbin change, like the chasmanthin - palmarin and jateorin - isojateorin changes already discussed, also involves position 8 will be given in Sect.IV of this thesis. Now if we consider the relatively simpler structures of decarboxy- and decarboxyiso-columbin (LXXXlll) it might be considered not unlikely that the more stable decarboxyisocolumbin (LXXXlll) has its hydrogen atom at position 8 trans to the methyl group at 9. Since decarboxyisocolumbin is obtained by direct decarboxylation of isocolumbin it follows that isocolumbin (LXXXll) must have the same configuration at position 8. It would seem not unreasonable to assume that configuration change at position 8, in columbin, chasmanthin and jateorin, is the same since the rotation changes observed on the isomerisation of columbin and a 3:1 mixture of chasmanthin and jateorin are about $+20^{\circ}$ and $+17^{\circ}$ respectively. The above conclusion is particularly interesting since it seems to indicate that, despite the major structural differences in chasmanthin (LXXXl) and columbin (LXXXll) and the difference in configuration which occurs in chasmanthin and jateorin,

a trans ring fusion of the lactone (B) system is always favoured thermodynamically.

Differences in the infrared absorption ($CHCl_3$) of the lactone (B) carbonyl of palmarin and isojateorin can be tentatively used to assign the configuration of the furanyl system attached to these molecules at position 12. These measurements seem to indicate that there is present in isojateorin (LXXXI) some form of interaction involving the lactone (B) carbonyl which is not apparent in palmarin (LXXXI). Geometrically such an interaction is only possible if the lactone (B) system exists in the chain conformation with the β-furanyl system in an axial position as in (LXXXIV;R_1=H, R_2= furanyl). A priori the lactone ring in the boat conformation with the furanyl group in a quasi- equatorial position (viz. LXXXV) might have been considered more probable.

On the basis of the above rather speculative considerations (LXXXIV; R_1=Furanyl, R_2=H) and (LXXXIV;R_1=H, R_2=Furanyl) are suggested as possible three dimensional representations of palmarin and isojateorin respectively. Undoubtedly the most important feature in these compounds is that they appear to be the first known diterpenoids containing a cis A:B ring junction, which must be created at some stage of their biogenesis prior to the formation of the unique 1:6 oxide bridge.

(B) Experimental

Melting Point Determinations.

All melting points are uncorrected and were determined on a Kofler block except where marked with an asterisk in which case evacuated capillaries were used.

Polarimetry.

These measurements were made at room temperature (15-25°) with a Hilger standard polarimeter, using the sodium D line. The specimens were dried *in vacuo* at *ca* 70°. Unless specified to the contrary, 1 to 3% solutions of the compounds in dry redistilled pyridine were used for the measurements.

Spectroscopy.

Infrared spectra were kindly determined by Mr. I. Orr and Mr. F. Gisbey on a Perkin-Elmer Model 13 double-beam spectrophotometer and a Unicam S.P.100 double-beam spectrophotometer. Nujol mulls were used, unless stated otherwise. Thanks are also due to Dr. G. Eglinton for guidance in the interpretation of these spectra.

Ultraviolet spectra were measured in absolute ethanol solution with the Unicam S.P.500 spectrophotometer.

Chromatography.

The fractional elution technique was invariably employed. The absorbents used were alumina, acid-washed, neutralised and standardized according to Brockmann and Schodder's method[123], and B.D.H. silica gel for chromatography. Where necessary specimens were dried by azeotropic distillation with benzene under reduced pressure prior to chromatography. Wherever used

in connection with chromatography or as a solvent for crystallisation the term "petrol" refers to the petroleum fraction of boiling point 60-80°.

Working-up

The phrase "in the usual way" as applied to working-up of reaction products, means either saturation with ammonium sulphate or addition of saturated ammonium sulphate solution followed by extraction with a water-immiscible solvent, ether unless stated otherwise. The combined extract is then washed several times with small volumes (ca 10%) saturated ammonium sulphate solution, once with a little water, dried over sodium sulphate and evaporated in vacuo. Where the phrase "carefully in the usual way" is used the dried solution is evaporated at 25° in vacuo.

Where working-up involves the separation into acidic and neutral fractions the following method was employed. The water-immiscible solution of the total organic product obtained in the usual way was washed with five portions of saturated sodium hydrogen carbonate solution (each containing about two moles of base). The combined alkaline extract was then washed twice with ether. The ether washings, so obtained, together with the original bicarbonate washed organic phase were washed, dried and evaporated in vacuo to give the neutral product. Acidification followed by working-up in the usual way furnished the acidic fraction.

Analysis.

Analysis were carried out by Mr. Cameron, Miss Mc.Millan and Miss Watt on specimens previously dried in vacuo at ca 90° overnight, unless specified otherwise. Observed and calculated values are expressed to the nearest 0.05%.

Extraction of the neutral bitter principles from the Colombo root.

The powdered root of Jateorhiza palmata Miers (400g.) was refluxed with ether (2 l.) for two hours. The cooled solution was filtered and the extraction repeated with three further portions (2 l.) of ether. The combined extracts were concentrated in vacuo to small volume and the precipitated solid (1.1g) collected. Evaporation of the mother liquors in vacuo afforded a gummy residue (6.5g.). Fractionation or chromatography of the latter failed to give a workable quantity of pure crystalline material.

Re-extraction of root (400g.) with boiling acetone (2 l.) afforded, after separation from alkaloidal material, a small yield of palmarin (ca 20mg.), identified by m.p. and mixed m.p.

Preliminary separation of columbin and "chasmanthin+" from the solid obtained by ether extraction of the Colombo root.

After trying several methods including chromatography over silica gel the following method was found to be most useful for large scale separations.

The crude solid (50g.) was refluxed with acetone (750ml.) for 15 min., cooled and then filtered affording an insoluble product (ca 3g.) m.p. 300°. Concentration of the acetone solution in vacuo to ca 270ml. followed by cooling and standing for 30 min. furnished a solid (2g.) m.p.* 185° which was primarily amorphous.

+ "chasmanthin" refers to the mixture of isomeric compounds chasmanthin, jateorin and palmarin.

Ethanol (250ml.) was then added to the acetone solution which was then further concentrated to ca 400ml. After standing several hours the crystalline material, (needles) (8.5g.); m.p.* 178-81° dec.; $[\alpha]_D$ = + 40° which separated was filtered off. This was washed with ethanol (ca 50ml.) and the combined mother liquors concentrated, in vacuo to ca 350ml. On standing overnight this solution deposited mixed crystals (needles and small cubes), (15.5g.), m.p.* 177-195°, $[\alpha]_D$ + 31.5°.

Further concentration of the mother liquors in vacuo to ca 270ml., ca 70ml. and ca 20ml. followed by standing for several days in each case respectively yielded the following crops of crystalline material:-

11g.	m.p.* 185-200°	sl.dec.	$[\alpha]_D$ + 15°
3g.	m.p.* 169-72°	dec.	
1.1g.	m.p.* 177-9°	dec.	$[\alpha]_D$ + 45°

Of the three major fractions the first ($[\alpha]_D$ + 40°) is essentially columbin whilst the third ($[\alpha]_D$ + 15°) contains a large proportion of "chasmanthin". Fractional crystallisation of the material ($[\alpha]_D$ + 31°) from acetone - alcohol affords two major crops which correspond to the columbin rich and "chasmanthin" rich fractions already described.

"Chasmanthin."

(1) By crystallisation and decarboxylation

The "chasmanthin" rich fraction, $[\alpha]_D$ + 15°, m.p.* 185-200° sl. dec. (10g.) was recrystallised three times from acetone chloroform to give a mixture of plates and rods (2.12g.) m.p.* 225-30° (s. 220°), $[\alpha]_D$ + 6°.

This product was heated at 250° for 5 min. (in an atmosphere of nitrogen) to decarboxylate any columbin present. The resulting glass crystallised from acetone-ethanol as a mixture of prisms and needles m.p.* 225-30° (s. 220°), $[\alpha]_D$ ± 0°.

(2) By decarboxylation.

This method was found to be most efficient in that reasonable yields of "chasmanthin", (ca 20-30%) could be obtained from material with $[\alpha]_D$ between +10° and +35°.

Thus a crude preparation, $[\alpha]_D$ + 34°, (8g.) after being heated in an oil bath at 225° for 10 mins. under nitrogen afforded crude "chasmanthin" from acetone-benzene, narrow plates (2.03g.) m.p. 243-48° (s. 230°), $[\alpha]_D$ - 1°.

Isolation of palmarin.

Repeated crystallisation of crude "chasmanthin", obtained by decarboxylation of crude columbin, afforded palmarin as cubic or hexagonal prisms, m.p. 254-56° (s. 245°), $[\alpha]_D$ + 12° (c, 1.15°), no ultraviolet absorption maxima (in dry dioxan) between 240 mμ. and 320 mμ. (ε = 12 at 284 mμ.), (Found: C, 64.4; H, 5.8. $C_{20}H_{22}O_7$ requires C, 64.2; H, 5.9%). In a C-Me determination palmarin was shown to contain 7.2% C-Me (the theoretical value for 1 C-Me is 4.0%). In a similar experiment isocolumbin was shown to contain 7.5% C-Me (the theoretical value for 1C-Me is 4.2%).

Treatment of palmarin with alkali.

Palmarin, m.p. 245-7°, (216mg.) in ethanol (2ml.) and N-aqueous sodium hydroxide (8ml.) was heated on the steam in a stream of nitrogen for 5 hrs. (2.3 moles of alkali were consumed). Working up in the usual way furnished an "acid"

fraction m.p. 235-42° (plates from benzene-petrol) which did not depress the m.p. of the starting material.

Attempts to resolve the "chasmanthin" mixture.

(1) By chromatography of the material obtained by decarboxylation.

The crude mixture (3.01g.) m.p. 226-40° was dissolved in 1: 4 acetone - benzene (50ml.) and chromatographed over alumina (90g.; grade III).

Elution with benzene-ether mixtures, with proportions of ether increasing to 100%, gave fractions A to F (82mg.). The m.p.s of these fractions from aqueous ethanol ranged from B with m.p. 180-91° (s. 170°) to F of m.p. 165-85°, $[\alpha]_D$ - 55° (chloroform). Fraction B did not depress the m.p. of decarboxyisocolumbin.

Elution with ether-acetone with proportions of acetone increasing to 33% afforded fractions G to V (2.39g.). These fractions crystallised from ethanol with m.p.s ranging from 230-48° (s. 210°) (O) to 242-48° (s. 230°) $[\alpha]_D$ + 10° (U).

By increasing the proportion of acetone to 100% and then using 9 : 1 acetone - methanol fractions W to Z (450mg.) were obtained with similar m.p.s, e.g. Z with m.p. 246-52° (s. 230°).

Fractional crystallisation of I, M, S and Z showed that these fractions were mixtures although no pure compounds could be obtained.

Isomerisation (see p.134) of fraction S m.p. 242-48° (s. 230°) afforded a product which was essentially palmarin, m.p. and mixed m.p. This clearly demonstrates that the

later fractions do not contain much jateorin which would be expected to have a more positive rotation than chasmanthin. It thus appears that the increase in m.p. and rotation from fraction H to fraction V is due to palmarin.

(2) By chromatography of the material obtained by crystallisation from acetone-chlorform. (see p. 131)

This method, although easily separating the columbin remaining in the product, produced no better separation than the previous experiment. This was concluded since isomerisation of the middle fractions, which had the highest m.p. 243-48° (s. 235°), afforded a mixture containing ca 50% of isojateorin.

(3) By a six stage triangulation of the material obtained by decarboxylation of the last crops from the preliminary acetone-alcohol fractionation. (see p. 130)

The most insoluble fraction (I) m.p. 245-55° (s. 240°) was shown to be palmarin by mixed m.p. and infrared spectrum. The infrared spectra of fractions (III), (V) and (VI) indicated that they were almost identical in composition. Proof of the complex nature of these fractions follows from the isomerisation of the most soluble fraction (VI) m.p. 225-35° (s. 200°) which furnished a complex mixture of palmarin and isojateorin.

Alkali isomerisation of "chasmanthin".

(After Wessely et al)[94]

Crude chasmanthin m.p. 226-240° (5.02g.), obtained by the decarboxylation method, in ethanol (70ml.) and N sodium

hydroxide (35ml.) was heated on the steam for 4 min., solution taking place almost immediately. The cooled solution was acidified to pH3, and the product which separated recrystallised from acetone-ethyl acetate to give hexagonal prisms (1.63g.) m.p. 243-49° (s. 237°). After two further crystallisations from acetone this product had m.p. 254-6° (s. 245°), $[\alpha]_D$ + 12° (C, 3.51) and was shown to be identical with natural palmarin by mixed m.p., rotation and infrared spectrum.

The mother liquors from the first crystallisation together with the ether extract of the reaction mixture afforded a further 3.3g. which was dissolved in 3 : 7 acetone - benzene (50ml.) and chromatographed over alumina (150g., grade III). Elution with benzene-ether mixture followed by ether-acetone mixtures up to 20% acetone afforded needles (300mg.) of crude decarboxyisocolumbin, m.p. and mixed m.p.

Eultion with ether-acetone, the proportion of acetone increasing from 25% to 35% furnished fractions (V) to (XII) (2.2g.) with m.p.s. ranging from 167-95° (IX) to 166-68° (99% melted) (XII).

The proportion of acetone was gradually increased and finally 10 : 1 acetone - methanol was used as eluant to give fractions (XIII) to (XIX) (700mg.) with m.p.s 167-73°.

Fractions (X) to (XII) were combined to give from methanol plates m.p. 165-8° (98% melted) $[\alpha]_D$ + 29° (C, 5.09). Recrystallisation of combined fractions (XIII) to (XIX) from methanol afforded isojateorin as plates m.p. 163-6° (98% melted), $[\alpha]_D$ + 30° (C, 3.99) (Found: C, 63.9; H, 6.3. $C_{20}H_{22}O_7$ requires C, 64.15; H, 5.9%).

In another experiment it was found that chromatography was not necessary. Thus fractional crystallisation of the isomerisation product from acetone-ethyl acetate afforded two crops which contained the bulk of the palmarin. Evaporation of the mother liquors in vacuo furnished a residue most of which was readily soluble in cold ethyl acetate. Crystallisation of this ethyl acetate soluble portion from methanol furnished isojateorin (plates from methanol) m.p. 165-7° (ca 98% melted) $[\alpha]_D$ + 29° (C, 3.21).

Acetylation of chasmanthin.

