A Thesis

entitled

"The Stereochemistry of the Colombo Root Bitter Principles"

submitted to the

University of Glasgow

for the degree of Doctor of Philosophy in the

Faculty of Science

by

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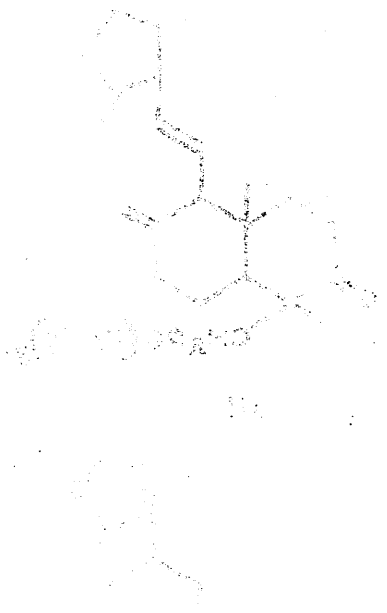
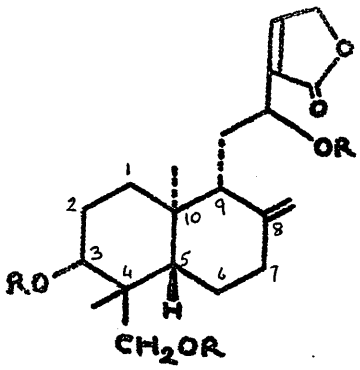
CONTENTS

Studies in the diterpenoid series, in recent years, have resulted in the elucidation of the structures of a number of compounds containing a furan ring and before discussing the stereochemistry of the Colombo root bitter principles a brief review of the chemistry of the labdane group of furan diterpene bitter principles will be considered.

I. REVIEW OF FURANOID DITERPENE BITTER PRINCIPLES.





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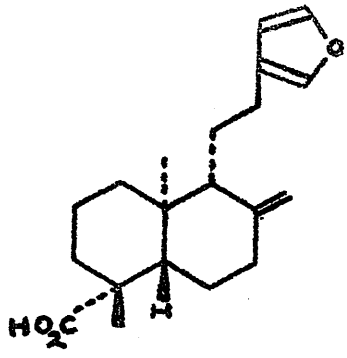

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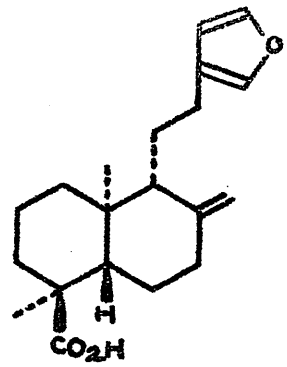

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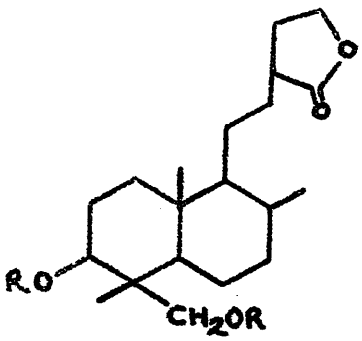

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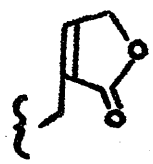

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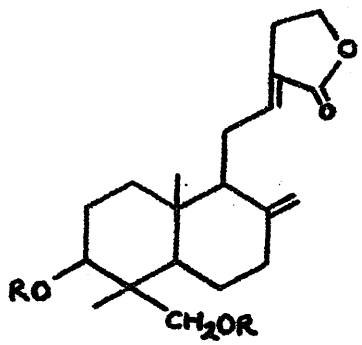

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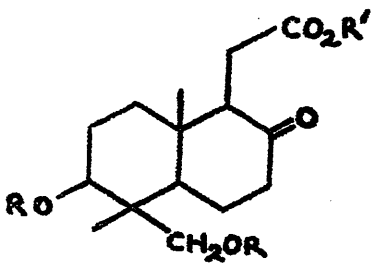

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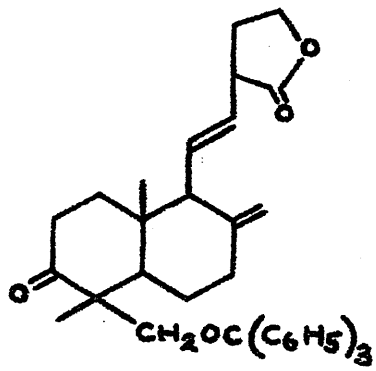

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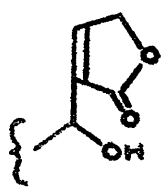
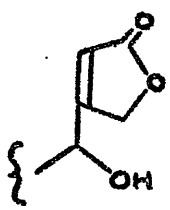


VIII a



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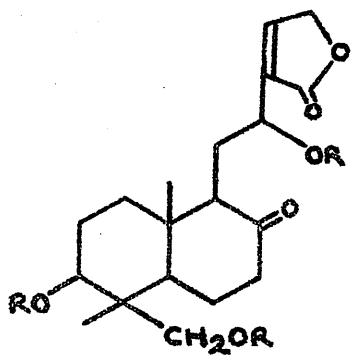


IX

I. Review of Furanoid Diterpene Bitter Principles.

Andrographolide,[1-3] $C_{20}H_{30}O_5$, a bitter substance isolated from Andrographis paniculata Nees, (1; R = H) although not a furanoid compound is closely related to daniellic (II)[4] and polyalthic (III)[5] acids.

It has been shown that andrographolide (I; R = H) contains three hydroxyl groups, a methylene group and an α ; β - unsaturated γ - lactone function. Selenium dehydrogenation gave 1 : 2 : 5 : 6 - tetramethylnaphthalene as would be expected from a compound with a hydroxyl at $C_{(3)}$ next to a tertiary centre.[6-8]

Andrographolide and its triacetate $C_{26}H_{36}O_8$ (I; R = $COCH_3$) on hydrogenation gave deoxytetrahydroandrographolide $C_{20}H_{34}O_4$ (IV; R = H) and the diacetate $C_{24}H_{38}O_6$ (IV: R = $COCH_3$) respectively, showing that one of the hydroxyl groups is allylic to a double bond.[8] When (I; R = $COCH_3$) was treated with aluminium amalgam an acetoxy group was eliminated giving (V a; R = $COCH_3$). This formulation for the desoxydiacetate was preferred but the alternative (V b: R = $COCH_3$) was not excluded.

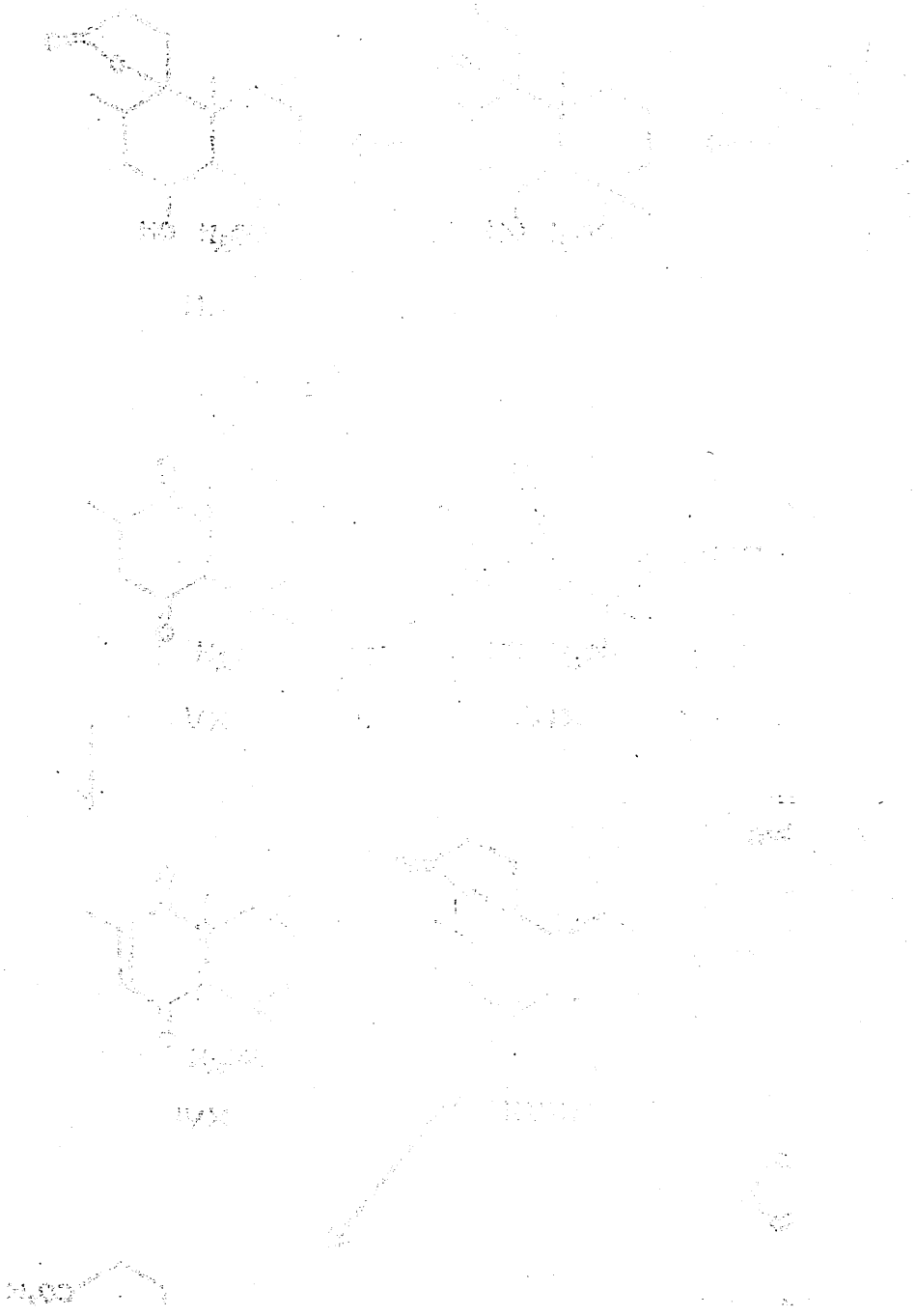
Oxidation of (I; R = $COCH_3$) and (V a; R = $COCH_3$) gave the keto-acid $C_{20}H_{30}O_7$ (VI; R = $COCH_3$, R^1 = H) which yielded 1 : 5 - dimethyl - 2 - naphthol on selenium dehydrogenation confirming the position of the original methylene group.

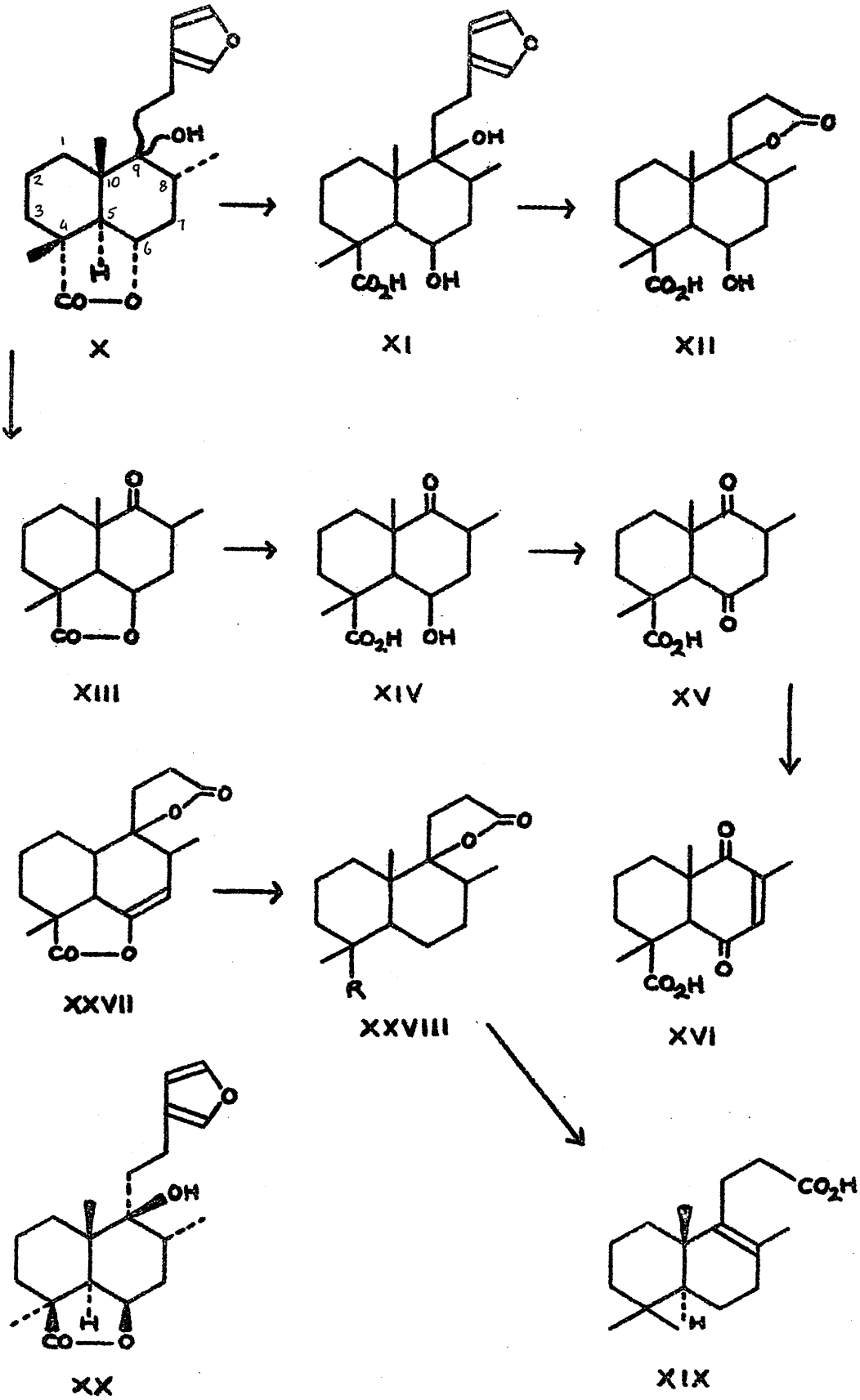
Andrographolide on treatment with trityl chloride in pyridine followed by chromium trioxide - pyridine oxidation gave a keto - anhydromonotrityl ether $C_{39}H_{40}O_4$ (VII). Acid hydrolysis of (VII) gave formaldehyde, by a retro - aldol reaction proving the relationship between the two remaining hydroxyl groups.

The nuclear magnetic resonance spectrum of andrographolide triacetate (I; R = $COCH_3$) confirmed the placing of the two tertiary C - methyl groups at $C_{(4)}$ and $C_{(10)}$ and favoured (VIII a) rather than Cava's original proposal (VIII b) as/

as the lactone system in the molecule.

The positive rotatory dispersion curves of (VI; R = $COCH_3$, R^1 = CH_3) and the nor - ketone (IX; R = $COCH_3$), derived by periodate cleavage of the diol obtained by treatment of andrographolide with osmium tetroxide, were given as further proof of the 2 - decalone system and indicated that the $C_{(10)}$ methyl group and the side chain were α .




X
XI
XII
XIII
XIV
XV
XVI
XVII
XVIII
XIX
XX
OH
CO2H
R

Marrubiin. $C_{20}H_{28}O_4$, the bitter principle of horehound (Marrubiin vulgare L.) has structure (X)[9,10].

The presence of a lactone system, a tertiary hydroxyl group, an inert oxygen function and two double bonds was established by conventional means.[11,12] Further spectroscopic measurements indicated that the two double bonds and the inert oxygen function were present as a furan ring and that marrubiin had a γ - lactone system.

Vigorous hydrolysis of marrubiin yielded the dihydroxy-acid $C_{20}H_{30}O_5$ (XI) and oxidation of this compound, with alkaline permanganate, gave the hydroxy lactone $C_{17}H_{26}O_5$ (XII). The five membered lactone ring (infrared absorption) in (XII) was shown not to involve the original carboxyl and hydroxyl and established the relative positions of the hydroxyl group and the furan system in marrubiin.

Dehydration of marrubiin, followed by ozonolysis gave the keto-lactone $C_{14}H_{20}O_3$ (XIII) which furnished the hydroxy - keto acid $C_{14}H_{22}O_4$ (XIV) on hydrolysis. Mild oxidation of (XIV) afforded the diketo acid $C_{14}H_{20}O_4$ (XV) which on dehydrogenation with selenium dioxide gave the ene - dione $C_{14}H_{18}O_4$ (XVI). The ultraviolet and infrared spectra of these compounds were in agreement with ths structure shown. The above experiments therefore establish the relative positions of the tertiary hydroxyl group and the lactonic hydroxyl group in the molecule.

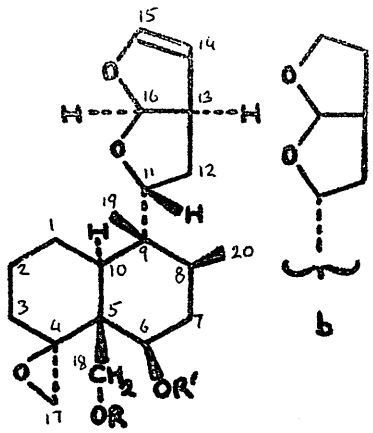
The complete carbon skeleton and the absolute stereochemistry at $C_{(5)}$ and $C_{(10)}$ in the molecule was established by conversion of the lactone (XII) into an unsaturated acid which had been previously obtained from ambreinolide.

The ketone derived from (XII), on treatment with acetic anhydride and sodium acetate, afforded the enol - lactone $C_{17}H_{22}O_4$ (XVII) which on hydrogenation gave the acid (XVIII; R = CO_2H). Rosenmund reduction to the aldehyde (XVIII; R = CHO) followed by Wolf-Kishner reduction gave the unsaturated acid (XIX) which was identical with the product obtained from ambreinolide.[14]

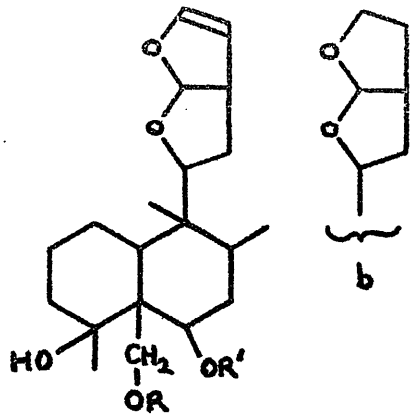
Since/

Since the hydroxy - keto acid (XIV) was stable to heat the carboxyl group must be attached to $C_{(4)}$. This is in agreement with the fact that marrubic acid (XI) resisted esterification and was decarboxylated on treatment with hot sulphuric acid. As the lactone system in marrubiin is five membered the lactonic hydroxyl must be located at $C_{(6)}$ in the molecule and the tertiary hydroxyl at $C_{(9)}$. Marrubiin is therefore formulated as (X) the only uncertainty being the position of substitution of the furan ring.