(a) After Wessely et al[94]

"Chasmanthin", $[\alpha]_D$ ± 0, (250mg.), fused sodium acetate (750mg.) and acetic anhydride (12ml.) were refluxed for 4 hrs. After working up two crystallisations from acetone-ethanol gave rossettes m.p. 265-68° (s. 255°), $[\alpha]_D$ + 19° (C, 1.30). After two further crystallisations the product had m.p. 272-74° (s. 265°), $[\alpha]_D$ + 26° (C, 1.99). (Found: C, 63.7; H, 6.0. $C_{22}H_{24}O_8$ requires C, 63.45; H, 5.8%)

(b) After Feist et al[95]

"Chasmanthin", $[\alpha]_D$ ± 0, (100mg.) was refluxed in acetic ahydride (3ml.) for 2½ hrs. Working up gave prisms from ethanol m.p. 271-76° (s.250°). The product on recrystallisation had m.p. 273-76° (s. 267°). (Found: C, 63.2; H, 5.4; OAc, 11.0. $C_{22}H_{24}O_8$ requires C, 63.45; H, 5.8; OAc, 10.35%). The product is therefore not a diacetate as claimed by Feist.[95]

Methylation of "chasmanthin".
(After Wessely et al[94])

Crude "chasmanthin", m.p. 210°, (450mg.), ethanol (6.3ml.) and 10% sodium hydroxide solution (5.3ml.) were heated on the steam bath to give a clear solution. To the cold stirred solution was added five portions of dimethyl sulphate (1.2ml.) and five portions of 10% sodium hydroxide solution (4.5ml.). The additions were made alternately at about 3 min. intervals; the solution remaining alkaline throughout the reaction. After stirring for a further 1 hr. the reaction mixture was carefully acidified. The product "methylchasmanthin" separated on standing and crystallised from aqueous acetone as rods (225mg.) m.p.* 259-63° (s. 258°).

Alkali treatment of methyl "chasmanthin".

"Methylchasmanthin", m.p.* 259-63° (s. 258°), (200mg.) in aqueous N sodium hydroxide (8ml.) was heated for 5 hrs. on a steam bath in a stream of nitrogen. The cooled solution was then acidified to pH 3 and the solid which separated, washed with water and crystallised from ethanol to give narrow plates (125mg.) m.p.* 261-63° (s. 259°), $[\alpha]_D$ + 50° (C, 1.71). The m.p. did not depress on mixing with the starting material and after four further crystallisations had the value 261-64° (s. 259°).

Methylation of palmarin.

Palmarin m.p.* 247-51° (s. 240°), (1.96g.) was methylated as described by Wessely et al.[90]. The product methylpalmarin crystallised from aqueous acetone as rods (1.46g.) m.p.* 254-57° (s. 252°).

Alkali treatment of methylpalmarin.

(1) Methyl palmarin m.p.* 254-57° (s. 252°) (190mg.) in aqueous N sodium hydroxide (8ml.) was heated for 5 hrs. on a steam bath in an atmosphere of nitrogen. Working up as described on p. 137 afforded from ethanol flat plates (115mg.) m.p.* 254-6° (s. 252°). After a further four crystallisations the product had $[\alpha]_D$ + 42° (C, 1.52), m.p. 257-59° (s. 254°) which did not depress with the starting material.

(2) In another similar experiment using a different sample of methylpalmarin 1.96 moles of N sodium hydroxide were consumed and the product recovered crystallised from aqueous acetone m.p. 252-54° (s. 245°), $[\alpha]_D$ + 39.5° (C, 2.44).

(3) In the same way using 10% methanolic potassium hydroxide for 6 hrs. under nitrogen on the steam methylpalmarin was recovered completely unchanged, m.p. and mixed m.p.

Treatment of methylpalmarin with sodium alkoxides.

Methylpalmarin (120mg.) was dissolved in 0.25 N sodium ethoxide (3ml.; 2.5 equiv.) and refluxed on the steam for 3 hrs. under nitrogen. The infrared spectrum of the product after careful evaporation of the solvent showed bonds at 1765 (lactone) 1590 and 1570 (split carboxylate) cm^{-1}. The residue was then taken up in butanol (5ml.) containing sodium (100mg.) and refluxed for $2\frac{1}{2}$ hrs. under nitrogen. Infrared examination as before showed only one band at 1573 (carboxylate anion) cm^{-1}.

Methylisojateorin.

isoJateorin m.p. 162-6°, $[\alpha]_D$ + 29°, (427mg.) was methylated in the same way as palmarin[90]. The crude product on recrystallisation from ethanol afforded flat rods (240mg.) m.p. 267-75° (s. 265°). An analytical sample had m.p. 275-8° (s. 272°), $[\alpha]_D$ + 58° (C, 1.40) (Found: C, 64.7; H, 6.35, $C_{21}H_{24}O_7$ requires C, 64.9; H, 6.2%.)

Anhydrides from merochasmanthinic acid methyl ether.

(1) The acid (see p. 141) was sublimed at 230°/10^{-2}mm. to give crude anhydride I which crystallised from chloroform-petrol as needles m.p. 253-55° (s. 170°), γ max. 1850, 1773, 924cm^{-1}.

(2) The acid (20mg.) in "Analar" acetic anhydride (0.5ml.) was refluxed for 1½ hrs.+ The solvent was carefully removed by azeotropic distillation with benzene in vacuo giving anhydride II as needles (15mg.) from chloroform.petrol m.p. 193-95° (s. 190°), γ max. 1860, 1785, 1762, 892cm^{-1}.

The same anhydride m.p. 195-96°, identified by mixed m.p. and infrared spectrum, was obtained in similar yield when the acid was treated with redistilled "Analar" acetyl chloride in a sealed tube at 100° for 3 or 15 hrs.

When anhydride II was heated at 230° for 10 mins. under nitrogen it had a m.p. 215-30° (s. 200°), with infrared spectrum clearly indicating a mixture of anhydride I and anhydride II.

+ Methylpalmarin was recovered completely unchanged after this treatment.

Attempted hydrogenation of hexahydropalmarinic acid over platinum.

(1) Hexahydropalmarinic acid m.p.* 207-8° (52mg.) in acetic acid (5ml.) was hydrogenated over a platinum catalyst in the microhydrogenator. After 18 hrs. no hydrogen appeared to have been absorbed. On working up the product crystallised from aqueous acetone as needles (20mg.) m.p. 211-12° (s. 210°) undepressed with the starting material.

(2) In another similar experiment 70% aqueous perchloric acid (0.1ml.) was added to the solvent. As before no hydrogen was absorbed. Unfortunately colloidal platinum prevented a sample of the starting material being recovered.

Treatment of hexahydropalmarinic acid with aqueous hydriodic acid.

The acid (87mg.) dissolved in 55% aqueous hydriodic acid (1ml.) was left overnight at room temperature. The product was worked up by pouring into water (8ml.) containing sodium hydrogen carbonate (1g.). The excess iodine was removed by saturating the alkaline solution with ammonium sulphate and extracting with ether. The alkaline solution was then worked up in the usual way to give an acidic product (50mg.) crystallising as needles from ethanol-petrol m.p. 211-13° (s. 208°) which did not depress with the starting material.

Action of concentrated sulphuric acid on benzilic acid and hexhydropalmarinic acid.

(1) Benzilic acid (35mg.) was dropped into sulphuric acid

(5ml.) at 0° in the microhydrogenator. After 2½ hrs. shaking at 17°, 18ml (0.5 mole) of gas had been evolved. On further shaking no more gas was evolved.

(2) Hexahydropalmarinic acid (44mg.) was treated with sulphuric acid in the microhydrogenator as above. After 15 hrs. at 17° and 2 hrs. at 50° the volume change corresponded to ca 0.04 moles.

Merochasmanthinic acid methyl ether (XXVII)

Methylpalmarin (2.08g.) was oxidized with aqueous potassium permanganate as described by Feist et al[98] to give a crude product (1.24g.). Crystallisation from acetone-benzene afforded the "mero acid"(240mg.) as needles m.p.* 248-51 dec.+, $[\alpha]_D$ + 46° (C, 1.06) (Found: C, 56.95; H, 5.85. $C_{16}H_{20}O_8$ requires C, 56.45; H, 5.95%)

Treatment of the bulked mother liquors in acetone with ethereal diazomethane furnished after three crystallisations from acetone-petrol the tris-nor acid methyl ester (XXVIII; R = Me) as fine needles m.p.* 260-63°, $[\alpha]_D$ + 60° ($CHCl_3$; C, 1.34) (Found: C, 60.5; H, 6.3; OMe, 14.8. $C_{19}H_{24}O_8$ requires C, 60.0; H, 6.35; OMe, 16.3%).

The remainder of the methylation product was carefully chromatographed over alumina (30g; grade III). Eultion with benzene, benzene-ether mixtures and ether afforded 13 fractions.

+ m.p.s of this compound taken in an evacuated capillary soften about 212°, melt with dec. ca 228° and then resolidify melting finally at ca 250°. When the m.p. is observed on the Kofler, m.p.s ca 230° are observed.

Combination of fractions (II)- (VI) and (IX) - (XIII) appeared to give the same compound plates from acetone-petrol m.p. ca 240-45° (subliming below m.p.), $[\alpha]_D$ + 64° (C, 1.21), $[\alpha]_D$ + 80° ($CHCl_3$; C, 3.38) (Found: C, 58.75; H, 5.9; OMe 22.85. $C_{18}H_{24}O_8$ requires C, 58.65; H, 6.55; OMe, 25.3%)

The infrared spectrum of this compound was identical with methyl merochasmanthinate methyl ether, plates from acetone m.p.* 255-57° obtained by methylation of pure merochasmanthinic acid methyl ether.

Selenium dioxide followed by potassium permanganate oxidation of methylpalmarin. (XXVI)

Methylpalmarin (445mg.) and selenium dioxide (2.5g.; ca. 20 moles) were refluxed in "Analar" acetic acid for 5 hrs. After removal of the solvent and selenium the residue was dissolved in chloroform and washed with water to remove the selenium dioxide. The residue obtained after evaporation of the chloroform in vacuo was dissolved in 8% methanolic potash (5ml.). To this well-stirred alkaline solution was added 5% aqueous potassium permanganate (150ml.) over $2\frac{1}{2}$ hrs.

The reaction mixture was then cooled to 0°, treated sulphur dioxide and worked up in the usual way. The crude product (148mg.) was methylated and chromatographed over silica gel (12g.). Elution with benzene-ether mixtures (50% to 100% ether) afforded fractions which were combined

to yield a product m.p. 298-301°, $[\alpha]_D$ + 51° ($CHCl_3$; C, 1.14), no ultraviolet absorption maximum from 210-270 m.μ. (Found: C, 59.4; H, 6.4; OMe 17.15. $C_{19}H_{24}O_8$ requires C, 60.0 H, 6.3; OMe 16.3. $C_{14}H_{18}O_6$ requires C, 59.6; H, 6.45%).

Other experiments using less selenium dioxide (2 moles and 4 moles) and refluxing for shorter periods (½ hr. and 1½ hrs.) and then treated as above afforded mixed products which did not depress the m.p. of methyl merochasmanthinate methyl ether.

Further Oxidations of methylpalmarin (XXVI) with potassium permanganate.

(1) The best yield of merochasmanthinic acid methyl ether was obtained as follows: methylpalmarin (1.07g.) in acetone (33ml.) was treated N sodium hydroxide (11ml.) on the steam bath for 7 mins. The remaining acetone was removed in vacuo and manganese sulphate (ca 50mg.) added to the alkaline solution. To the cold well-stirred alkaline solution was then added, in a dropwise manner over 20 mins., 5% aqueous potassium permanganate (210ml.). After stirring at room temperature for a further 2 hrs. the reaction mixture was cooled to 0° and treated with sulphur dioxide. The product was then carefully worked up by saturation with salt and ether extraction in the usual way. The gummy product crystallised from acetone-benzene to give the "mero acid" (XXVII) as flatish rods (407mg.) m.p. 230-36°. An additional crop of rather crude acid (80mg.; total yield ca 50%) m.p. 210-15° was obtained on concentration of the mother liquors.

(2) Reversed addition.

Using the same proportions of reactants as described above the alkaline solution of methylpalmarin was added with stirring to the potassium permanganate solution over 25 mins. Working up afforded a 30% yield of acid (XXVII) as the only crystalline product. Filtration of the reaction mixture, before sulphur dioxide treatment, followed by the usual working-up procedure demonstrated that the acid (XXVII) was still in solution at the end of the reaction.

(3) Under strongly alkaline conditions.

To methylpalmarin (180mg.) in 25% aqueous potassium hydroxide (33ml.) at 47° was added with stirring over 20 mins. 4.3% aqueous potassium permanganate (100ml.) containing 14% potassium hydroxide. Working up in the usual way, after a further 40 min., afforded as the only crystalline product a 3% yield of acid (XXVII).

(4) Under similar conditions to the oxidation of (XXX)[105]

Methylpalmarin (194mg.) in acetone (6ml.) was treated with 5% aqueous potassium hydroxide (3ml.; 6 equiv.) on the steam bath for 8 mins. The remaining acetone was removed in vacuo and a solution containing potassium permanganate (1.70g.) and potassium hydroxide (450mg.) in water (83ml.) added. After heating for 1 hr. on the steam the product was carefully worked up in the usual way. The gummy product in ether was treated three times with petrol. The insoluble fractions so obtained crystallised to give acid (XXVII) (ca 25 mg.) identified by m.p. mixed m.p. and infrared spectrum.

The ether-petrol soluble fraction on standing in water crystallised to give a product (7mg.) m.p. 153-7° (300°). On recrystallisation from water it gave flat plates of the acid (XXXIII) m.p. 165-85°, resolidifying after 10 mins. at 210° to rods m.p. ca 285°, ν max.(CCl_4) 1715cm^{-1}.

Sublimation at 190°/10^{-2}mm. of the above gave the anhydride (XXXIV) m.p. (without crystallisation) 303-6°, ν max. 1800, 1755cm^{-1}.

(5) Under neutral conditions (magnesium sulphate)

Methylpalmarin (178mg.), acetone (6ml.) and N sodium hydroxide (2ml.) were heated for 7 mins. on the steam and the acetone removed in the usual way. To this stirred solution heated on the steam bath was added 15% aqueous magnesium sulphate (40ml.)[+] followed by 5% potassium permanganate solution (40ml.). After 1 hr. on the steam bath careful working up afforded the acid (XXVII) (12mg.), m.p. 224-33° as the only crystalline product.

Attempts to oxidize merochasmanthinic acid methyl ether (XXVII)

In the following experiments the only product which could be obtained crystalline was the unchanged starting material, identified by m.p. and infrared spectrum.

[+] This solution acts as a buffer precipitating the excess hydroxide ions as magnesium hydroxide while the "monosodium salt of methyl palmarin" stays in solution.

(1) Using method employed by Buchi et al[105] to oxidize (XXX)

The acid (XXVII) (43mg.), 1.9% aqueous potassium hydroxide (3.8ml; 10 moles) and potassium permanganate (85mg; 2 atoms of "oxygen") were heated for 2 hrs. on the steam. Careful working up furnished 27mg. which crystallised to give unchanged starting material (17mg.)

(2) Using conditions similar to those employed in the oxidation of (XXXV)[106]

To the acid (XXVII) (22mg.), in 60% aqueous potassium hydroxide (0.35ml.), at 40°, was added in a dropwise manner with shaking over 20 mins. a solution of potassium permanganate (45mg.) and potassium hydroxide (180mg.) in water (0.9ml.). After a further 10 mins. at 40° the reaction mixture was cooled to 0° and carefully worked up in the usual way to give starting material (14mg.).

(3) Under neutral conditions.

The acid (XXVII) (25mg.) was dissolved in $\frac{N}{10}$ sodium hydroxide (0.77ml; 1 mole) to give the mono-sodium salt, pH ca. 5, and water (2ml.) containing a few crystals of manganese sulphate added. Potassium permanganate (31mg.) dissolved in water (0.6ml.) and 0.522N hydrochloric acid (0.38ml.) were made up to 1.33ml., giving a solution which contained 4 atoms of "oxygen" and the exact quantity of acid required to neutralise the potassium hydroxide formed as the permanganate was reduced. This solution was added in drops to the stirred solution of the half neutralised acid

at 60°. After 2 hrs. less than 0.45 atoms of "oxygen" had been consumed. At 100°, 3.5 atoms of "oxygen" were consumed over 2½ hrs. Careful working up of the product furnished 10mg. of starting material.

In another experiment 24 "oxygen" equivalents of this permanganate solution were added to the acid (XXVII) over a period of 14 hrs. Careful working up afforded a product which could not be crystallised.

(4) Using nitric acid.

The acid (XXVII) (10mg.) was refluxed for 20 mins. with concentrated nitric acid (1ml.). The nitric acid was evaporated at 100° *in vacuo* to give a 40% recovery of starting material.

Reduction of merochasmanthinic acid methyl ether (XL) with lithium aluminium hydride.

The acid (290mg.) was extracted from a Soxhlet thimble with ether over a period of *ca* 20 hrs. into a boiling solution of lithium aluminium hydride (900mg.) in ether (300ml.). The reaction was carefully worked up in the usual way (see p.151). The crude product (190mg., by hand extraction) was extracted with boiling ether. The ether soluble fraction crystallised from acetone-petrol to give the diol-hemiacetal (XLI: R_1 = H, R_2 = H) as flat blades (31mg.) m.p. 143-8°. On recrystallisation it had m.p. 178-80°(s. 170°) (Found: C, 61.15; H, 8.0. $C_{16}H_{26}O_6$ requires C, 61.16; H, 8.35%)

An attempt to acetylate the above crude reaction product using acetic anhydride-pyridine ("Analar" reagents) overnight at room temperature, followed by evaporation of the solvents at $40^{\circ}/10^{-1}$mm. and careful chromatography failed to give a crystalline product.

Another attempt using "Analar" acetic ahydride and fused sodium acetate for 2 hrs. at 140° was also unsuccessful.

Attempted tosylation of the crude reduction product from merochasmanthinic acid methyl ether (XL)

(1) The tetrahydrofuranohemiacetal (XLIII)

To the gum (70mg.) in "Analar" pyridine (7.5ml.) was added in one portion pure recrystallised p-toluene sulphonyl chloride (464mg.). After $2\frac{1}{2}$ hrs. the product was carefully worked up as described on p.152 to give a gum (ca 60mg.) which was dissolved in benzene and chromatographed over alumina (3g.; grade III).

Elution with benzene (300ml.) afforded two gummy fractions (5mg.) and two partially crystalline fractions (9mg.). Elution with 2% and 4% ether-benzene (150ml.) afforded the remainder of the partially crystalline material (16mg.). Further elution with benzene-ether mixtures, ether, ether-acetone mixtures and finally 4 : 1 acetone-methanol gave non crystalline products (8, 5, 3 and 14mg. respectively).

The semicrystalline fractions crystallised from acetone-petrol to give the hemiacetal (XLIII), short rods,

m.p. 203-5°, no absorption maximum in the ultraviolet from 205-340mμ, ε = 300 at 220mμ, (Found: C, 64.65; H, 7.9. $C_{16}H_{24}O_5$ requires C, 64.85; H, 8.15%).

An alternative approach, in which a solution of pure p-toluene sulphonyl chloride (300mg.; 4 moles) in "Analar" pyridine (0.7ml.; 100 drops) was slowly added (5 drops every 15 mins.) to a well-stirred solution of the gum (67mg.) in "Analar" pyridine (1.8ml.) did not improve the yield of the hemiacetal (XLIII)

(2) Hemiacetal ditosylate (XLI: $R_1 = T_s$, $R_2 = T_s$)

The crude gum (129mg.) in "Analar" pyridine (0.7ml.; 60 drops) was slowly added (4 drops every 5 mins.) to a well-stirred solution of pure p-toluene sulphonyl chloride (1.04g.; ca 10 moles) in "Analar" pyridine (0.9ml.).

After stirring for a further 45 mins. the reaction was carefully worked up in the usual way (p. 152) to give a product (121mg.) which was dissolved in 1 : 1 benzene - petrol (8ml.) and chromatographed over alumina (4g.; grade V).

Elution with petrol containing from 50 to 90% benzene (6 fractions) afforded gummy material (22mg.). Elution with benzene and benzene containing up to 8% ether (7 fractions) afforded partially crystalline material (60mg.). Solvent mixtures of benzene and ether (16 to 100%) (4 fractions) gave non crystalline material (7mg.). The remainder of the product (21mg.) was eluted with acetone and 4 : 1 acetone - methanol and could not be crystallised.

The early partially crystalline fractions were combined and crystallised from acetone to give the ditosylate (XLI: $R_1 = T_s$, $R_2 = T_s$) as needles (43mg.) m.p. 169-70°, λ max. 226mμ. (ε 27,200) (Found: C, 57.95; H, 5.9; S, 9.45 . $C_{30}H_{38}O_{10}S_2$ requires C, 57.8; H, 6.15; S, 10.3%).

Attempted acetylation of hemiacetal ditosylate (XLI; $R_1 = T_s$, $R_2 = T_s$)

To the compound (20mg.) in "Analar" pyridine (0.5ml.) at 0° was added "Analar" acetic anhydride (0.5ml.). The temperature of the reaction mixture was allowed to rise slowly to room temperature. After standing overnight at room temperature the solvents were removed at 25° at 0.1mm. using benzene as an azeotrope. The residue crystallised from acetone-petrol, as needles (8mg.) m.p., and mixed m.p. with starting material, 169-70°.

Oxidation of hemiacetal ditosylate (XLI; $R_1 = T_s$, $R_2 = T_s$) with chromium trioxide.

The ditosylate (3.28mg.) in "Analar" acetic acid (1ml.) was treated with chromium trioxide in "Analar" acetic acid (0.0332N; 1ml.; ca. 3 moles). After standing overnight at room temperature 0.98 atoms of "oxygen" had been consumed. The product was carefully worked up in the usual way and filtered in benzene solution through a short silica column (150mg.). The total product was dissolved in "Analar" carbon tetrachloride and its infrared spectrum determined. γ max. 1777, 1368, 1180 cm^{-1}.

Reduction of palmarin (XLVI) with lithium aluminium hydride.

Palmarin (1.944g.) in dry tetrahydrofuran (ca. 200ml.) was added dropwise over 3 hrs. to a well-stirred suspension of lithium aluminium hydride (4.0g.) in dry tetrahydrofuran (150ml.). The reaction mixture was then refluxed with stirring for 1 hr. The excess reagent was decomposed with ethyl acetate, 50% aqueous ammonium sulphate (150ml.) added, and the product carefully worked up in the usual way using ether as solvent. The infrared spectrum of the crude product (1.86g .) showed no carbonyl absorption.

Attempts to characterise this product, by acetylation using acetic anhydride and pyridine followed by working up in the usual way and careful chromatography, gave oily fractions which could not be crystallised.

Sodium metaperiodate oxidation of the above crude reduction product.

The crude product (1.86g.) in methanol (70ml.) and water (35ml.) was treated with 0.2 M aqueous sodium metaperiodate (70ml.). Titration showed that after standing overnight 0.9 moles of periodate were consumed; there was no further uptake. The product was carefully worked up in the usual way, any excess of the reagent being removed by careful washing, to give the crude ketol mixture containing (Ll) and (L; R = H, R_1 = H) as a colourless oil (1.41g.), $[\alpha]_D$ - 47° (C, 1.69) unchanged after prolonged standing in pyridine, ν max. 3380 (broad), 1740 (shoulder), 1710, 1502, 1156cm^{-1}.

Tosylation of crude mixture containing ketols (LI) and (L; R = H, R_1 = H).

To the gum (120mg.) in "Analar" pyridine (6ml.) was slowly added a solution of recrystallised p-toluene sulphonyl chloride (700mg.; 12 moles) in "Analar" pyridine (1ml.). After 30 mins. the deep red solution was poured into saturated aqueous sodium carbonate (ca 20ml.) and left for 1 hr. Careful working up afforded a clear gum (130mg.) which was dissolved in benzene and chromatographed over alumina (4g.; grade III).

The first four fractions eluted with benzene afforded the monotosylate (LII) (see p.157) (30mg.) as needles from benzene-petrol, m.p. 160-64°, identified by m.p., mixed m.p. and infrared spectrum. Further elution with benzene, benzene-ether and ether-acetone mixtures furnished gums (14mg., 8mg., 25mg. respectively) which could not be crystallised.

Base treatment of crude ketol mixture to give keto-triol (L; R = H, R_1 = H).

(1) Preliminary experiments on a small scale

Base	Proportion H_2O : ROH	Normality	% mole alkali	Time mins.	% Yield crystalline keto-triol (approx.)
$NaHCO_3$	2 : 5	0.03	30	5	10
$NaHCO_3$	2 : 5	0.03	150	200	45
KOH	2 : 2	0.02	100	180	10
KOH	1 : 3	0.02	30	20	40
KOH	1 : 7	0.02	20	20	40
KOH	1 : 50	0.3	100	5	45

(2) On a preparative scale.

The crude ketol mixture (1.41g.) in ethanol (13ml.) and water (2ml.) was treated with 2% methanolic potassium hydroxide (1.1ml.) for 20 mins. and then without acidification carefully worked up in the usual way. Crystallisation from acetone-benzene gave the keto-triol (L; R = H, R_1 = H) as flat plates (722mg.) m.p. 187-91° (s. 176°). Recrystallisation from ethanol-benzene gave m.p. 196-8° (previous decomposition from 170°), $[\alpha]_D$ - 40° (C, 1.26), (Found: C, 65.25; 65.65; H, 7.15, 7.55. $C_{19}H_{26}O_6$ requires C, 65.1; H, 7.5%).

In another experiment the crude ketol mixture (59.4mg.) in methanol (1ml.) was treated with 2% methanolic potassium hydroxide (1ml.). Complete solution was obtained in 5 mins., and water (10ml.) was then added. After a further 20mins. 33% aqueous sulphuric acid (0.2ml.) was added and the formic acid produced determined by the standard method used for "Acetyl values". Using a similar blank the ketol mixture was shown to contain ca 15% of formate; the formic acid was characterised as sodium formate; identified by its infrared spectrum (potassium chloride disc).

Quantitative infrared analysis (1% solutions in pyridine) of the carbonyl absorption of this compound and cholestanone shows that it contains one carbonyl group.

Microhydrogenation of the keto-triol (L; R = H, R_1 = H) in "Analar" acetic acid and methanol over 10% palladised charcoal showed that 2.1 and 2.3 moles of hydrogen were respectively consumed. In neither case could a

crystalline compound be obtained from the hydrogenation, even after acetylation.

On standing overnight at room temperature with 2.2 moles of bromine in "Analar" acetic acid containing a trace of hydrogen bromide, the crude tetrahydro derivative of keto triol (L; R = H, R_1 = H) consumed 2.0 moles of bromine.

More vigorous basic treatment of keto-triol (L; R = H, R_1 = H)

The compound (2.0mg.) was suspended in 5% aqueous sodium hydroxide (0.1ml.) and warmed on a water bath at 100°. Within a few seconds solution took place with a development of a deep violet colour which became very intense within ca. 40 sec. In 0.1 N aqueous sodium hydroxide the colour appeared after ca. 1 min., the maximum intensity developing within ca. 3 min. In both cases this colour remained on cooling but not on dilution with water or ethanol.

A series of reactions were carried out in which the temperature, time and concentration of alkali were varied. After the reaction the alkaline solution was diluted with ethanol and the ultraviolet spectrum recorded. In some cases the spectrum was remeasured after acidification.

The product obtained after $2\frac{1}{2}$ mins. at 100° was worked up in the usual way and treated with excess diazomethane in methylene chloride. The ultraviolet absorption of this product in ethanol and ca. 0.02 N ethanolic sodium hydroxide were recorded.

The ultraviolet maxima of these products and dimedone are recorded below.

Normality of aqueous NaOH	Time mins.	Temperature °C	λ max. (ε x 10^{-3}) alkaline EtOH	λ max. (ε x 10^{-3}) acidic EtOH	ε alkali / ε acid
1.2	1	98	290 (ε 9.0) 340 (ε 2.0)	271 (ε 7.2)	1.25
1.2	2½	98	290 (ε 15.6) 340 (ε 4.6)	270 (ε 11.1)	1.40
1.2	4	98	290 (ε 12.5) 335 (ε 3.5)		
1.2	6	98	290 (ε 14.0) 335 (ε 3.5)		
1.2	8	98	290 (ε 13.0) 335 (ε 3.5)		
1.2	15	98	290 (ε 12.0) 335 (ε 3.3)	266 (ε 8.7)	1.41
1.2	15 (nitrogen)	98	290 (ε 7.0) 335 (ε 1.3)	266 (ε 5.0)	1.40
1.2	24 hrs.	15	290 (ε 3.1) 335 (ε 0.7)	271 (ε 2.6)	1.20
0.1	3	98	290 (ε 4.9)		
0.1	6	98	290 (ε 5.4)		
0.1	12	98	290 (ε 6.1)		
0.1	2 hrs.	98	290 (ε 10.0)		
Dimedone			282 (ε 23.3)	255 (ε 17.5)	1.62
Product + diazomethane			258 (ε 6.7)	258 (ε 6.7)	1.00

Attempted acetylation of keto-triol (L; R = H, R_1 = H).

Several attempts were made using pyridine-acetic ahydride ("Analar" reagents) overnight. (Polarimetric control gave no indication of an optimum reaction time). In no case, however, despite careful working up and chromatography over silica, could a crystalline product be obtained.

Tosylation of keto-triol (L; R = H, R_1 = H).

To the compound (202mg.) in "Analar" pyridine was added recrystallised p-toluene sulphonyl chloride (1.00g.; ca 9 moles). After 3 hrs. (polarimetric control indicated that this was the optimum time) the product was carefully worked up and chromatographed as described before (p.152). Recrystallisation from benzene-petrol gave the monotosylate (LII) as needles (90mg.) m.p. 163-5°, λ max. 223mμ (ε 14,800) (Found: C, 63.95; H, 6.0; S, 6.8. $C_{26}H_{30}O_7S$ requires C, 64.2; H, 6.2; S, 6.6%).

Quantitative infrared absorption measurements in 1% carbontetrachloride solution using cholestanone as a standard showed that the tosylate contained one carbonyl group.