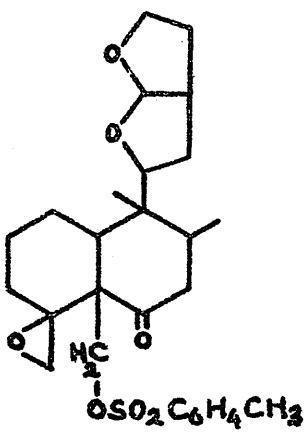
On the basis of molecular rotation differences and conformational arguments Cocker[13] has deduced the complete stereochemistry of the molecule as (XX) but his arguments, as discussed by Rigby[10] are in some ways questionable. Later, evidence for the formulation (X) was supplied by Castine and Wheeler[13a].



XXIa



XXIIa



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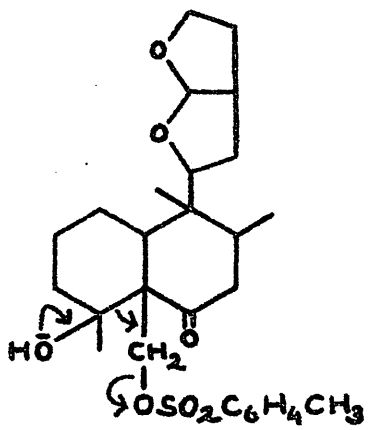

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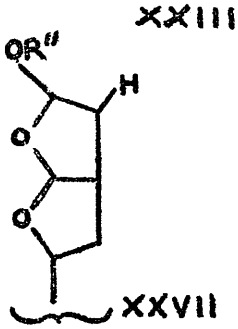

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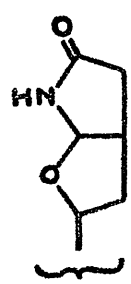
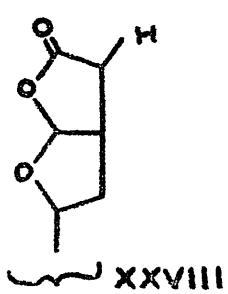
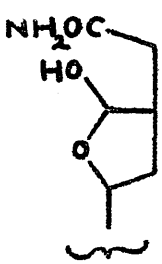


XXX



XXVIII



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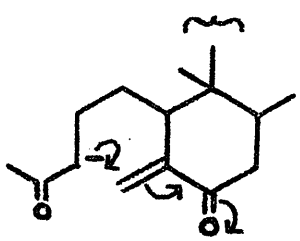
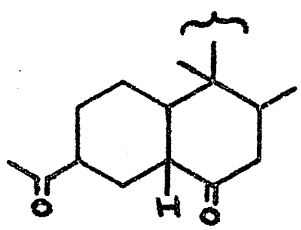


XXV



XXVI

The constitution and stereochemistry of Clerodin, $C_{24}H_{34}O_7$, the main bitter principle of the Indian bhat tree (Clerodendron infortunatum) has been established by X-ray crystallography as (XXI a; R = R^1 = $COCH_3$).[15]

Early investigations of the compound were carried out by Banerjee,[16] Chaudhury and Dutta[17] and the following chemical data, obtained by Barton and his colleagues,[18,19] supports the assigned constitution.

Mild alkaline hydrolysis of clerodin (XXI a; R = R^1 = $COCH_3$) gave deacetylclerodin $C_{20}H_{30}O_5$ (XXIa; R = R^1 = H) reconverted into clerodin on acetylation. Hydrogenation of clerodin and deacetylclerodin afforded the dihydro - derivatives $C_{24}H_{36}O_7$ (XXI b; R = R^1 = $COCH_3$) and $C_{20}H_{32}O_5$ (XXI b; R = R^1 = H) respectively, the latter furnishing (XXI b; R = R^1 = $COCH_3$) on acetylation.

Reduction of clerodin and dihydro-clerodin with lithium aluminium hydride gave the triols $C_{20}H_{32}O_5$ (XXII a; R = R^1 = H) and $C_{20}H_{34}O_5$ (XXIIb; R = R^1 = H) which on acetylation gave the diacetates (XXII a; R = R^1 = $COCH_3$) and (XXII b; R = R^1 = $COCH_3$) respectively. The remaining hydroxyl group in these molecules could not be acylated easily and was stable to chromic acid indicating that it was tertiary. Its formation is explained by cleavage of an ethereal ring, a 1, 2 epoxide being most likely.

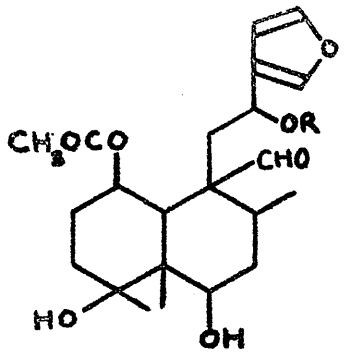
Deacetyldihydroclerodin (XXI b; R = R^1 = H) and deacetyltetrahydro-clerodin (XXII b; R = R^1 = H) on treatment with ethyl chloroformate in pyridine, gave the cyclic carbonates $C_{21}H_{30}O_6$ (XXI b; R, R^1 = CO) and $C_{21}H_{37}O_6$ (XXII b; R, R^1 = CO) respectively. The infrared carbonyl frequencies (1735 $cm.^{-1}$) of these compounds indicated a six membered ring, confirming that the acetate residues of clerodin are attached to a 1, 3 - glycol system.

Deacetyldihydroclerodin (XXI b; R = R^1 = H) on treatment with toluene - p - sulphonyl chloride in pyridine followed by chromic acid oxidation gave the ketone $C_{27}H_{36}O_7$ S (XXIII). Deacetyltetrahydroclerodin (XXIIb; R = R^1 = H) with the same treatment afforded the unstable ketone (XXIV) which, under the action of mild base, furnished/

furnished, via (XXV), the diketone $C_{20}H_{30}O_4$ (XXVI). This series of reactions established the relationship between the 1, 3 - glycol system and the 1, 2 epoxide grouping in clerodin.

Clerodin when dissolved in acetic acid gave the adduct $C_{26}H_{38}O_9$ (XXVII; $R = R^1 = R^{11} = COCH_3$) which on treatment with aqueous acetic acid afforded the hemi - acetal $C_{24}H_{36}O_8$ (XXVII; $R = R^1 = COCH_3$, $R^{11} = E$) (the second major compound from Clerodendron infortunatum) This hemi - acetal was smoothly oxidised by chromic acid to the γ - lactone $C_{24}H_{34}O_8$ (XXVIII; $R = R^1 = COCH_3$) proving the presence of a five-membered vinyl - ether system in clerodin.

The relationship of the seventh oxygen atom in clerodin to the vinyl ether system was defined as follows. The γ - lactone (XXVIII; $R = R^1 = COCH_3$) on treatment with ammonia furnished the amide (XXIX; $R = R^1 = COCH_3$) which on dissolution in acetic acid at room temperature gave the γ - lactam (XXX; $R = R^1 = COCH_3$). The normal fate of the amide in acetic acid would have been reversion to its lactonic progenitor and lactamisation can only be explained if the 'alkyl' oxygen of the γ - lactone (XXVIII; $R = R^1 = COCH_3$) has an ethereal oxygen attached to it.



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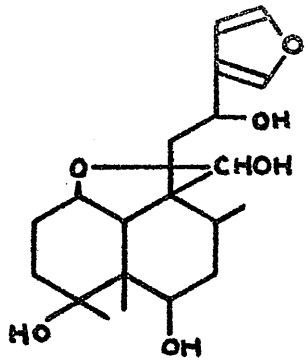

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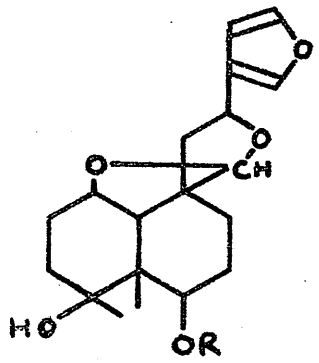

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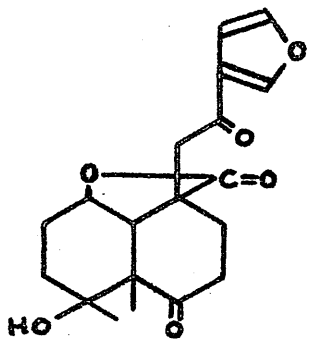
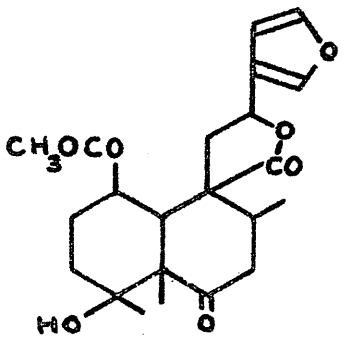
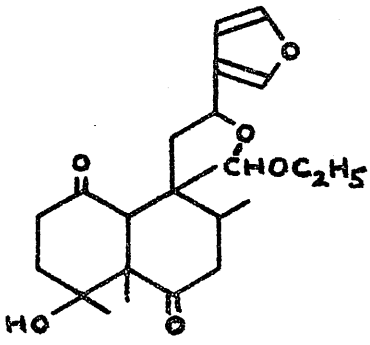


XXXIV



XXXV



XXXVI

Cascarillin, $C_{22}H_{32}O_7$, the bitter principle from cascarilla bark (Croton eleuteria Schwartz) has structure (XXXI; R = H)[38].

The infrared and ultraviolet spectra established the presence of a carbonyl group and a furan ring and a positive hydroxamic acid test indicated an ester grouping.

The compound did not give an anomalous Cotton curve showing that the ester was the only carbony present in the molecule.

Hydrolysis of cascarillin with aqueous alkali gave the hemi-acetal (XXXII) and hydrolysis with alcoholic alkali the acetal (XXXIII; R = H). Acetic acid was also formed during hydrolysis establishing an acetate residue in the molecule.

Acetylation of cascarillin gave a triacetate (XXXI; R = $COCH_3$) whose infrared spectrum showed that a gem - dimethyl group was absent in the molecule. Acetylation of (XXXIII; R = H) gave the hydroxy-acetate (XXXIII; R = $COCH_3$) which gave a naphthalene derivative on dehydrogenation.

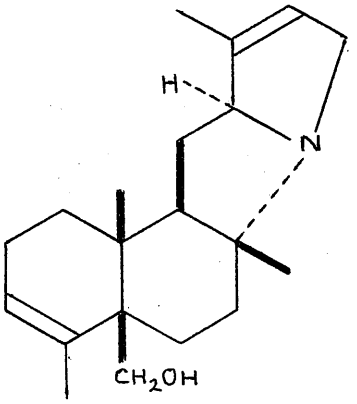
The nuclear magnetic resonance spectrum and the mass spectrum of cascarillin were consistent with the presence of an aldehyde and a 2 : 4 - dinitrophenylhydrazone was obtained.

Chromic acid oxidation of (XXXII) gave (XXXIV) with a γ - lactone and a β - furyl ketone system establishing a 1 : 3 relationship between the aldehyde group and the acetate and the presence of a hydroxyl α to the furan ring. Chromic acid oxidation of cascarillin gave the compound (XXXV). The presence of a γ - lactone in this molecule established the relationship between the aldehyde and the hydroxyl α to the furan. These experiments also indicated that a tertiary hydroxyl was present in the molecule.

Treatment/

Treatment of cascarillin with acidified ethanol gave the $C_{(19)}$ ethyl ether which on hydrolysis and oxidation gave the diketone (XXXVI), which was not a β - diketone. The two secondary hydroxyls in the molecule must occupy positions which do not bear a 1 : 3 relationship to one another.

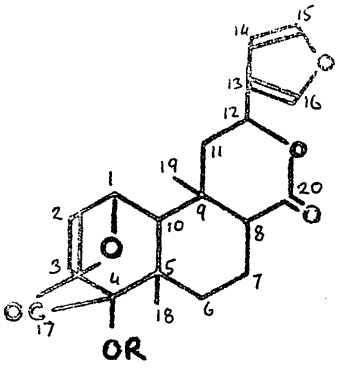
Thelepogine[39,40], isolated from thelepogan elegans, has been shown by X - ray methods (on the derived methiodide) to have the structure (XXXVII) and though not a terpene it is related biogenetically (see page 106) to the compounds described in this section.



XXXVII

II. COLOMBO ROOT BITTER PRINCIPLES.

1 (a) STRUCTURES OF COLUMBIN, CHASMANTHIN, JATEORIN AND PALMARIN.



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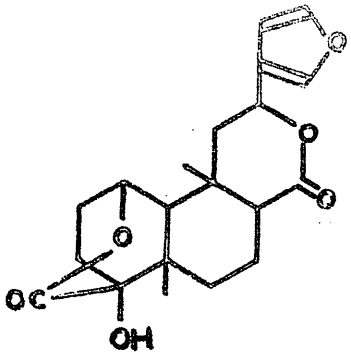

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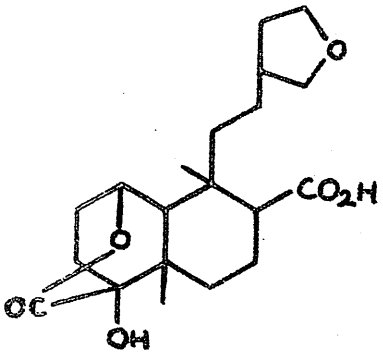
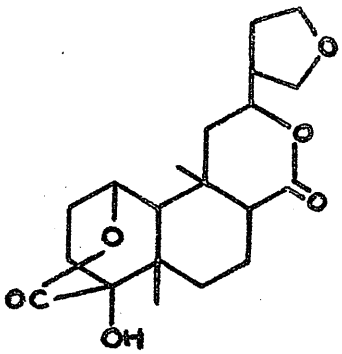


III



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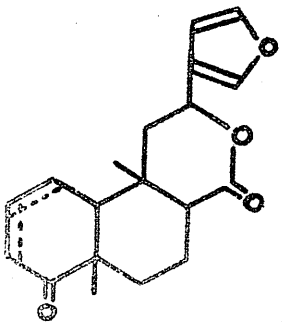

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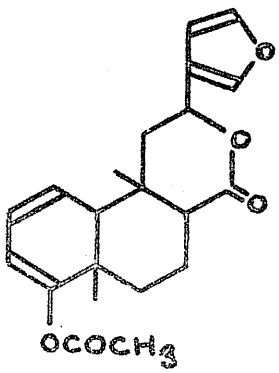


VI

Structure of Columbin.

Columbin, $C_{20}H_{22}O_6$, the major bitter principle of Colombo root (Jatrorrhiza palmata Miers) is a lactonic diterpenoid with structure (I; R = H)[20].

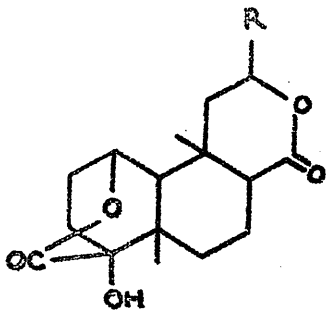
The bitter principles of the Colombo root were extensively investigated by the schools of Wessely[22-27] and Feist[28-33] and by standard methods columbin (see also Cava[21]) was shown to contain two lactones, a tertiary hydroxyl which is somewhat acidic, three double bonds and an inert oxygen function.

On treatment with mild alkali columbin (normal series) consumed 1 mole of base to give isocolumbin (iso series) both of these compounds affording the same acetate (I; R = $COCH_3$) and methyl ether (I; R = CH_3) on treatment with acetic anhydride - sodium acetate and alkaline dimethyl sulphate respectively. A second mole of base is consumed on more vigorous alkaline treatment but no defined product was isolated.

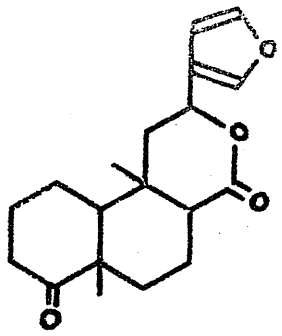
Complete hydrogenation of columbin (and isocolumbin) afforded an octahydro-derivative $C_{20}H_{30}O_6$ (III) and a hexahydro - derivative $C_{20}H_{28}O_6$ (IV), the former was a monocarboxylic acid indicating that hydrogenolysis had occurred during hydrogenation.

A characteristic property of columbin, which disappears on conversion to dihydrocolumbin $C_{20}H_{24}O_6$ (II), is the loss of 1 mole of carbon dioxide on melting furnishing decarboxycolumbin $C_{19}H_{22}O_4$ (V) which has a keto - group (infrared, cyclohexanone) and no hydroxyl group (this compound is obtained as a mixture of $\alpha:\beta$- and $\beta:\gamma$ - unsaturated forms.). These facts suggest the presence of a $\beta:\gamma$ - unsaturated - α - hydroxy lactone in columbin (lactone A). This was confirmed by formation of the diene $C_{21}H_{24}O_5$ (VI) on decarboxylation of acetylisocolumbin (I; R = $COCH_3$) which had spectroscopic properties consistent with its formulation.

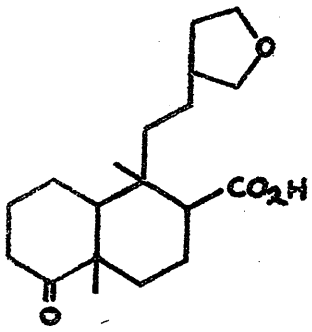
The spectroscopic properties of columbin and its derivatives indicate the presence of a furan system which was confirmed as follows. Treatment of dihydrocolumbin/



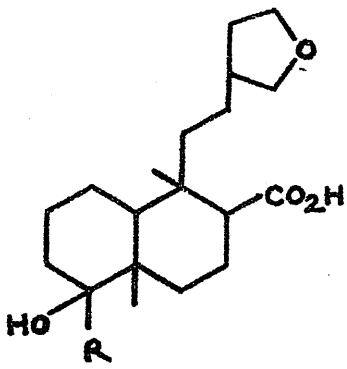
VII



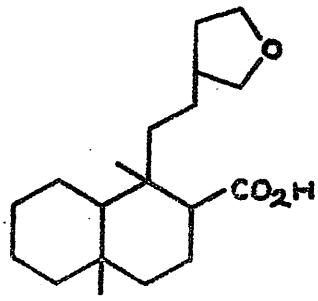
VIII



IX



X



XI

dihydrocolumbin (II) with ozone gave the acid $C_{17}H_{22}O_7$ (VII: R = CO_2H) and the keto-acid $C_{18}H_{22}O_8$ (VII; R = $COCO_2H$), formation of the latter proving that the furan ring in columbin is β substituted.

Hydrogenation of decarboxycolumbin (V) and dihydrodecarboxycolumbin (VIII) gave the octahydro - acid $C_{19}H_{30}O_4$ (IX) showing that lactone A is not involved in hydrogenolysis. These experiments confirm that the alkyl oxygen atom of one of the lactones (lactone C) in columbin must be attached allylically to the furan system.