On quantitative hydrogenation the tosylate consumed 2.0 moles of hydrogen. The resulting tetrahydro derivative on standing overnight with 2.2 moles of bromine in acetic acid took up 2.0 moles of bromine.

Ozonolysis of palmarin (LVI).

Palmarin (1.01g.) in ethyl acetate (400ml.) was treated with ozone at 0° for 3½ hrs. (i.e. till the ultraviolet absorption at 211mμ reached a minimum, ε = 1100). Addition of water followed evaporation at 40° in vacuo furnished a gummy product (the ozonide) This gum on standing overnight in water (ca 8ml.) was transformed into a crystalline acid. After heating for 15 mins. on the steam and cooling the crystals were filtered off. Recrystallisation from acetone-ethanol containing a little water furnished the trisnor acid (LVII) as well-formed rods (620mg.) m.p. 260-64° (s. 240°). The methyl ester crystallised from ethanol m.p. 266-68° (s. 255°). (Found: C, 58.5; H, 6.65. $C_{18}H_{22}O_8 \cdot C_2H_6O$ requires C, 58.25; H, 6.85%).

Reduction of the trisnor acid (LVII) with lithium aluminium hydride.

The recrystallised acid (240mg.) in dry tetrahydrofuran (40ml.) was slowly added over 2 hrs. to a well-stirred boiling suspension of lithium aluminium hydride (750mg.) in boiling dry tetrahydrofuran (20ml.). After refluxing the well-stirred suspension for a further 2 hrs., one third of the solvent was evaporated and the product carefully worked up using ethyl acetate, 50% aqueous ammonium sulphate (25ml.) and ether in the usual way.

The crude product (170mg.) showed a very slight carbonyl absorption in the infrared but crystallised from benzene-methanol to give the triol hemiacetal (LVIII), short rods, m.p. 211-13°, (no infrared absorption from 1850-1600cm^{-1}) (Found: C, 59.2; H, 8.6. $C_{17}H_{28}O_7$ requires C, 59.3; H, 8.2%).

Constant ether extraction of the aqueous phase and washings gave the remainder of the product (70mg.) which crystallised as before giving 34mg., m.p. 211-13° (s. 208°).

Sodium metaperiodate oxidation of the triol hemiacetal(LVIII)

To the compound (56mg.) in water (3ml.) was added sodium metaperiodate (145mg.; ca 4.5 moles) which dissolved to give a clear solution. Within ca 15 mins., however, the product started to crystallise from the reaction medium.

After standing overnight titration showed that 1.98 moles of metaperiodate had been consumed. The product was carefully worked up in the usual way, any excess of the reagent being removed by suitable washing, to give the formate hemiacetal (LXII), needles from acetone-petrol (35mg.) m.p. 196-7° (S. 193°) (Found: C, 62.1; H, 6.95. $C_{16}H_{22}O_6$ requires C, 61.9; H, 7.15%).

Acetylation of the formate hemiacetal (LXII)

The compound (25mg.) in "Analar" pyridine (1.5ml.) was treated with "Analar" acetic anhydride (0.7ml.) and left

overnight at room temperature. Using benzene as an azeotrope the solvents were evaporated at 20° at 0.01mm. The residue crystallised from acetone-petrol to give the formate acetate (LXII; OH = OAc) as well formed needles (17mg.) m.p. 157-59° (S. 147°), ν max. (CCl_4) 1752 and 1726 (double intensity) cm^{-1}.

Attempted oxidation of the formate hemiacetal (LXII) with silver oxide.

(cf. Berkley, et al)[124]

A finely divided suspension of silver oxide in aqueous alkali was obtained by adding silver nitrate (6.8mg.) in water (0.06ml.) in three portions with stirring to 20% aqueous sodium hydroxide (0.05ml.). To this was added the compound (6.5mg.) in dioxan (0.15ml.) in 10 portions with stirring over 7 mins. After vigorous agitation for 1 hr. the product was carefully worked up in the usual way. Crystallisation from acetone-petrol afforded a crude product (ca 1mg.) m.p. 169-72° (s. 164°) ν max. (CCl_4) 3624, 1725cm^{-1}.

In a blank experiment 7.4 mg. of the formate hemiacetal afforded a product (2mg.) m.p. 175-84° (s. 165°) which did not depress the m.p. of the product of the above reaction.

Oxidation of the formate hemiacetal (LXII) using chromium trioxide.

The compound (22mg.) was treated with chromium trioxide in "Analar" acetic acid (0.078N; 12.5ml.,

ca 6.5 atoms of "oxygen") for 2 days at room temperature. Titration showed that under these conditions 1.24 atoms of "oxygen" were consumed.

The excess reagent was destroyed with methanol and most of the acetic acid removed at 25° at 0.01mm. On the addition of water (ca 4ml.) the inorganic material dissolved leaving the desired product as well-formed needles. Recrystallisation from methanol gave the formate lactone (LXIII; R = Fr), as rods, (13.5mg.) m.p. 221-25° (s.210°) ν max. (CCl_4) 1745, 1734 and 1727cm^{-1}.

Working up of the aqueous washings in the usual way furnished a neutral product (2.5mg.) identical with the previous material, m.p. and mixed m.p.

Hydrolysis of the formate lactone (LXIII; R = Fr).

To the compound (9.3mg.) in dioxan (0.25ml.) was added 9% methanolic potassium hydroxide (0.08ml.; ca 5 moles) and water (2 drops).

After 18 hrs. at room temperature saturated aqueous ammonium sulphate (1ml.) was added and the alkaline solution thoroughly extracted with ether. After washing, drying and evaporation in vacuo at 25° in the usual way a neutral product was obtained (1mg.), rods from acetone m.p. 225-40° (s. 217°).

The reaction mixture was then acidified and carefully worked up in the usual way to give an acid fraction

(negligible) and a neutral fraction (3mg.). The latter recrystallised from methanol to give the hydroxy lactone (LXIII; R = H) as stout rods (1.1mg.) m.p. 245-47° (s.237°) ν max. (in CCl_4) 3633, 1749 and 1727cm^{-1}.

The ultraviolet absorption of the mother liquors from the latter neutral fraction showed a slight shoulder between 225 and 240mμ (total ε = ca 1400 at 225mμ).

Acetylation of the hydroxy lactone (LXIII; R = H).

The compound m.p. 245-47° (s. 237°) (3mg.) in "Analar" pyridine (0.2ml.) and "Analar" acetic anhydride (0.2ml.) was left for 48 hrs. at room temperature. The solvents were diluted with benzene and removed azeotropically at 20° at 0.01mm. The residue crystallised in good yield from methanol to give the acetate lactone (LXIII; R = Ac) as fine needles, m.p. 215-16° (s. 200°), no ultraviolet absorption maximum from 210-255mμ (ε = 800 at 224mμ), ν max. (CCl_4) 1757 (double intensity), 1730cm^{-1}.

Basic hydrolysis of the formate hemiacetal (LXII)

The formate (9mg.) in methanol (0.45ml.) was treated with 9% methanolic potassium hydroxide (0.30ml.; ca 15 moles) and the solution left overnight at room temperature. Careful working up in the usual way afforded after two recrystallisations from methanol-ethyl acetate the hemiacetal ketol (LXIV) as hexagonal prisms (1.5mg.) m.p. 191-94° (s. 188°) which depressed

the m.p. of the starting material, ν max. (CCl_4) 3624 and 1725cm^{-1}.

Chromium trioxide oxidation of hemiacetal ketol (LXIV)

The compound (1.02mg.) was treated with chromium trioxide in "Analar" acetic acid (0.4 N; 1ml; ca 5.5 atoms of "oxygen") for 40 hrs. at room temperature. Titration showed that 2.5 atoms of "oxygen" had been consumed. Working up carefully afforded the crude product which was dissolved in 3% methanolic potassium hydroxide (0.1ml.) and then diluted with ethanol (10ml.). The ultraviolet spectrum of this solution was measured against a similar blank.

Initial observations indicated a peak at 280-85mμ the intensity of which slowly increased with time. On prolonged standing however this peak disappeared and a more sharply defined peak at 267 mμ remained as shown.

Time hr.	λmax. mμ	ε max. (±1000) (assume 75% extraction)
1	280-85	2300
1.5	280-85	2380
2.5	280-85	2520
5.5	280	2680
18	267	2600

On acidification of the products obtained after 5.5 hrs. and 18 hrs. in 0.03% ethanolic potassium hydroxide the intensity over the range 240 to 290 mμ definitely dropped at least 25% in both cases. No further reliable facts as to the nature of the absorption curve could be obtained owing to a little contamination with hydrogen chloride.

Permanganate oxidation of methylisojateorin (LXXIII, R = Me)

Methylisojateorin m.p. 270-74° (s. 266°) (130mg.) was oxidised with permanganate according to the conditions of Feist et al.[98] The crude product (92mg.) crystallised from acetone-benzene as flat needles (11mg.) m.p. 222-30°, undepressed on mixing with authentic merochasmanthinic acid methyl ether m.p. 228-32° (s. 223°). Both acids had identical infrared spectra.

The gummy mother liquors in acetone were treated with excess etheral diazomethane overnight. The product crystallised from acetone as plates (13mg.) m.p. 230-50° which recrystallised from acetone as stout hexagonal rods m.p. 255-59° (s. 238°). This product was identical with authentic methyl merochasmanthinate methyl ether (LXXIV), m.p., mixed m.p. and infrared spectrum.

Hydrogenation of palmarin (LXXIII; R = H)

Palmarin, m.p. 254-5°(s. 245°), $[\alpha]_D$ + 12°, (322mg.) was hydrogenated in "Analar" acetic acid (115ml.); (ca 2.9 moles) of hydrogen was rapidly consumed.

After filtration of the catalyst and evaporation of the acetic acid *in vacuo* the residue was separated into acid and neutral fractions.

The neutral fraction (*ca* 35mg.) crystallised from acetone-petrol as rectangular rods (25mg.) m.p. 275-80°, $[\alpha]_D$ + 8° (C, 1.43). Recrystallisation from acetone afforded pure tetrahydropalmarin (LXXVI), rectangular rods, m.p. 322-25° (s. 310°), $[\alpha]_D$ + 16° (C, 0.95) (Found: C, 63.25; H, 6.85. $C_{20}H_{26}O_7$ requires C, 63.5; H, 6.95%)

Acidification of the bicarbonate extract afforded a crystalline solid (200mg.), $[\alpha]_D$ + 35° (C, 1.23) which was slowly crystallised from aqueous ethanol to give hexahydropalmarinic acid (LXXV) as rosettes of rods (135mg.) m.p. 214-16° (221°), $[\alpha]_D$ + 41° (C, 2.16). This product was recrystallised quickly from a larger volume of solvent to give thin rods, m.p. 222-25° (229°), (Found: C, 63.05; H, 7.5. $C_{20}H_{28}O_7$ requires C, 63.15; H, 7.4%)

Hydrogenation of isojateorin (LXXIII; R = H)

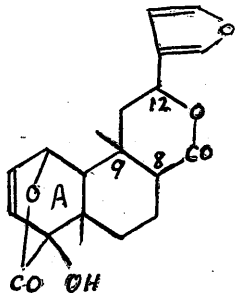
isoJateorin m.p. 165-67° (180°), $[\alpha]_D$ + 29°, (590mg.) in "Analar" acetic acid (200ml.) was hydrogenated over 10% palladised charcoal (110mg.). The uptake of hydrogen was 108ml. (*ca* 2.7 mole).

The product was separated into acidic and neutral fractions as in the hydrogenation of palmarin. By boiling with ethyl acetate the neutral fraction was separated into

a soluble (57mg.) and insoluble (60mg.) fractions. The soluble fraction had $[\alpha]_D$ + 37° (C, 1.70) and crystallised from acetone as stout rectangular rods m.p. 282-88° (s. 265°). Crystallisation of the insoluble material twice from acetone gave stout rods of tetrahydroisojateorin (LXXVI) m.p. 318-20° (s. 315°), $[\alpha]_D$ + 39° (C, 0.95) (Found: C, 63.45; H, 7.15. $C_{20}H_{26}O_7$ requires C, 63.5; H, 6.95%). Mixed m.p., infrared spectra and rotation clearly demonstrate that this compound is different from tetrahydropalmarin

The crude acidic fraction (265mg.) obtained in the same was as the corresponding compound from palmarin had m.p. 213-19° (s. 207°), $[\alpha]_D$ + 36.5° (C, 2.02). Two quick crystallisations of this product from aqueous ethanol afforded clusters of short rods (95mg.) m.p. 214-17° (222°), $[\alpha]_D$ + 41° (C, 2.08).

This product hexhydroisojateorinic acid (LXXV) was shown to be identical with hexahydropalmarinic acid (m.p., mixed m.p., rotation and infrared spectrum.).



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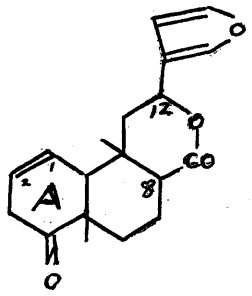

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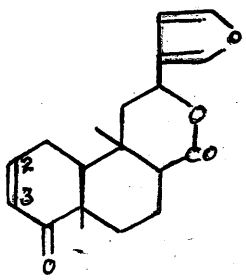

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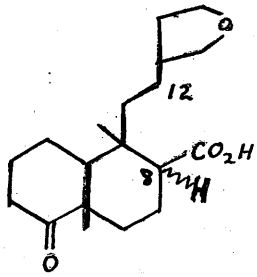

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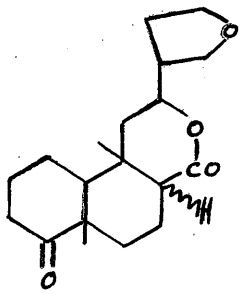


(V).

4. Some Transformation Products of Columbin

(A) Discussion

Although the constitution of columbin (1) as deduced by Barton and Elad[101] is certainly correct there remain several details to be rigorously established. Firstly there exists no chemical evidence which excludes position 12 as that which undergoes epimerisation during the columbin - isocolumbin transformation. Furthermore a chemical proof for the methyl group which has been allocated to position 9 is desirable. Finally since the biogenesis of columbin must involve some rearrangement of the normal diterpenoid biogenetic precursor, its stereochemistry and absolute configuration are of great theoretical importance.

The columbin - isocolumbin isomerisation takes place under the mild alkaline conditions which bring about the chasmanthin - palmarin transformation. The nature of the change may be discussed in the following way. On melting[89] columbin (1) loses one mole of carbon dioxide and is converted into decarboxycolumbin (11). The latter on hydrogenation affords[92] octahydrodecarboxycolumbinic acid (111) the structure of which has been rigorously proved by dehydrogenation (see p. 21). Since under the mild isomerisation conditions decarboxycolumbin (11) is converted[89] into a product identical with that obtainable by decarboxylation of isocolumbin, it follows that the isomerisation does not involve any position in ring A. Consideration of this together with the structures of columbin (1) and decarboxycolumbin (11) leads one to the conclusion that the only positions which would be expected to be labile to base are 8 and 12. As has been discussed previously (see p.117) the decision between these two possibilities requires only a comparison of the octahydro-acids (111) from decarboxycolumbin and decarboxyisocolumbin since these compounds lose their asymmetry at $C_{(12)}$ during

hydrogenolysis. Unfortunately although Wessely found the preparation of the crystalline octahydro-acid from decarboxycolumbin relatively simple all his attempts to prepare a similar product from decarboxy*iso*columbin were unsuccessful.[92] Wessely's decarboxy-*iso*columbin appeared to be a variable mixture and it was felt that resolution of this might facilitate further reactions.

When pure *iso*columbin was decarboxylated the product obtained was a definite mixture with properties similar to those described by Wessely.[89] Fractionation using crystallisation or chromatography separated one pure compound of mp. 213-14° and $[\alpha]_D$ -129° and another fraction of m.p. 202-4°, $[\alpha]_D$ -103° which was obviously a mixture. The problem of the nature of these products resolved itself when the ultraviolet absorption of the latter and the other intermediate fractions were determined. These results showed that the product of $[\alpha]_D$ -129° was essentially a pure $\beta:\gamma$ -unsaturated ketone whereas the products of more positive $[\alpha]_D$ contained up to 40% of $\alpha:\beta$ -unsaturated ketone ; i.e. λ max.(by difference) 235m.μ. (ε *ca* 3500) . This observation was subsequently confirmed by Cava[115] who found that further basic treatment of decarboxy*iso*columbin furnished the pure $\alpha:\beta$ -unsaturated ketone which he called ψ -decarboxy*iso*columbin (1V). The question now arises as to why such a mixture arises on decarboxylation of *iso*columbin while similar treatment of columbin affords essentially a pure product. This can best be explained by assuming that the conformation of decarboxy -columbin favours a 1:2 double bond e.g. (11) whereas the conformation of decarboxy*iso*columbin is such that a 2:3 double bond as depicted in (1V) is equally favoured.

Contrary to Wessely's experience,[92] it was found that careful working up of the product, obtained by hydrogenation of any of the

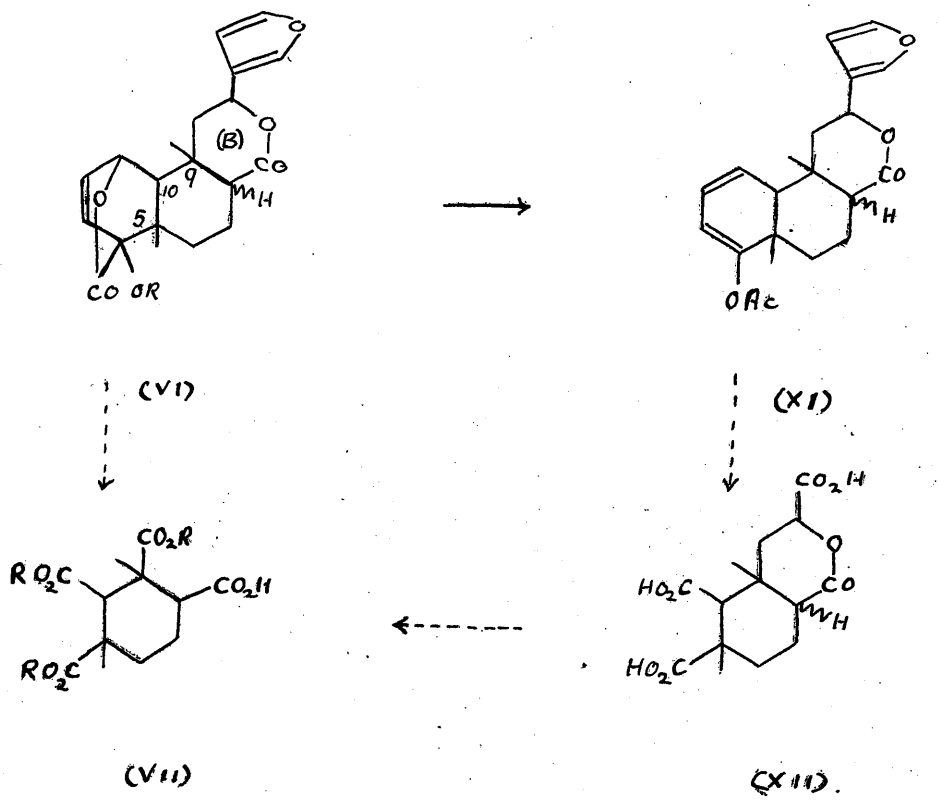
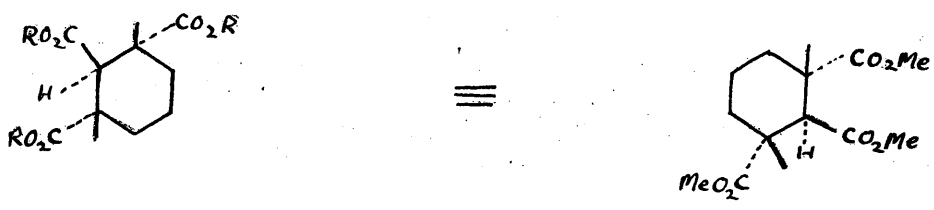


Fig. A



decarboxy*iso*columbin specimens, furnished two crystalline products. These were the octahydro-acid $C_{19}H_{30}O_4$, (111) which was the major product, and the neutral hexahydro-compound $C_{19}H_{28}O_4$ (V). In both cases these compounds were clearly different from the corresponding hydrogenation products of decarboxycolumbin.[92] The non-identity of the octahydro-acids therefore completely confirms that decarboxycolumbin and decarboxy*iso*columbin must differ in stereochemistry at position 8 as originally proposed by Barton and Elad.[101]

We next consider the remaining aspects of the columbin problem as listed on page 167. Figure A depicts two schemes by which it would appear possible to convert columbin or *iso*columbin (V1;R=H) to the tetracarboxylic acid (V11;R=H). Methylation of the latter followed by selective hydrolysis should afford the mono-carboxylic acid (V11;R=Me) decarboxylation of which should lead to the trimethyl ester (V111;R=Me). This ester (V111;R=Me) is of particular interest since, if it is not identical with methyl esters (1X) or (X) obtained from abietic acid (p. 33) and agathene dicarboxylic acid (p. 15) respectively, it should be accessible by synthesis. Consequently if a trimethyl ester such as (V111;R=Me) could be isolated by degradation of columbin, and its stereochemistry confirmed, then this would completely confirm not only the presence of methyl group at position 9 but also the absolute configuration and stereochemistry at position 5, 9 and 10 in columbin. Furthermore if the trimethyl ester (V111; R=Me) were shown to be identical with the corresponding product from abietic acid (1X) this would provide excellent supporting evidence for the biogenetic scheme proposed for columbin on page 126.

The preparation of (V11;R=H) was first attempted using potassium permanganate on a solution of *iso*columbin (V1) in which the lactone (B)

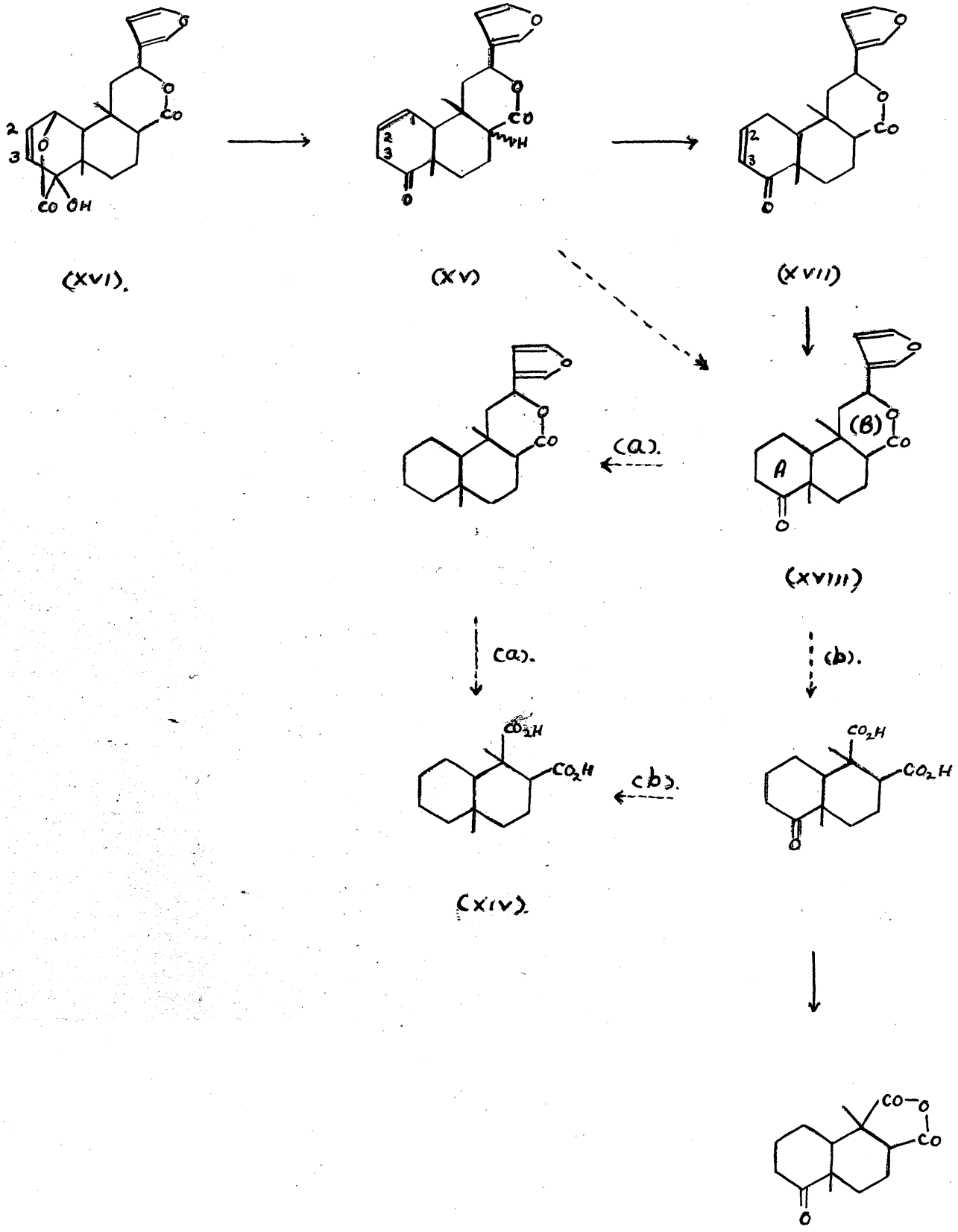


Fig. B.

system was open. Under these conditions there was obtained an amorphous acidic solid which could not be crystallised as such or after conversion to its methyl ester. Consequently the alternative approach via decarboxyacetyl*iso*columbin (Xl) was next investigated. Modification of Wessely's method[89] of acetylating *iso*columbin (Vl;R=H) furnished a better yield and improved the quality of the desired starting material acetyl*iso*columbin (Vl;R=Ac). This was then decarboxylated smoothly in the usual way to give decarboxyacetyl-*iso*columbin (Xl). The next stage, aimed at getting (Xll), was the key one and this was attempted by ozonolysis of decarboxyacetyl - *iso*columbin in methylene chloride solution at -60°. Unfortunately the product so obtained was amorphous* and could not be crystallised even after methylation and chromatography.

Having met with little success in the above scheme attention was turned to other possible methods of degrading columbin under controlled conditions to give a relatively simple product, e.g. the decalin dicarboxylic acid (XlV) capable of being synthesised. Two possible routes to this compound are shown in Figure B. These schemes involve selective hydrogenation of the nuclear double bond in decarboxycolumbin (XV) followed by (a) reduction of the carbonyl group and alkaline potassium permanganate oxidation of the furanyl side chain or (b) a reversal of this sequence.

Whereas selective reduction of the 2:3 double bond in columbin (XVl) is readily achieved a similar specific hydrogenation of the 1:2 ethylenic linkage in decarboxycolumbin (XV) could not be achieved despite considerable effort. It follows from this that the 1:2

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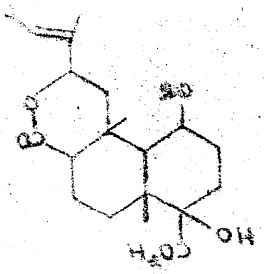
* More recent experience with ozonolysis products suggests that such an amorphous product may have been undecomposed ozonide, further treatment of which might have produced a crystalline product.

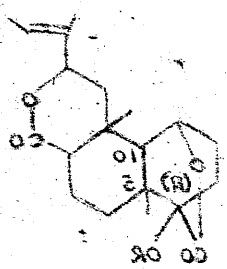
double bond in decarboxycolumbin (XV) must be much less accessible than the 2:3 double bond in columbin (XVI). In the light of our previous conclusion, that the change in conformation caused by isomerisation of decarboxycolumbin (XV) at position 8 favoured the 2:3 position for the ring A double bond rather than the 1:2 position, the solution to our problem of selective hydrogenation was relatively simple. Thus when decarboxycolumbin (XV) was hydrogenated in ethanolic sodium ethoxide over palladised charcoal one mole of hydrogen was smoothly consumed, presumably by (XVII), and there was obtained in high yield dihydrodecarboxyisocolumbin $C_{19}H_{24}O_4$ (XVIII). The very hindered nature of the carbonyl group in this compound was clearly demonstrated by its very weak reaction to Zimmerman's test[116] and also by the observation that it would not form a ketal.

Owing to the hindered nature of the carbonyl group in (XVIII) some experience of Barton et al's[117] modification of the Wolff-Kishner procedure for hindered ketones was first gained by carrying out a successful reduction of α-onoceradienedione. Unfortunately, application of this technique or the milder original version[118] to dihydrodecarboxyisocolumbin (XVIII) failed to produce any significant amounts of crystalline material. From the appearance of the complex hydroxyl and carbonyl absorption of the products it was evident that either incomplete reduction of the carbonyl group had taken place or in some way the vigorous alkaline conditions had irreversibly opened the lactone (B) system.