The formation of 1 : 2 : 5 trimethylnaphthalene on zinc dust distillation of columbin[29] suggested an appropriately substituted bicyclic nucleus in the molecule.

The compound (X; R = CH_3), obtained from octahydrodecarboxycolumbinic acid (IX) by reaction with 1 mole of methyl magnesium iodide, on treatment with lithium aluminium hydride followed by selenium dehydrogenation gave the same naphthalene derivative.

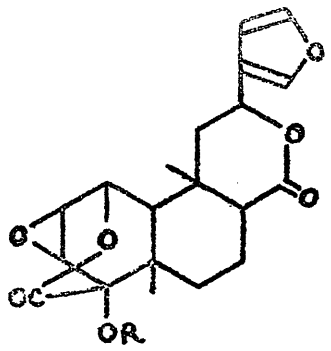
The Wolf-Kishner reduction product (XI) of octahydrodecarboxycolumbinic acid (IX) afforded on selenium dehydrogenation 1 - methyl - 2 naphthoic acid, whose carboxyl group fixed the position of the carbonyl in lactone C at $C_{(8)}$ in the molecule.

Finally, selenium dehydrogenation of (X; R = H), obtained by acid catalysed hydrogenation of (IX) gave 1, 5 - dimethyl - 2 - naphthoic acid. The 5 - methyl group in the naphthalene nucleus must have arisen during dehydrogenation by migration of a tertiary methyl group to the position of attachment of the hydroxyl group. This confirms that the lactone A carbonyl, and the tertiary hydroxyl, in columbin must be attached to $C_{(4)}$ with a tertiary methyl group at the adjacent $C_{(5)}$.

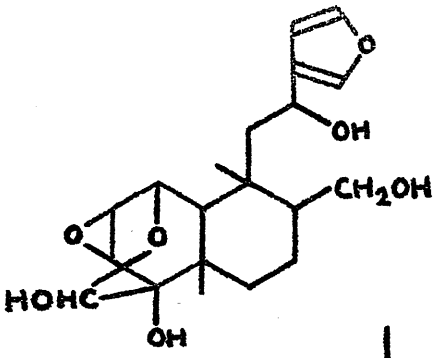
Kuhn - Roth oxidation of columbin indicated two C - methyl groups and the second/

second methyl group was placed at $C_{(9)}$ since this explained the formation of o - cresol on dehydrogenation better than alternative with the methyl group at $C_{(10)}$.

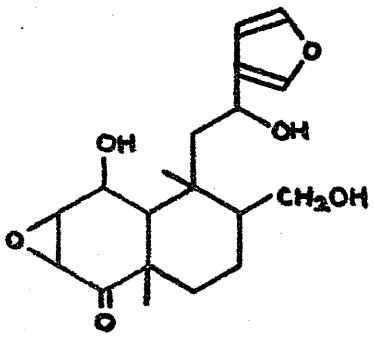
On the above evidence columbin was formulated as (I; R = H) and that $C_{(8)}$ and not $C_{(12)}$ was involved in epimerisation during the columbin - <u>iso</u>-columbin change was shown by the non-identity of the octahydro-acids derived from decarboxycolumbin and decarboxy<u>iso</u>columbin[34].



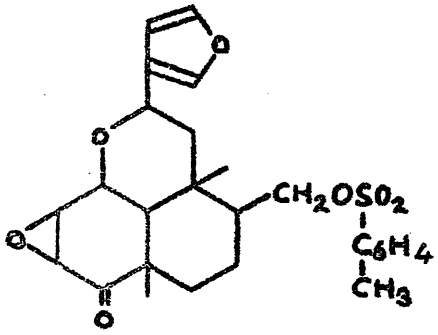
XII



XIII



XIV



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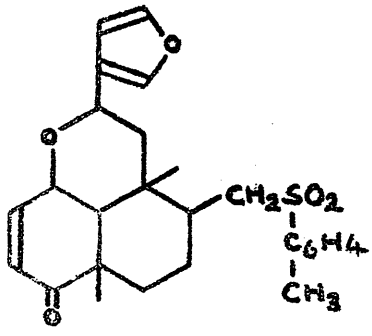
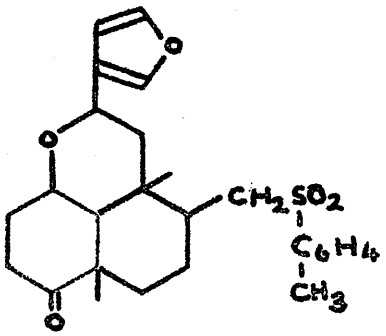


XVI



XVII

Structures of Palmarin, Chasmanthin and Jateorin[35,36]

As well as columbin, $C_{20}H_{22}O_6$, the Colombo root contains the bitter principles palmarin, chasmanthin and jateorin $C_{20}H_{20}O_7$.

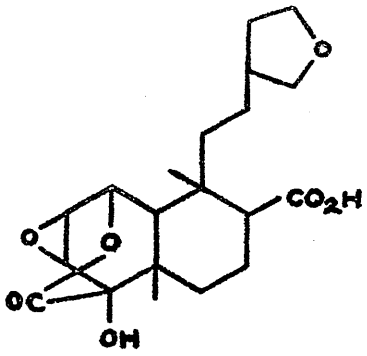
Palmarin has been isolated from the root extract only in small amounts and can be readily purified but chasmanthin, the second major bitter constituent of the Colombo root, has not been isolated in a pure state. For this reason early work on these compounds gave confused results[23,27 - 33].

Remaining amounts of columbin can only be removed from chasmanthin by decarboxylation and treatment of the purified product with mild alkali afforded palmarin and a new bitter principle named isojateorin. It was proposed that isojateorin was formed by isomerisation of jateorin which occurred naturally in admixture with chasmanthin (the palmarin isolated from the root was probably an artefact arising during work up of the root extract). The relationship between chasmanthin and palmarin and between jateorin and isojateorin was considered to be similar to the columbin - isocolumbin relationship.

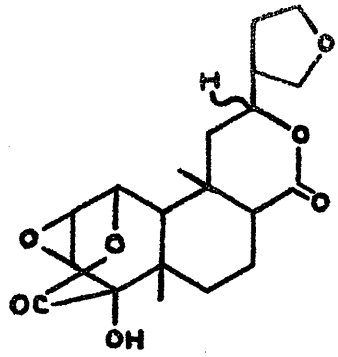
Isojateorin, $C_{20}H_{22}O_7$, was assigned the structure (XII; R = H) as follows. Isojateorin was reduced with lithium aluminium hydride to the hemi-acetal (XIII) which on periodate cleavage and mild alkaline hydrolysis afforded the trihydroxy - ketone (XIV). Treatment of the latter with toluene - p - sulphonyl chloride in pyridine furnished an anhydro-monotoluene - p - sulphonate (XV). This compound was considered to arise from the initially formed bis - toluene - p - sulphonate by solvolysis to give a carbonium ion α to the furan ring, which then undergoes intramolecular cyclisation with the neighbouring hydroxyl group.

Reduction of (XV) with chromous chloride[37] to (XVI) followed by hydrogenation of the $\alpha:\beta$ - unsaturated ketone gave (XVII). The same compound was obtained when dihydroisocolumbin (II) was subjected to a similar reaction sequence (the last two steps in this case were not required).

The/



XVIII



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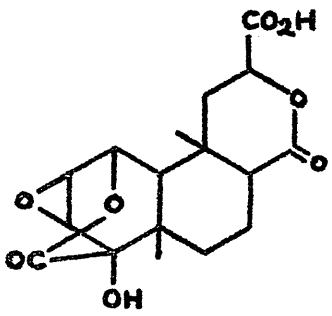
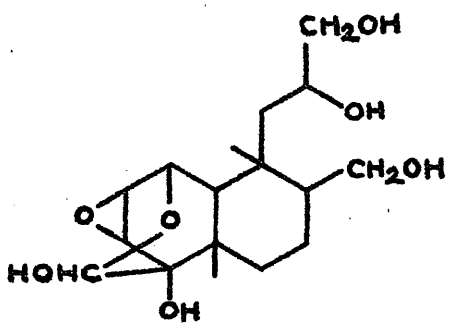
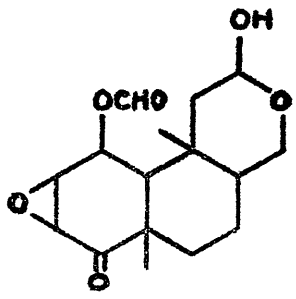


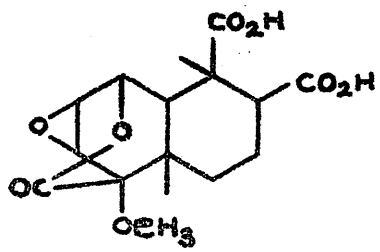
XX



XXI



XXII



XXIII

The relationship between isojateorin and palmarin was established by isolating the hydrogenation products of these compounds. Isojateorin and palmarin, like columbin, afford acidic and neutral compounds on hydrogenation and it was found that they gave the same hexahydro - compound (XVIII) but different tetrahydro - compounds (XIX). This proves that isojateorin and isocolumbin (and hence columbin) have the same stereochemistry and palmarin (and chasmanthin) the opposite stereochemistry at $C_{(12)}$.

In agreement with its structure palmarin was found to contain a furan ring (infrared), two lactones and a tertiary hydroxyl. That the furan ring was mono-substituted followed from the isolation of trisnorpalmarinic acid (XX) on treatment of palmarin with ozone.