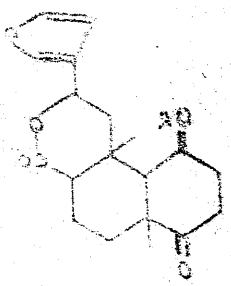
It is considered that a weak Zimmerman colour, such as is shown by dihydrodecarboxyisocolumbin (XVIII), indicates a low degree of enolisation and hence it follows that one may be able to oxidize the furanyl side chain of (XVIII) without affecting the ketonic carbonyl in ring A. Consequently (XVIII) was oxidized using Feists mildly alkaline potassium permanganate conditions[98] to give a non crystalline

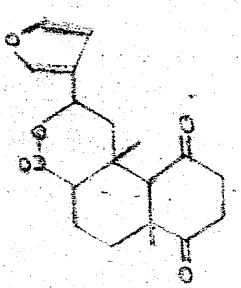


(XXI)

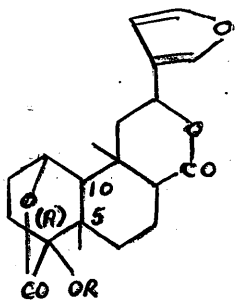


(XX)

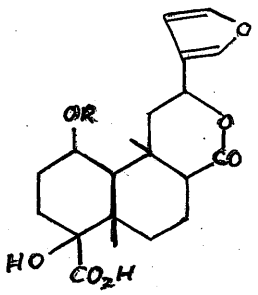




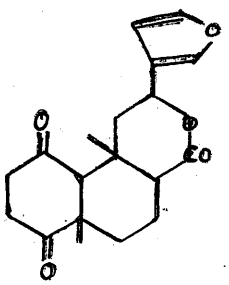
(XXII)



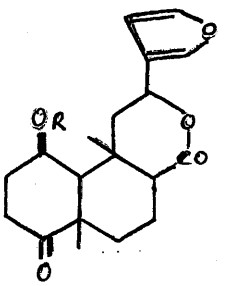
(XX).



(XXI)



(XXIII).



(XXII)

acidic product. The infrared spectra of the fractions obtained by fractional sublimation of the latter acidic product seemed to indicate oxidation of the ring A system. These spectra (nujol) showed bands at 1850 and 1780 (succinic anhydride) and 1740 - 1700 (carboxyl) $cm.^{-1}$ thus demonstrating that, although the desired oxidation of the side chain had occurred, some undesired oxidation of the carbonyl in ring A must also have taken place to account for the carboxyl absorption. Enolisation of the 4-keto group of (XVlll) was presumably the cause of this over-oxidation, consequently in an attempt to eliminate this a further oxidation was carried out in neutral conditions at 0°C. In this experiment the lactone (B) system was open, the excess alkali neutralised with carbon dioxide and aqueous potassium permanganate added in an atmosphere of carbon dioxide. Despite these precautions the product could not be obtained crystalline and although fractional sublimation furnished a small crystalline fraction with the carbonyl and succinic anhydride absorption expected of (XlX), the bulk of the sublimate was non crystalline and acidic.

Owing to the lack of success of the above schemes designed to reveal the complete structure and stereochemistry of columbin, the more conservative approach (XX;R=H) to (XXlll) was conceived in which the principles of conformational analysis[114] and molecular rotation differences[119] could be applied. Thus examination of the optical rotations of (XXl;R=H, R=Ac and R=Bz) and (XXll;R=H, R=Ac and R=Bz), and the stability of (XXlll) to base should afford conclusions on the absolute configuration of columbin and also the probable stereochemistry at $C_{(5)}$ and $C_{(10)}$.

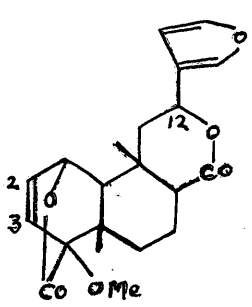
It was considered initially that rather vigorous conditions might be necessary to hydrolyse the bridged lactone ring (A) system consequently some model experiments were carried out on methyldihydro-

(XXIV).

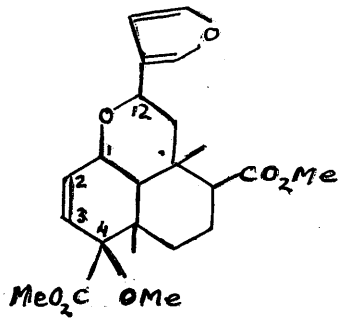
(XXV)

(XXVI).

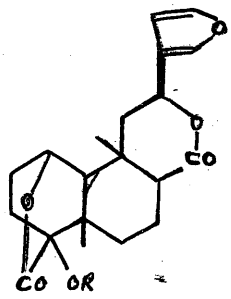
(XXVII).



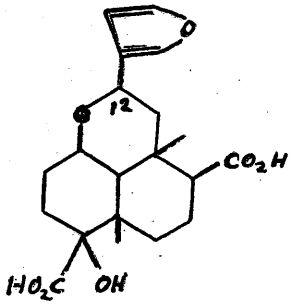
(XXIV).



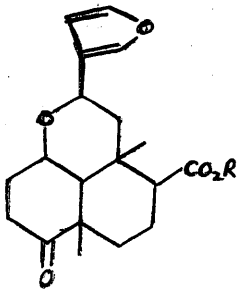
(XXV).



(XXVI).



(XXVII).



(XXVIII).

isocolumbin (XX;R=Me). These experiments were not particularly successful and therefore attention was concentrated on hydrolysis of the desired compound dihydroisocolumbin (XX;R=H) which fortunately went more readily. Thus under relatively vigorous alkaline conditions in an atmosphere of nitrogen dihydroisocolumbin, $C_{20}H_{24}O_6$, (XX;R=H) consumed two moles of base and furnished in good yield a crystalline acid $C_{20}H_{26}O_7$, which was presumed to be (XXI;R=H). The products obtained from normal acetylation or lead tetraacetate oxidation of this acid could not be crystallised. Oxidation, however, with chromium trioxide in pyridine[120] furnished a crystalline product $C_{19}H_{24}O_5$. The properties of this compound were not those of the expected diketone (XXIII); thus the product was obviously acidic since it consumed one mole of base by titration and formed a crystalline mono-methyl ester $C_{20}H_{26}O_5$. Further evidence regarding the nature of the oxygen functions in the acid $C_{19}H_{24}O_5$ and its methyl ester can be obtained by examination of their respective spectral properties. Thus the infrared spectrum (nujol) of the acid showed bands at 3400 - 2500 (associated hydroxyl of carboxyl), 1697 (superimposed carboxyl and cyclohexanone) and 1505 (furan) $cm.^{-1}$. In addition the acid and its methyl ester show two maxima in their ultraviolet absorption, one at 211 to 212 m.μ. (ε ca. 5400) and the other at 286 to 287 m.μ. (ε = 118 and 55 respectively), suggestive of a furan ring system and one or two carbonyl groups.

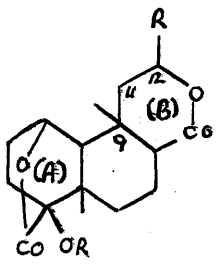
In the light of the above work an experiment of Wessely's,[93] which had hitherto defied explanation, assumed a new importance. Thus it was claimed that on prolonged alkaline treatment under nitrogen methylisocolumbin $C_{21}H_{24}O_6$ (XXIV) consumed irreversably two moles of base to give an acidic product, methylation of which furnished a small yield of the incompletely characterised dimethyl ester $C_{23}H_{30}O_7$. By repitition of this work these observations

were confirmed and the crystalline methyl ester completely characterised by analysis, which showed 3 methoxyl groups, optical rotation and infrared spectrum. The latter in CCl_4 showed bands at 1730 (carbomethoxyl) and 1505 (furan) $cm.^{-1}$ but no hydroxyl. It thus appears that of the seven oxygen atoms in $C_{23}H_{30}O_7$, four are present in the two carbomethoxyl groups, one is present in a simple methoxyl group and another in a furan ring. The remaining oxygen atom, since it cannot be present as a carbonyl or hydroxyl function, must be present in an inert ether linkage. Of these functions the furan ring system, and methoxyl are present in the starting material, methyl<u>iso</u>columbin (XXIV), while the two carbomethoxyl groups are most likely to be derived from the carbonyl groups of the two lactone systems. It appears therefore that the inert ether function in $C_{23}H_{30}O_7$ must be related to the alkyl-oxygens of the two lactones at $C_{(1)}$ and $C_{(12)}$ in its precursor methyl<u>iso</u>columbin (XXIV). On the basis of the above facts and conclusions the dimethyl ester $C_{23}H_{30}O_7$ would appear to be best represented by (XXV) in which the potential hydroxyl groups in the starting material at $C_{(1)}$ and $C_{(12)}$ have interacted to form a rigid tetrahydropyran system. That there must be hydrogenolysable carbon-oxygen bonds at $C_{(12)}$ and $C_{(1)}$ as depicted in formulation (XXV) for the dimethyl ester $C_{23}H_{30}O_7$ can be deduced in the following way. Under carefully controlled conditions the dimethyl ester in acetic acid containing 0.4% hydrogen chloride, (two promote hydrogenolysis) was hydrogenated over platinum in a microhydrogenator. This reaction was carried out twice 4.7 and 4.8 moles of hydrogen being consumed respectively. Since it is known that the dimethyl ester must contain 3 double bonds it follows that 1.8 moles of hydrogen must have been utilized for hydrogenolytic fission prior to hydrogenation. Under the conditions used it is known (see p.182) that a furan system, containing an ethereal oxygen atom

attached to the α-carbon atom of a substituent [that is a system analagous to that depicted in (XXV)] , consumes 1.3 moles of hydrogen for hydrogenolysis. The remaining 0.5 molesof hydrogen used in the hydrogenolysis of the dimethyl ester must therefore be associated with carbon-oxygen bonds allylic to the 2:3 double bond. Of the two such bonds depicted in formulation (XXV) there can be no doubt about the position of the methoxyl group attached to position 4, whereas the other ethereal oxygen attached to position 1 , remains to be proved. Assuming that there is only one hydrogenolysable function, for example the methoxyl attached to $C_{(4)}$, then the consumption of 0.5 moles of hydrogen for hydrogenolytic cleavage requires that hydrogenation and hydrogenolysis must take place at equal rates. From the known ease of hydrogenation of the 2:3 double bond, and the hindered nature of the tertiary methoxyl group attached to $C_{(4)}$, it would appear reasonable to assume* that hydrogenolytic cleavage would be much slower, and hence much less than 0.5 of a mole of hydrogen would be consumed. The observed uptake of hydrogen for hydrogenolysis can only be explained if there is another oxygen function attached to the alternative position at $C_{(1)}$as depicted in (XXV). The above argument therefore conclusively establishes that the cyclic ether function present in the dimethyl ester $C_{23}H_{30}O_7$ must be attached to positions 1 and 12.

By analogy with the hydrolysis of methyl<u>iso</u>columbin (XXIV) the dicarboxylic acid $C_{20}H_{26}O_7$ previously mentioned (p.178) which was obtained by hydrolysis of dihydro<u>iso</u>columbin (XXVI;R=H) is formulated as (XXVII). In agreement with its formulation the latter on hydrogenation in acetic acid over palladised charcoal

* It should be noted that a reasonable estimate of the hydrogenolysis of the $C_{(4)}$ methoxyl group under these conditions could be relatively easily obtained by comparing the hydrogen uptake of methyl<u>iso</u>columbin (XXIV) and dihydromethyl<u>iso</u>columbin (XXVI;R=Me). Unfortunately no time was available for this experiment.



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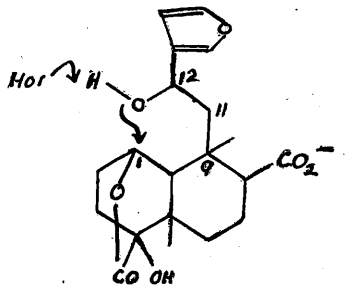

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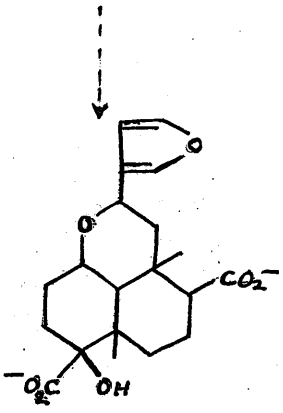
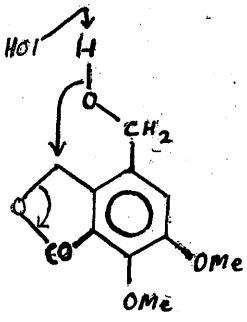
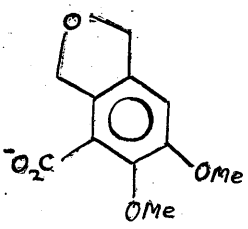


(xxxi).

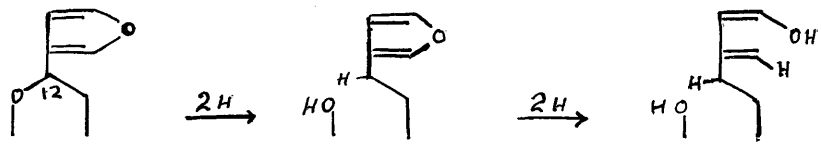


(xxxii).

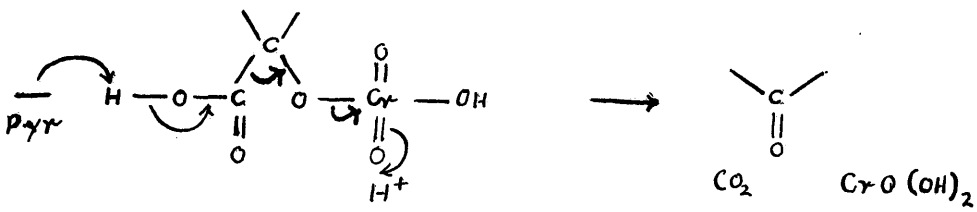


(xxxiii).

absorbed 1.8 moles of hydrogen indicating that no hydrogenolysis took place under these conditions. However when the dicarboxylic acid (XXVll) was hydrogenated in acetic acid containing 0.4% hydrogen chloride, 3.3 moles of hydrogen were consumed indicating that 1.3 moles of hydrogen had been used for hydrogenolysis. This can be explained by assuming that under the reaction conditions not only the $C_{(12)}$- oxygen bond but also a proportion of the carbon-oxygen bond of the furan ring is hydrogenolysed[121] as shown below:



The keto acid, $C_{19}H_{24}O_5$, obtained by oxidation of the dicarboxylic acid, $C_{20}H_{26}O_7$, (XXVll) must be formulated as (XXVlll; R=H) which is complete agreement with its spectral properties discussed on p.178. Although the oxidative degradation of an α-hydroxy carboxylic acid under the mild[119] chromium trioxide pyridine conditions may seem a little unexpected at first this is not difficult to explain mechanistically viz.



As additional evidence in support of structure (XXV) (XXVll) and (XXVlll) it is now proposed to demonstrate how these compounds may be derived by a simple but acceptable two stage mechanism viz. (XXlX; R= β-furanyl, R'=H) to (XXXl). In the first stage one mole of base is selectively consumed and the mono-sodium salt (XXX) formed. Such a selective opening of the lactone (B) system is

quite feasible and can be readily demonstrated experimentally. In the intermediate (XXX) rotation about the $C_{(9)}$ - $C_{(11)}$ bond is completely unhindered thus enabling the $C_{(12)}$ hydroxyl group to assume the ideal position for the key step in the mechanism which is a nucleophilic displacement at $C_{(1)}$ of the alkyl-oxygen bond of the lactone (A) system. Whether the hydrogen atom attached to the $C_{(12)}$ hydroxyl is removed as a proton before or after cyclisation cannot be deduced from the present evidence. The latter step in this mechanism is completely analagous to the hydroxymethylphthalide rearrangement, recently investigated by Newbold et al,[122] which involves the transformation under basic conditions of the hydroxy-lactone (XXXll), into the compound (XXXlll) containing a cyclic ether ring and a carboxyl group. The reacting functional groups in (XXX) and (XXXll) are very similar in that both contain ideally situated hydroxyl groups and strongly hindered lactone carbonyl functions. It thus appears that, under these rather special steric and geometric conditions, the normal nucleophilic attack by hydroxyl ion on the lactone carbonyl is slowed down to such an extent that the intramolecular nucleophilic displacement of the alkyl-oxygen by the hydroxyl group becomes the predominant reaction.