Reduction of the trisnor - acid (XX) with lithium aluminium hydride gave a hemi-acetal (XXI) which consumed 2 moles of periodate to furnish the keto - formate (XXII) with bands at 1710 (cyclohexanone), 1730 and 1148 $cm.^{-1}$ (formate) in the infrared. Methylpalmarin (XII; R = CH_3) was oxidised with potassium permanganate to the dicarboxylic acid (XXIII), which furnished a five membered anhydride, and trisnorpalmarinic acid (XX). These reactions prove that the side chain in palmarin is the same as that in columbin.

Dehydrogenation of hexahydropalmarinic acid (XVIII) with selenium afforded 1 : 2 : 5 - trimethyl naphthalene and 1 : 5 - dimethyl - 2 - naphthoic acid and Kuhn-Roth oxidation of palmarin gave two C - methyl groups. Formation of these products indicate the same substitution of groups, in a bicyclic nucleus, as columbin.

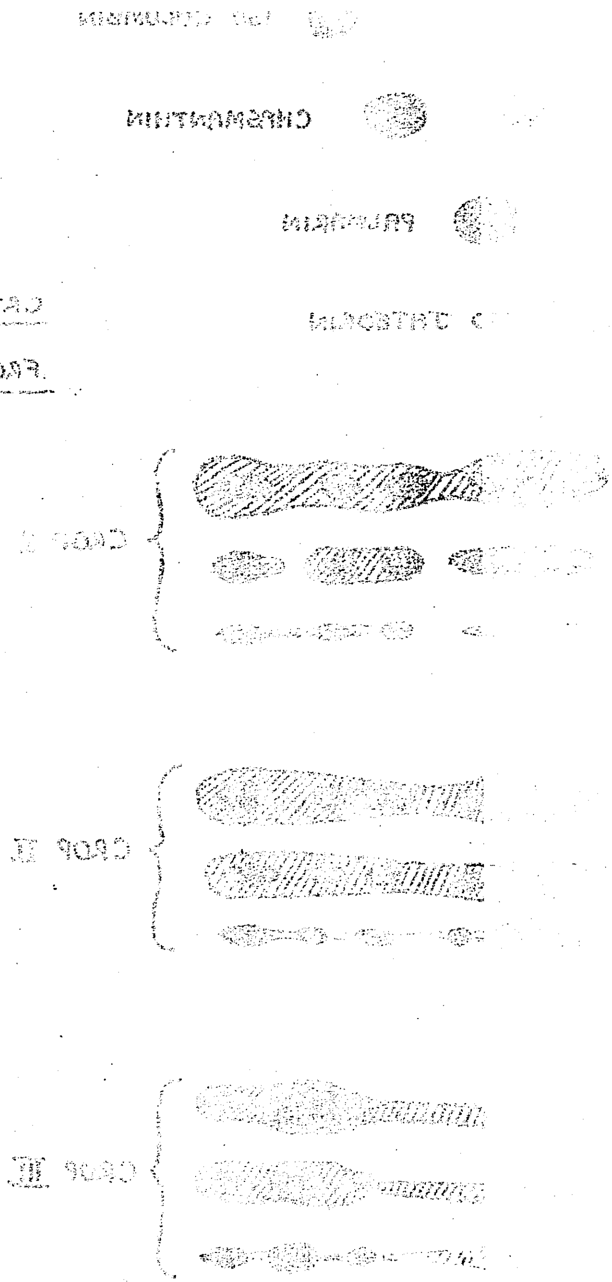
Some other features of the palmarin molecule are worth mentioning. The high carbonyl frequency in the infrared of the ring A lactone (1775 $cm.^{-1}$) indicate a γ - rather than a δ - lactone in the molecule but presumably the strain factors in the 2 : 3 - epoxy - 1 : 4 - (boat) δ - lactone are responsible for/

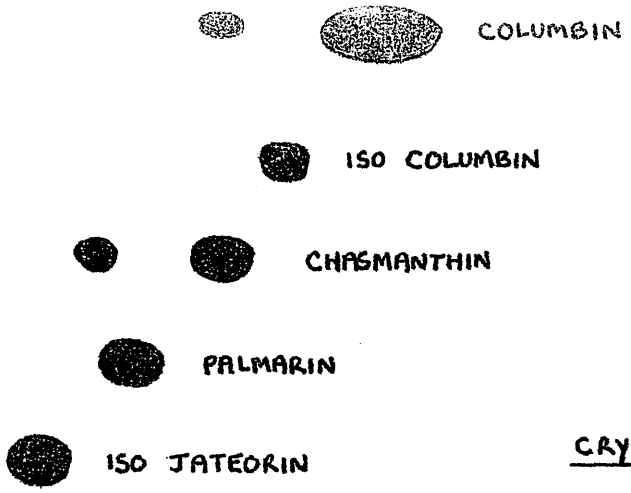
for this abnormally high frequency. The epoxide ring in palmarin was not sensitive to acid or to lithium aluminium hydride and the misleading assumption in early work on this compound was that this oxygen atom was part of a five - or six - membered ethereal ring.

It also should be stated that nuclear magnetic resonance studies in the palmarin series were found to be consistent with the formulation (XII; R = H).

(b) MINOR ROOT CONSTITUENTS.

CHROMATOPLATES OF CRYSTALLINE FRACTIONS OBTAINED FROM THE SOLID ROOT EXTRACT





CHROMATOPLATES OF CRYSTALLINE FRACTIONS OBTAINED FROM THE SOLID ROOT EXTRACT

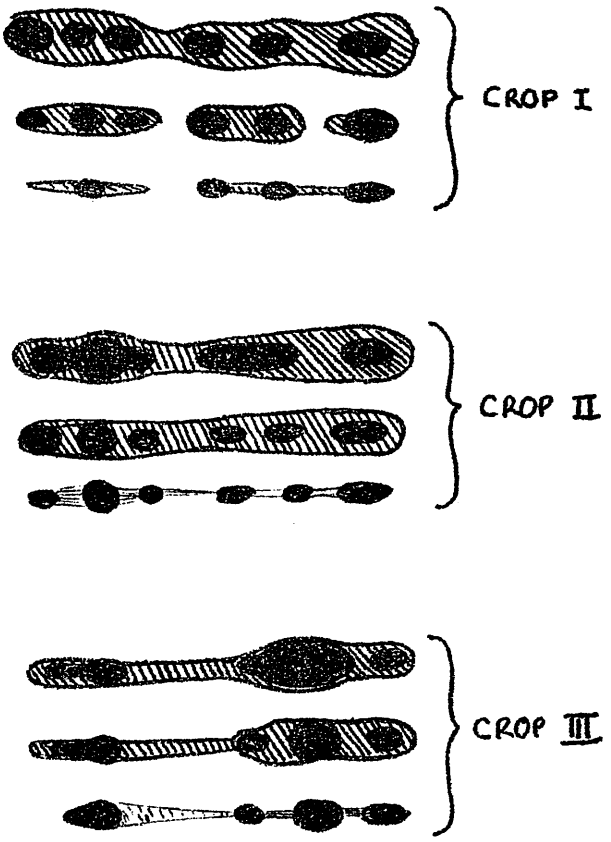


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Fig. 1.

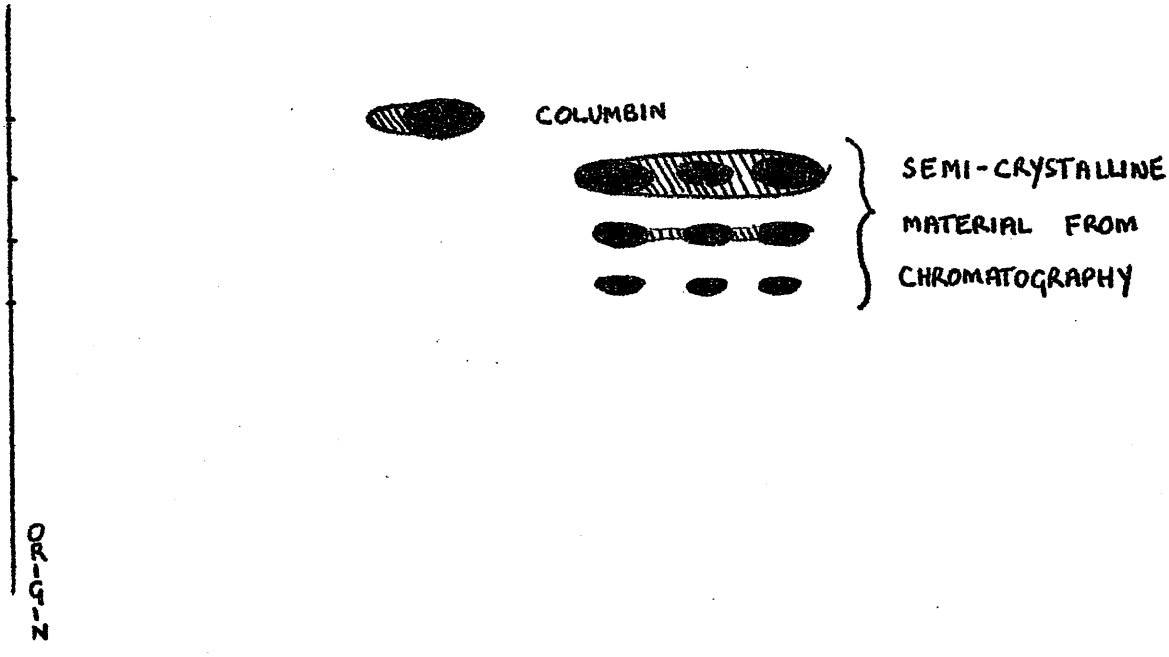

COLUMBIN
SEMI-CRYSTALLINE
MATERIAL FROM
CHROMATOGRAPHY
ORIGIN

FIG. 2.

(b) Minor Root Constituents.

a. Discussion.

Extraction of the Colombo Root (Jatrorrhiza palmata Miers) with ether and subsequent concentration affords a crude solid fraction and ether residues.

The crude solid fraction is the source of columbin, palmarin, chasmanthin and isojateorin discussed in the previous section and attempts were made, using the technique of thin layer chromatography to determine whether any minor compounds of interest, perhaps related to the main bitter principles, were also present.

Initial crystallisation of the solid fraction from acetone - alcohol afforded three crops of crystalline material which were found to contain columbin, isocolumbin, chasmanthin, palmarin, jateorin and isojateorin. These compounds were detected by running the material obtained by crystallisation along with authentic specimens of columbin, isocolumbin, chasmanthin (containing jateorin) palmarin and isojateorin on chromatoplates. No indications of any other compound were found in the first three crystalline fractions (Fig.1).

The mother liquors, after the first three crops had been removed, on chromatography on alumina (III) in benzene gave a semi-crystalline which contained three compounds (as judged by three spots on a chromatoplate Fig.2) which had not been previously detected. Further extensive chromatography and fractional crystallisation resulted in the isolation of one of these compounds in a pure state.

This material was isolated in the form of pale yellow needles m.p. 221 - 3°, $[\alpha]_D$ - 88° (pyridine) and the colour of this compound persisted after chromatography, charcoal treatment and sublimation.

The compound had bands in the infrared (nujol) at 1508 and 3150 $cm.^{-1}$ and at ($CHCl_3$) 1750, 1712 and 1680 $cm.^{-1}$ which indicated furan and possibly lactone and unsaturated carbonyl systems and the ultraviolet spectrum had bands at λ max. 215 - 216 mμ., (inflexion) ε, 6950 and λ max. 218 mμ., ε, 7090 which are consistent/

consistent with a furan and an $\alpha:\beta$ - unsaturated ketone in the molecule. By analogy with the other Colombo root bitter principles the molecule could have a lactone - furan system of the same type, the other functional groups being incorporated in a decalin nucleus. The nuclear magnetic resonance spectrum shows bands at 3.52 τ indicating a β substituted furan, 9.18 and 9.11 τ showing two C - methyl groups, 8.01 and 7.96 τ possibly due to a methylene group adjacent to a carbonyl system and 3.29 τ which could arise from the α proton in an $\alpha:\beta$ - unsaturated ketone system.

Mass spectroscopic measurements indicate a molecular weight of 328 - 330 for the molecule and as yet no molecular formula consistent with both this value and C and H analysis has been obtained. On the basis of the molecular weight a molecular formula $C_{19}H_{20}O_5$ (328), $C_{19}H_{18}O_5$ (326), $C_{20}H_{26}O_4$ (330) or $C_{20}H_{24}O_4$ (328) seems to be indicated (assuming a ca C_{20} diterpene skeleton). However none of these are within 1% C of the percentage C found in the molecule.

While attempts were still being made to assign a molecular formula to the molecule it was noticed that the nujol spectrum of the new compound was practically identical to that of decarboxyisocolumbin but it was soon realised that this was their only similarity. Decarboxyisocolumbin is a colourless compound m.p. 213 - 14°, $[\alpha]_D$ - 130° with a molecular weight of 314 ($C_{19}H_{22}O_4$), 12 - 16 less than the new compound. The analytical values for decarboxyisocolumbin (C, 72.6; H, 7.05%) are not too close to those of the new compound. Further the two compounds separate on a chromatoplate. Decarboxyisocolumbin itself is known to exist as a mixture of $\alpha:\beta$ - and $\beta:\gamma$ - unsaturated forms and gives two spots on a chromatoplate, the new compound gives only one spot on a chromatoplate which does not correspond to either (though quite close to one of them) of the decarboxyisocolumbin spots. The ultraviolet spectrum of the compound, mentioned earlier, however does indicate an $\alpha:\beta$ - unsaturated ketone in the molecule.

The difference in molecular weight of the two compounds suggests a difference of $-CH_2$, $-CH_3$ or $-O$ in the molecules. A 2 : 3 epoxide (as in palmarin/

palmarin) cannot be accommodated on the spectral evidence and to incorporate an extra carbon atom is difficult on biogenetic grounds and particularly having regard to the similarity of the infrared spectrum to that of decarboxy<u>iso</u>columbin.

The situation was not improved by comparing the hydrogenation products of the two systems. Decarboxy<u>iso</u>columbin and the new compound give, on hydrogenation the dihydro - compounds m.p. 215 - 216° and 240 - 2° respectively. Molecular weight determinations on the compound m.p. 240 - 2° again gave a molecular weight in the region of 330 (compared with 316 for dihydrodecarboxy<u>iso</u>columbin) and its infrared spectrum showed differences to that of dihydrodecarboxy<u>iso</u>columbin though it was similar in the carbonyl region.

Treatment of the original compound with mild alkali had no effect, the material being recovered unchanged, in good yield, suggesting (assuming a relationship with columbin) that we are dealing with the <u>iso</u> - series.

At the moment the marked similarity of the infrared spectra of the compound and decarboxy<u>iso</u>columbin makes it very difficult to accommodate functional groups, other than those present in decarboxy<u>iso</u>columbin, in the molecule and suggests that their structures must be very closely related. The difference in molecular weight of the two systems cannot be explained easily and no worthwhile suggestion as to the structure of this compound can be made at present in view of the small amount of chemical data available and the fact that time did not permit a fuller investigation.

The other two compounds, occurring with the material described above, were isolated as a mixture m.p. 210 - 15° and were partially separated into components m.p. 200 - 7° and m.p. 213 - 15°. The infrared spectra of the two materials (nujol) were similar to that of the compound described above, in particular they showed furan absorption (1508 and 3150 $cm.^{-1}$) and carbonyl absorption at 1725 and 1690 $cm.^{-1}$ Neither of the compounds, however, was isolated in a pure state and further investigation was not attempted.

b) Experimental.

Thin Layer Chromatography

(The method used was that described by Stahl[41])

A slurry of silica gel (30 gms.) (Merk Kieselgel G.) in water (15 ml.) was spread evenly on glass plates to give a layer of silica ca 0.25 mm. thick. The silica was allowed to dry at room temperature overnight and the general method of using the chromatoplates is as follows.

A solution (.05 -.01 ml.) of the material under investigation (1 mg.) in solvent (0.5 ml.) was dropped on the plate, as a single spot, about an inch from one end and allowed to dry. The plate was placed in a container, in a vertical position, with the end having the spot of material immersed in solvent (usually benzene) to a depth of half an inch. The plate was removed from the container, when the solvent front had run up about 3/4 of its length, and allowed to dry at room temperature. Development was carried out by spraying the plate with a solution of ceric sulphate (2 gms.) in sulphuric acid (6 N, 100 ml.) and heating in an oven at ca 110° for about an hour. The material showed up on the plate as a black spot and small amounts of secondary components in a mixture could be detected easily.

Investigation of the Solid Root Extract.

The solid root extract (200 gms.)(supplied by Messrs. Stafford Allen, London and obtained by extraction of the powdered Colombo root with ether and subsequent concentration) was heated with acetone (2 l.) until it just refluxed. The 2 l. level was marked on the flask and ethanol (1.4 l.) added in portions allowing the 2.5, 3, and 3.4 l. levels to be marked. The total volume was reduced to 3 l. and ethanol (600 ml.) added. The volume was again reduced to 3 l. and ethanol (1 l.) added, then reduced to 3.5 l. and ethanol (500 ml.) added. Standing at room temperature gave a crop of solid material (45 gms.) I. A further crop of material (47 gms.)II was obtained by adding ethanol (300 ml.), reducing the volume to 3 l. and standing overnight. The mother liquors on standing for about 1 week gave a third crop of material (60 gms.) III.

The/

The solid material I was mainly columbin but isocolumbin, chasmanthin, palmarin, jateorin, and isojateorin were detected on chromatoplates (Fig.1).

The solid material II contained more chasmanthin and palmarin than the previous but no unknown compounds were detected (Fig.1).

The material III was substantially isocolumbin but also contained the other known root constituents as minor components as shown by the distribution of spots on a chromatoplate (Fig.1).

After the third crop of solid material had been removed the mother liquors were evaporated in vacuo giving a brown oil (ca 50 gms.) This material on chromatography on grade (III) alumina in benzene afforded a semi-crystalline fraction (2.3 gms.) which gave three spots on a chromatoplate, none of which corresponded to the known Colombo root constituents (Fig.2). The remainder of the material on the column was eluted with ethyl acetate - methanol and contained a mixture of the known root bitter principles.

The semi-crystalline fraction on further chromatography and fractional crystallisation, using ethyl acetate - petrol, gave a mixture of two compounds m.p. 210 - 15° and a single compound (300 mg.) m.p. 221 - 3°. The mixture m.p. 210 - 15° was partially separated by crystallisation from ethyl acetate - petrol giving two components m.p. 200 - 7° and 213 - 15° with ν max. (nujol) 1508 and 3150 cm.$^{-1}$ (furan) and 1725 and 1690 cm.$^{-1}$ The single compound on further crystallisation gave m.p. 222 - 3°, $[\alpha]_D$ - 88° (C, 1.2); ν max. (nujol) 1508 and 3150 cm.$^{-1}$ (furan) and 1675, 1695, 1725 and 1600 cm.$^{-1}$; ν max. ($CHCl_3$) 1750, 1712 and 1680 cm.$^{-1}$; λ max. 215 - 216 m.μ. (inflexion) ε , 6950 and λ max. 218 m.μ . ε ,7090. (Found: C, 71.75; H, 6.7: C, 71.15: H, 6.7: C, 71.5; H, 6.6% and for sublimed material C, 72.05; H, 6.4%.) Mol. wt. found 328 - 330 (mass spectroscopic).