(β) Experimental

For general experimental see p. 128.

Columbin (1).

The product, crystallising in needles m.p.* 178-81° dec. $[\alpha]_D$ + 40°, obtained in page /3/ is essentially columbin. Repeated recrystallisation of this material affords pure columbin of $[\alpha]_D$ ca + 50°. In most cases this step is not required since the contaminants are very readily removed after isomerisation or decarboxylation.

iso Columbin (1)

(cf. Wessely et al)[89]

Crude columbin (2.00g.), $[\alpha]_D$ + 38°, m.p.* 179-81° dec., was isomerised as described on page /34. Recrystallisation of the crude product from aqueous ethanol afforded pure isocolumbin (1.17g.), $[\alpha]_D$ + 74.5° (C, 2.02) m.p.* 180-83° dec..

Decarboxyisocolumbin (11)

(cf. Wessely et al)[89]

isoColumbin (2.21g.), $[\alpha]_D$ + 72°, was heated at 220° in a silicone bath for 15 mins. under an atmosphere of nitrogen. Recrystallisation of the product from chloroform-ethanol gave rods (1.40g.) m.p.* 200-5° (s. 185°)

The total product was dissolved in chloroform-benzene (1 : 20; 200ml.) and chromatographed over silica

(120g.). Elution with benzene containing up to 20% ether afforded 10 fractions (1.15g.) of m.p. above 198°. By slowly increasing the proportion of ether in the benzene to 75% there was obtained a further eight fractions (500mg.) of m.p. between 187-197°.

Crystallisation of fractions 1 to 10 from chloroform-ethanol and then acetone gave a product (400mg.) m.p.* 213-14°, $[\alpha]_D$ -129° (C, 3.48). Fractions 11-18 were combined to give a product m.p. 202-4° (s. 190°), $[\alpha]_D$ - 103° (C, 1.12). Below the ultraviolet spectra of decarboxyisocolumbin $[\alpha]_D$ -129° and the mother liquors from decarboxyisocolumbin $[\alpha]_D$ -103° are compared with that of decarboxycolumbin.

	1 ($\beta:\gamma$-) Decarboxy-columbin $[\alpha]_D$ -19°	2 ($\beta:\gamma$-) Decarboxy-isocolumbin $[\alpha]_D$ -129°	3 ($\alpha:\beta + \beta:\gamma$-) crude mother liquors from material $[\alpha]_D$ -103°	(3 - 1)	(2 - 1)
$\lambda m\mu$	ε	ε	ε	ε	ε
210	6270	7230	7440	1170	960
215	5870	6230	6920	1150	860
220	3570	4330	5960	2390	760
225	1770	2540	5250	3480	770
230	785	1610	4920	4135*	825
235	580	1370	4710	4130*	790
240	515	1170	4250	3735	665
245	433	895	3560	3127	462
255	289	425	2480	1191	136
280	198	258	1350	1152	60

Hydrogenation of decarboxyisocolumbin (II).

Decarboxyisocolumbin m.p. 207-9°, $[\alpha]_D$ -125°, (185mg.) was hydrogenated in "Analar" acetic acid (30ml.) over 10% palladised charcoal (64mg.); 3.6 moles of hydrogen were rapidly absorbed.

After filtration of the catalyst and evaporation of the acetic acid in vacuo the residue was separated into acid and neutral fractions.

The neutral fraction hexahydrodecarboxyisocolumbin (V) (44mg.) crystallised from acetone-petrol as needles m.p. 182-3°, $[\alpha]_D$ + 67° (c, 0.75 $CHCl_3$). (Found: C, 71.00; H, 9.15. $C_{19}H_{28}O_4$ requires C, 71.20; H, 8.80%).

Acidification of the bicarbonate extract followed by careful working up in the usual way furnished crude octahydrodecarboxyisocolumbinic acid (III) (160mg.). On standing in acetone-petrol this slowly crystallised as rosettes of fine needles m.p. 130-3°, $[\alpha]_D$ + 59° (c, 1.24 $CHCl_3$). (Found: C, 70.75; H. 9.2. $C_{19}H_{30}O_4$ requires C, 70.75; H, 9.4%).

Attempted oxidation of isocolumbin (VI ; R = H)

isocolumbin $[\alpha]_D$ + 72° (175mg.) in hot acetone (4ml.) was heated with N sodium hydroxide solution (0.6ml.; 1.2 moles) for 2 mins. on the steam.

Aqueous potassium permanganate (3.3%, 31ml. ≡ 1.1 theoretical) was slowly dropped from a burette into the well-stirred solution under an atmosphere of carbon dioxide. The time of addition was 2 hrs. and stirring

was continued for another 2hrs. whence there was still a slight excess of the oxidant.

Working up in the usual way furnished an amorphous acidic solid (100mg.). Neither this nor its methyl ester could be obtained crystalline.

Acetylisocolumbin (VI ; R = Ac)

(cf. Wessely et al[89])

isoColumbin (4.03g.) $[\alpha]_D$ + 75°, "Analar" acetic anhydride (ca. 125ml.) and fused sodium acetate (14g.) were heated in a silicone bath at 145° for 2 hrs. This method was found to be much more efficient than that of Wessely et al[89] who refluxed for 6 hrs. The product crystallised from acetone-alcohol as needles (3.10g.), m.p.* 214-15° dec., $[\alpha]_D$ + 22.5° (c, 3.17).

Decarboxyacetylisocolumbin (XI)

(After Wessely et al[89])

Acetylisocolumbin (2.75g.) afforded decarboxyacetyl-isocolumbin (XI) (2.21g.) as needles from aqueous ethanol m.p.* 164-65° (s. 162°), $[\alpha]_D$ - 333° (c, 3.12).

Ozonolysis of decarboxyacetylisocolumbin (XI)

The dried compound (100mg.) in dry methylene chloride (30ml.) was treated with ozone at -60°. After 10 mins. the furan and diene absorption had disappeared (ε = 1900 at 212 mμ and ε = 730 at 270 mμ) Water (1ml.) was added and the suspension heated on the steam for 10 mins.

The product could be precipitated from acetone solution using benzene, chloroform or ether. Fractional precipitation appeared to produce two amorphous products of m.p. 145-50° dec. and 175-235°.

In another similar experiment the crude ozonolysis acid (300mg.) was chromatographed over silica (18g.) using 10% acetone in benzene as eluant. The proportion of acetone was slowly increased to 30% and 20 fractions were taken. Fractions 5-11 (110mg.) were combined and repeatedly precipitated from acetone using ether to give an amorphous solid (19mg.) m.p.* 150-7° (s. 140°).

Dihydrodecarboxyisocolumbin (XVIII)
(cf. Barton et al[125])

Under a wide variety of conditions many unsucessful attempts were made to selectively hydrogenate decarboxycolumbin.

Sodium (10.1g.) was dissolved in dry ethanol (330ml.) and to this solution was added 10% palladised charcoal (2.00g.). When the catalyst was saturated decarboxycolumbin (7.76g.), m.p. 144-5° was added and the hydrogenation uptake noted, 1.1 moles being absorbed. The product crystallised from methanol as flat rods (6.12g.) m.p. 214-18°. Sublimation followed by recrystallisation gave a m.p. 215-16°, $[\alpha]_D$ + 30° (c, 2.82 in $CHCl_3$) (Found: C, 72.0; H, 7.4. $C_{19}H_{24}O_4$ requires C, 72.1; H, 7.65%).

Attempted ketal formation of (XVIII).

(cf. Sarett et al[120])

The compound (200mg.), p-toluene sulphonic acid (5mg.), ethylene glycol (0.25ml.) and "Analar" benzene (30ml.) were refluxed in a constant water separator. After 24 hrs. and 48 hrs. samples were withdrawn and carefully worked up under mildly alkaline conditions. In both cases the infrared spectrum of the product was identical with the starting material.

In another attempt using twice as much ethylene glycol, five times as much p-toluene sulphonic acid and dry toluene as solvent no reaction seemed to have taken place after 60 hrs.

Attempted Wolff-Kishner reduction of dihydrodecarboxyisocolumbin (XVIII).

(1) Sealed tube

Sodium (200mg.) was dissolved in dry ethanol (2ml.) in a micro Carius tube. To this solution was added the compound (103mg.) and anhydrous hydrazine (1 ml.). The tube was sealed and heated 15 hrs. at 185°. The product was worked up in the usual way, the hydrazine being removed by acid washing to give as the product, a clear gum (76mg.).

Careful chromatography over silica (4g.) using benzene-petrol mixtures, benzene and benzene-ether mixtures up to 40% ether furnished 15 fractions. Fractions 3, 4 and 5 contained small quantities (ca 3mg. in all) of a product crystallising in needles m.p. 145-65°. Despite a carefully

repeated experiment there was obtained no increase in the yield of this product.

(2) Barton modification[117]

The standard conditions were applied successfully to α-onoceradienedione but despite careful working up, followed by careful chromatography no crystalline product could be obtained when this method was applied to (XVIII).

The infrared spectra of the various chromatographic fractions showed hydroxyl and complex carbonyl absorption (1750-1700 cm^{-1}).

Oxidation of dihydrodecarboxyisocolumbin (XVIII) with potassium permanganate.

(1) At 20-30°

The compound was oxidized according to the method used by Feist et al[98] on methylpalmarin. The product obtained could not be crystallised, either before or after fractional sublimation.

(2) In a carbon dioxide atmosphere at 0°

The compound (190mg.) in acetone (4ml.) and N sodium hydroxide (1ml.; 3 moles) was heated for 3 mins. on the steam. Water (6ml.) and manganese sulphate (ca 5mg.) were added and the solution stirred at 0° in an atmosphere of carbon dioxide; the pH was 8.5. To this cooled well-stirred solution potassium permanganate solution (4%; 30ml.) was added from a burette over 90 mins.

The product was carefully worked up in the usual way and separated into acidic and netural fractions. The latter was negligible and the former was a clear gum (120mg.) which could not be crystallised. Fractional sublimation of the gum at 10^{-2}mm. furnished three fractions. Fraction 1 (ca 2mg.) obtained at 98° showed peaks at 1850, and 1765 cm^{-1} and appeared to crystallise slowly on standing. Fractions 2 and 3 obtained at 120° and 130° would not crystallise and showed strong absorption at 1720-1690 and 3400-2450 cm^{-1} in addition to the above bands.

A careful repeat of this experiment using the theoretical amount of more dilute potassium permanganate solution (2%) gave no better results.

Dihydroisocolumbin (XXVI; R = H)

(cf. Barton and Elad[101])

isoColumbin (5.23g.), $[\alpha]_D$ + 68° in ethyl acetate (500ml.) was hydrogenated over 1% palladium calcium carbonate (1.2g.), prepared as described by Vogel[126]. Within 40 mins. hydrogenation was complete, 1 mole having been absorbed. The catalyst was removed by filtration and the solvent evaporated to give dihydroisocolumbin (XXVI; R = H). This crystallised from aqueous ethanol as well-formed rods (4.23g.) m.p.* 228-31°; $[\alpha]_D$ + 32° (c, 3.41).

Methyldihydroisocolumbin (XXVI; R = Me)

(In collaboration with Dr. Dov Elad.)

(a) Dihydroisocolumbin (1.00g.), ethanol (10ml.) and aqueous sodium hydroxide (12%; 5ml.) were heated on the steam bath

until all was dissolved. Dimethyl sulphate (6 x 2.6 ml.) and aqueous sodium hydroxide (12%; 6 x 10ml.) were added alternately, the temperature being kept at 40 to 50°. Crystallisation of the product from ethanol gave dihydromethylisocolumbin (XXVI; R = Me) (300mg.) m.p.* 236-38°, $[\alpha]_D$ + 65° (c, 1.14, $CHCl_3$) (Found: C, 67.2; H, 6.85; OMe, 8.3. $C_{21}H_{26}O_6$ requires C, 67.35; H, 7.0; 1 OMe, 8.2%).

(b) Methylisocolumbin (m.p.* 215-17° dec.; 650mg.) (see p.196) in 19 : 1 ethyl acetate acetone (250ml.) was hydrogenated over 1% palladised calcium carbonate[126] (200mg.). The product was identical (m.p. and mixed m.p.) with that described above.

Attempted hydrolysis of methyldihydroioscolumbin (XXVI; R = Me)

The compound (87mg.) was treated with N sodium hydroxide (4ml.) as described below ; 1.95 moles of alkali were consumed.

Working in the usual way furnished a very small neutral fraction and a gummy acidic material (80mg.). Treatment of the latter with methanol furnished needles (ca 5mg.) which softened at 130° with much frothing. Recrystallisation did not alter this.

Dihydroisocolumbinic acid (XXVII)

isoDihydrocolumbin (993mg.) suspended in N sodium hydroxide (40ml.) was heated for 5 hrs. at 100° in a vigorous

stream of nitrogen. Complete solution was obtained in 20 mins. and titration at the end of the reaction demonstrated that two moles of base had been consumed.

The product was separated into acid and neutral fractions. The latter was very small and shown to be unchanged starting material by m.p. and mixed m.p.. The acidic fraction dihydroisocolumbinic acid (XXVII) (900mg.) crystallised from acetone-benzene in stout cubes (462mg.) m.p.* 220-22° d. (s. 218°), $[\alpha]_D$ + 21° (c, 1.91) (Found: C, 63.75; H, 6.75. $C_{20}H_{26}O_7$ requires C, 63.5; H, 6.95%)

An attempt to acetylate this compound using acetic anhydride and pyridine overnight at 20° gave a gummy product which could not be crystallised despite chromatography.

Microhydrogenation of dihydroisocolumbinic acid (XXVII)

(1) Using palladised charcoal in acetic acid

The acid, m.p.* 217-19° dec. (14.3mg.) was added to presaturated palladium charcoal (11mg.) in "Analar" acetic acid (5ml.). The uptake of hydrogen is shown below.

Time (hr., min.)	0,20	0,43	1,15	1,35	2,20	3,25	4,25	11,57	20,35
Moles of H_2 absorbed	0.02	0.12	0.21	0.27	0.43	0.67	0.83	1.61	1.76

(2) Using platinum oxide in acetic acid containing hydrogen chloride.

The acid m.p.* 217-19° dec. (13.5mg.) was added to presaturated Adam's catalyst (10mg.) in "Analar" acetic acid (5ml., containing 1% 10 N hydrochloric acid).

Time (hr., min.)	0,20	0,45	0,58	1,15	1,25	1,40	2,00	2,35	2,50
Moles of H_2 absorbed	0.63	1.15	1.99	2.60	2.96	3.18	3.25	3.29	3.30

Oxidation of dihydroisocolumbinic acid (XXVII) using chromium trioxide in pyridine.

(cf. Sarett et al[120])

Chromium trioxide (400mg.) was added in small portions over 15 mins. to "Analar" pyridine (4ml.) cooled to 10°. The acid (241mg.) in "Analar" pyridine (1.5ml.) was then added to this yellow complex and the whole vigorously shaken for 5 mins. giving a homogeneous light brown sludge.

After some 15 hrs. the reaction mixture was poured into dilute acid solution and the product worked up to give acidic and neutral fractions. The latter was negligible and the former crystallised from benzene-petrol to give the keto-acid (XXVIII; R = H) as needles (94mg.) m.p. 204-7°, $[\alpha]_D$ + 16.5° (c, 2.14 $CHCl_3$), λ max. 286mμ (ε = 118) (Found: C, 69.2, 69.1; H, 7.55, 7.3. $C_{19}H_{24}O_5$ requires C, 68.65; H, 7.3%).

An attempt to make an oxime of this ketone using hydroxylamine hydrochloride in pyridine produced a product which could not be crystallised.

Keto-acid (XXVIII; R = H) was recovered unchanged after 1½ hrs. at 100° in 5% methanolic potassium hydroxide.

Methylation of keto-acid (XXVIII; R = H)

The acid m.p. 204-6° (75mg.) in benzene-ether was treated with excess distilled ethereal diazomethane for 10 mins. The crystalline product, being contaminated with an amorphous material, was chromatographed in benzene-petrol (1 : 1) over silica. All the fractions crystallised from methanol as needles m.p. 157-8° but these were still slightly contaminated. Further crystallisation from petrol furnished needles of the methyl ester (XXVIII; R = Me) m.p. 159-60°; $[\alpha]_D$ + 21° (c, 1.24; $CHCl_3$), λ max. 212mμ (ε = 5,300) and 287mμ (ε = 55) (Found: C, 69.85; H, 7.9; OMe, 9.55. $C_{20}H_{26}O_5$ requires C, 69.35; H, 7.55; OMe, 8.95%).

Methylisocolumbin (XXIV)
(After Wessely et al[93])

isoColumbin, $[\alpha]_D$ + 69° was methylated as described to give on recrystallisation from acetone-alcohol methylisocolumbin (XXIV) m.p. 215-17°, $[\alpha]_D$ + 60° (c, 0.98 in $CHCl_3$).

Hydrolysis of methylisocolumbin (XXIV)
(After Wessely et al[93])

Methylisocolumbin (165mg.) in ethanol (1 ml) was

heated at 100° with $^{N}/_{10}$ sodium hydroxide (15ml.) for 10 hrs. under nitrogen; 1.9 moles of alkali were consumed.

The product was carefully worked up and separated into acidic and netural fractions in the usual way. The neutral fraction (15mg.) was identical (m.p. and mixed m.p.) with the starting material. The acidic fraction in benzene was left overnight with excess distilled ethereal diazomethane solution to give the dimethyl ester (XXV) as plates (70mg.) m.p. 118-20°, $[\alpha]_D$ + 219° (c, 2.78 in $CHCl_3$) (Found: OMe, 21.5%. $C_{23}H_{30}O_7$ requires 3OMe, 22.2%.)

Although the m.p. of this ester was identical with that quoted by Wessely[93] it was found that the product was improved by chromatography over alumina (III) using 2 : 5 ether - benzene as eluant. Thus there were obtained flat rods m.p. 136-37° (s. 135°), $[\alpha]_D$ + 223° (c, 2.63 in $CHCl_3$).

Microhydrogenation of dimethyl ester (XXV)

The ester (16.3mg.), m.p. 136-7° was added to presaturated Adam's catalyst (12mg.) in "Analar" acetic acid (5ml.; containing 1% 10N hydrochloric acid). The molar uptake of hydrogen is noted below.

Time (hr., min)	0,19	1,02	1,17	1,37	1,55	3,24
Mole H_2 absorbed	1.71	3.75	4.20	4.53	4.62	4.80

In a repeat experiment 4.65 moles of hydrogen were absorbed. The combined products (32mg.) in benzene were carefully chromatographed over alumina (III) but no crystalline product could be obtained.

REFERENCES

1. Eschenmoser, Ruzicka, Jeger and Arigoni, Helv., 1955, 38, 1890.
2. Vesterberg, Ber., 1903, 36, 4200.
3. Simonsen and Barton, "The Terpenes", Vol. III, Cambridge University Press.
4. Barton, "Chemistry of Carbon Compounds", Vol. IIB, Chapter 15, Rodd Elsevier Publishing Co.
5. Willstatter and Oppé, Annalen, 1911, 378, 1.
6. Fischer and Lowenberg, Annalen, 1929, 475, 183.
7. Ruzicka, Siedel and Engel, Helv., 1942, 25, 621.
8. Hosking and Brandt, Ber., 1936, 69, 780.
9. Zeiss and Grant, J. Amer. Chem. Soc., 1957, 79, 1201.
10. Hosking and Brandt, Ber., 1935, 68, 1311.
11. Buchi and Pappas, J. Amer. Chem. Soc., 1954, 76, 2963.
12. Lederer, Mercier, and Perot, Bull. Soc. Chim., 1947, (5) 14.
13. King and Jones, J., 1955, 658.
14. Cocker and Halsall, J., 1956, 4262.
15. Djerassi and Marshall, Tetrahedron, 1957, 1, 238.
16. Ruzicka and Lardon, Helv., 1946, 29, 912.
17. Ruzicka and Bernold, Helv., 1941, 24, 1167.
18. Burn and Rigby, J., 1957, 2964.
19. Hardy, Rigby and Moody, J., 1957, 2955.
20. Dietrich and Lederer, Helv., 1952, 35, 1148.
21. Cocker, Edward and Holley, Chem. and Ind., 1955, 772.
22. Barton and Elad, J., 1956, 2085, 2090.
23. Barton and Overton, J., 1955, 2639.
24. Wenkert, Chem. and Ind., 1955, 282.
25. Campbell and Todd, J. Amer. Chem. Soc., 1942, 64, 928.
26. King, King and Topliss, J., 1957, 573.
27. Bredenberg, Acta. Chem. Scand., 1957, 11, 932.
28. Brandt and Thomas, J., 1952, 2442.

28(a). Barltrop and Rogers, Chem. and Ind., 1957, 20.

29. Ruzicka and Sternbach, Helv., 1940, 23, 124.

30. Harris and Sanderson, J. Amer. Chem. Soc., 1948, 70, 2081.

31. Ukita, Tsumita and Utsugi, Pharm. Bull. (Jap.) 1955, 3, 441.

32. Harris, Whalley and Robertson, Symposium on Terpenoid Compounds, Glasgow, July, 1957.

33. Ruzicka, de Graff and Muller, Helv., 1932, 15, 1300.

34. Haworth, J., 1932, 2717

35. Ruzicka, Sternbach and Jeger, Helv., 1941, 24, 504.

36. Stork and Schulenberg, J. Amer. Chem. Soc., 1956, 78, 250.

37. Ruzicka and Kaufmann, Helv., 1941, 24, 939.

38. Harris and Sanderson, J. Amer. Chem. Soc., 1948, 70, 339.

39. Humber and Taylor, J., 1955, 1044.

40. King and King, J., 1953, 4158.

41. Woodward and Eastman, J. Amer. Chem. Soc., 1950, 72, 399.

42. King, Godson and King, J., 1955, 1117.

43. Beer, Dutton, Jaquiss, Robertson and Savige, J., 1956, 4850.

44. Campbell and Tood, J. Amer. Chem. Soc., 1942, 64, 928.

45. King, King and Topliss, Chem. and Ind., 1956, 113.

46. Brandt, New Zealand J. Sci. Technol., 1952, 34 B, 46.

47. Bottomley, Cole and White, J., 1955, 2624.

48. Djerassi, Riniker and Riniker, J. Amer. Chem. Soc., 1956, 78, 6362.

49. Djerassi, Cais and Mitscher, J. Amer. Chem. Soc., 1958, 80, 247.

50. Mossettig and Nes. J. Org. Chem., 1955, 20, 884.

51. Djerassi, Smith, Lippman, Figdor and Herran, J. Amer. Chem. Soc., 1955, 77, 4801.

52. Haworth, Jubb and McKenna, J., 1955, 1983.

53. Djerassi, Bendas and Sengupta, J. Org. Chem., 1955, 20, 1046.

54. Djerassi and Bendas, Chem. and Ind., 1955, 1481.

55. Haworth and Johnstone, J., 1957, 1492.

56. MacMillan, Cross, Grove and Mulholland, Proc. XVIth Congress of Pure and App. Chem., Paris, 1957, 265.

57. Bredenberg, Acta Chem. Scand., 1957, 11, 927.

58. Birch, "Perspectives in Organic Chemistry", Chap.6, Todd, Interscience Publishers, 1956.

59. Ruzicka, Experientia, 1953, 357.

60. Popjak, "Chemistry, Biochemistry and Isotopic Tracer Technique", R.I. Chem. Monograph No. 2, 1955.

61. Bloch and Rittenberg, J. Biol. Chem., 1942, 145, 625.

62. Clayton and Bloch, J. Biol. Chem., 1956, 218, 305.

63. Dauben and Richards, Chem. and Ind., 1955, 94.

64. Popjak, Arch. Biochem. Biophys., 1954, 48, 102.

65. Tchen and Bloch, J. Biol. Chem., 1957, 226, 931.

66. Olson, Lindberg and Bloch, J. Biol. Chem., 1957, 226, 941.

67. Cox, King and King, Proc. Chem. Soc., 1957, 290.

68. Djerassi, Mills and Villotti, J. Amer. Chem. Soc., 1958, 80, 1005.

69. Mazur, Weizmann and Sondheimer, J. Amer. Chem. Soc., 1958, 80, 1007.

70. Tavormina, Gibbs and Huff, J. Amer. Chem. Soc., 1956, 78, 4498.

71. Braithwaite and Goodwin, Biochem. J., 1957, 67, 13P.

72. Tchen, J. Amer. Chem. Soc., 1957, 79, 6344.

73. Cornforth, Cornforth, Popjak and Youhotsky-Gore, Biochem. J., 1957, 66, 10P.

74. Admur, Rilling and Bloch, J. Amer. Chem. Soc., 1957, 79, 2646.

75. Birch, Seminar at Glasgow University, Dec., 1957.

76. Birch, English, Massy-Westrope and Herchel Smith, J, 1958, 369.

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

78. Cornforth, Youhotsky-Gore and Popjak, Biochem. J., 1957, 65, 94.

79. Kappeler, Stauffacher, Shinz and Eschenmoser, Helv., 1954, 37, 957.

80. Stork and Burgstahler, J. Amer. Chem Soc., 1955, 77, 5068.

81. Wenkert, Chem.and Ind., 1955, 282.

82. Weisner, Armstrong, Bartlett and Edwards, J. Amer. Chem. Soc., 1954, 76, 6068.

83. Roberts, Lee and Saunders, J. Amer. Chem. Soc., 1954, 76, 4501.

84. Cookson and Travett, J., 1956, 3121.

85. Briggs, Cawley, Loe and Taylor, J., 1950, 955.

86. Manske and Holmes, "The Alkaloids", Vol.IV, 92, Academic Press, New York, 1955.

87. Manske and Holmes, ibid, IV, 95.

88. Wittstock, Poggendorf's Ann., 1830, 19, 298.

89. Wessely, Dinjaski, Isemann and Singer, Monatsh., 1935, 66, 87.

90. Wessely, Schonol and Isemann, Monatsh, 1936, 68, 21.

91. Wessely, Isemann, Singer and Schonol, Annalen, 1936, 522, 41.

92. Wessely, Munster and Schonol, Monatsh., 1936, 68, 313.

93. Wessely and Jentzsch, Monatsh., 1937, 70, 30.

94. Wessely and Schonol, Monatsh., 1938, 71, 10.

95. Feist, Rintelen and Kuntz, Annalen, 1935, 517, 119.

96. Feist, Kuntz and Brachvogel, Annalen, 1935, 519, 124.

97. Feist, Kuntz and Brachvogel, Annalen, 1935, 521, 184.

98. Feist and Brachvogel, Annalen, 1936, 522, 185.

99. Feist, Brachvogel and Volksen, Annalen, 1936, 523, 289.

100. Feist and Volksen, Annalen, 1938, 534, 41.

101. Barton and Elad, J., 1956, 2085, 2090.

102. Cocker, Cross, Duff, Edward and Holley, J., 1953, 2540.

103. Schmidlin and Wettstein, Helv., 1953, 36, 1241.
Plattner, Ruzicka, Heusser and Angliker, Helv., 1947, 30, 395.

104. Haggis and Owen, J., 1953, 389.

105. Buchi and Goldman, J. Amer. Chem. Soc., 1957, 79, 4741.

106. Kenyon and Symons, J., 1953, 2129.

107. Holker, Holker, McGookin, Robertson Sargeant and Hathway, J., 1957, 3746.

108. Tipson, J. Amer. Chem. Soc., 1952, 74, 1354.

109. Reynolds and Kenyon, J. Amer. Chem. Soc., 1950, 72, 1597.

110. Feiser and Heymann, J. Amer. Chem. Soc., 1951, 73, 5252.

111. Doering and Aschner, J. Amer. Chem. Soc., 1949, 71, 838.

112. Pearson and Dillon, J. Amer. Chem. Soc., 1953, 75, 2439.
Bonhoeffer, Geib and Reitz, J. Chem. Phys., 1939, 7, 664.
113. Skell and Hauser, J. Amer. Chem. Soc., 1945, 67, 1661
Lindstead, Owen and Webb, J. 1953, 1211.
114. Barton and Cookson, Quart. Revs., 1956, 10, 44.
115. Cava and Soboczenski, J. Amer. Chem. Soc., 1956, 78, 5317.
116. Barton and de Mayo, J., 1954, 887.
117. Barton, Ives and Thomas, J., 1955, 2056.
118. Wolff, Annalen, 1912, 394, 90.
Kishner, J. Russ. Phys. Chem. Soc., 1911, 43, 582.
119. Klyne, J., 1953, 3072.
120. Poos, Arth, Beyler and Sarett, J. Amer. Chem. Soc., 1953, 75, 422.
121. Smith and Fuzek, J. Amer. Chem. Soc., 1949, 71, 415; see also 52.
122. Blair and Newbold, J., 1954, 3935.
Blair, Logan and Newbold, J. 1956, 3608.
123. Brockman and Schodder, Ber., 1941, 74B, 73.
124. Berkley, Farrar, Knowles and Raffelson, J. Amer. Chem. Soc., 1954, 76, 5017.
125. Barton, Delflorin and Edwards, J., 1956, 530.
126. Vogel, "Practical Organic Chemistry", Longmans, 1956, 891.