Attempted/

Attempted Epimerisation of the Compound m.p. 221 - 3°.

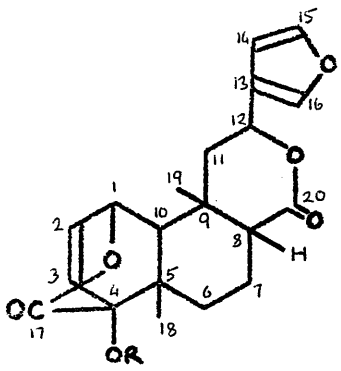
The compound (10 mg.) was treated with N sodium hydroxide on the steam bath for 5 minutes. Acidification gave material (8 mg.) with identical infrared spectrum to that of the starting material.

Hydrogenation of the Compound m.p. 221 - 3°.

The compound (50 mg.) in ethyl acetate ('Analar', 10 ml.) was hydrogenated over 1% palladised charcoal until 1 mole of hydrogen had been absorbed. The catalyst was removed by filtration through kieselguhr and evaporation of the ethyl acetate gave a solid residue (50 mg.). This material on crystallisation twice from ethyl acetate - petrol gave a compound (15 mg.) m.p. 234 - 6°, which showed two spots on a chromatoplate one of them corresponding to the starting material. The material (15 mg.) was run on a 1m.m. silica plate, the band corresponding to the starting material being removed. Crystallisation of the material, obtained by washing the silica with ethyl acetate, gave the dihydro-compound (8 mg.) m.p. 240 - 2°, ν max. (nujol) 1675 and 1725 cm.$^{-1}$ and 1508, 3150 cm.$^{-1}$ (furan) (Found: C, 70.0; H, 6.15%) Mol.wt. found 330 - 2 (mass spectroscopic).

2. STEREOCHEMISTRY OF COLUMBIN, CHASMANTHIN, JATEORIN AND PALMARIN.

FOR THE SAKE OF CLARITY COMPOUNDS DERIVED FROM COLUMBIN (NORMAL SERIES) WILL BE DRAWN WITH $C_{(8)}$ H β AND THOSE DERIVED FROM ISOCOLUMBIN (ISO SERIES) WITH $C_{(8)}$ H α.



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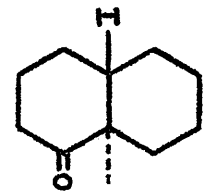

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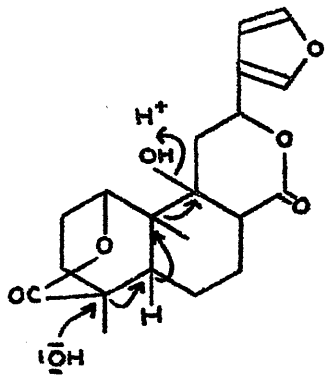

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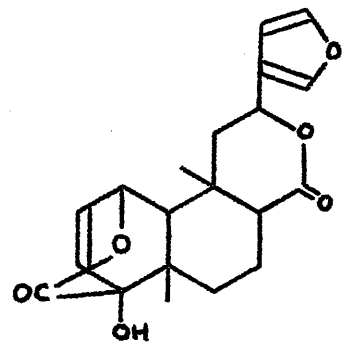

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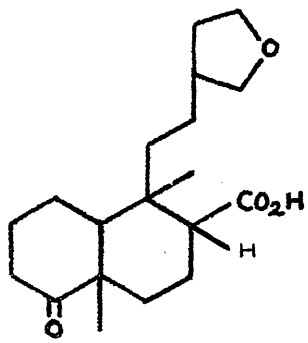

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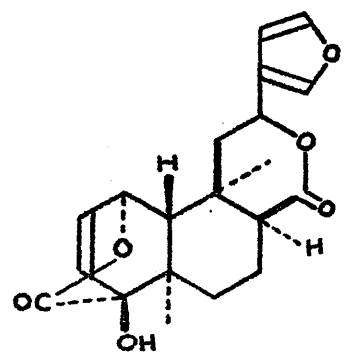


VI

2. The Stereochemistry of Columbin, Chasmanthin, Jateorin and Palmarin.

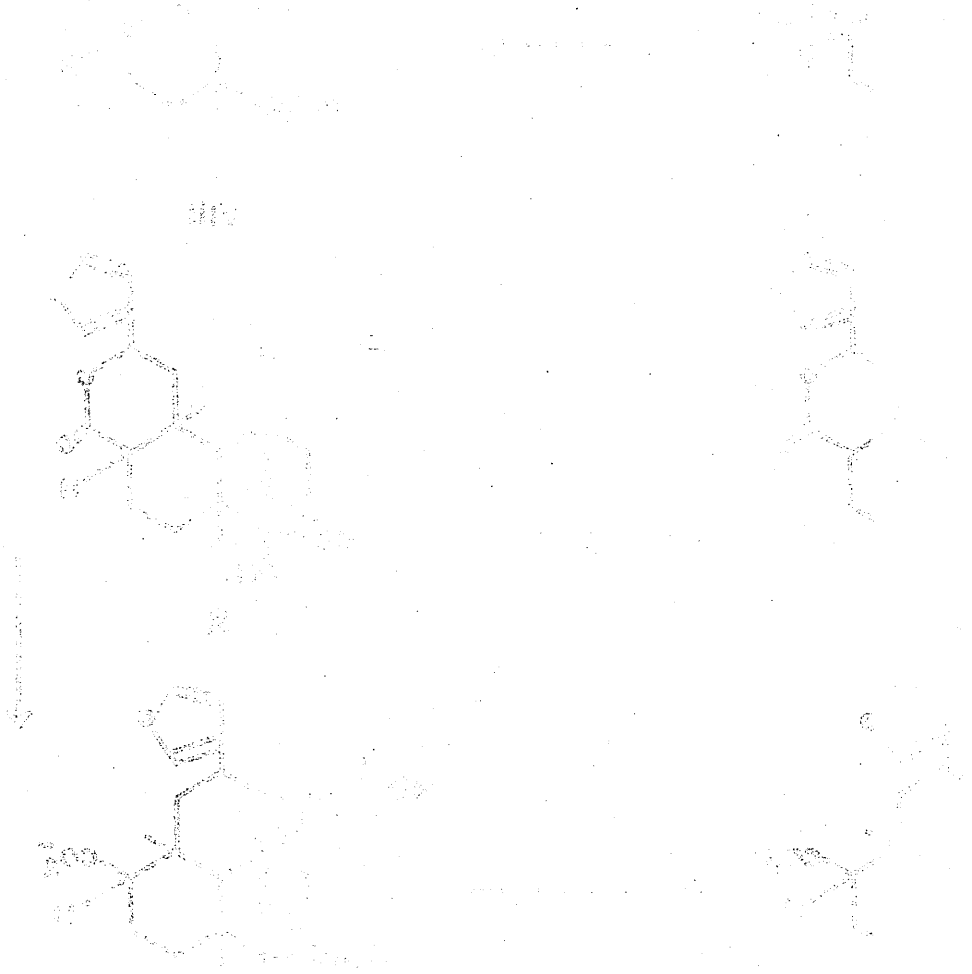
(a) Discussion.

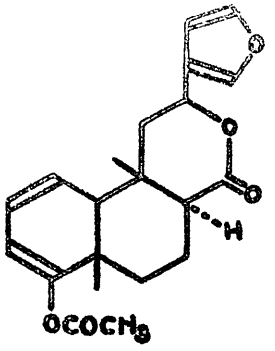
Previously published proposals concerning the stereochemistry of columbin are scant and can be summarised as follows.

Barton and Elad[20] have suggested that the columbin - isocolumbin change involves epimerisation of the hydrogen atom, α to the lactonic carbonyl group, at $C_{(8)}$. This has been confirmed by Wylie[34] who showed that since octahydrodecarboxycolumbinic acid (IV) and octahydrodecarboxyisocolumbinic acid (IV; $C_{(8)}$ α H) were different compounds epimerisation must involve $C_{(8)}$ and not $C_{(12)}$. Since trans fusion of two six membered rings is generally thermodynamically more stable than cis fusion it seems likely that in columbin (I; R = H) the B/C ring fusion is cis. Barton and Elad have further suggested that (II) - (III) could represent the biogenetic derivation of columbin, a scheme which might have stereochemical implications.

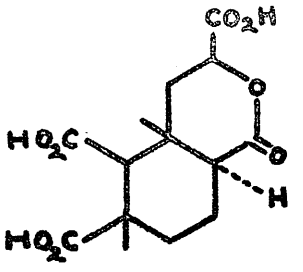
Cava[42] and his colleagues have compared the optical rotatory dispersion curves of octahydrodecarboxycolumbinic acid (IV) and (+) - trans - 9 - methyldecalone (V) and because of their similarity have postulated the absolute stereochemistry of columbin as (VI), utilising the biogenetic proposal of Barton and Elad. The lactone bridge in ring A was placed on the same side of the molecule as the 18-methyl group to account for its resistance to hydrolysis and the α 19-methyl group followed from the proposed biogenetic scheme. It is worth noting that this structure presupposes a precursor of 'unnatural' stereochemistry, i.e. one having a $C_{(10)}$ α methyl group. Furthermore the comparison of the optical rotatory dispersion curves of (IV) and (V) depend on columbin having an A/B trans fusion. It will be shown that the A/B fusion in columbin is in fact cis and that the precursor has the 'natural' configuration at $C_{(10)}$.

Since/

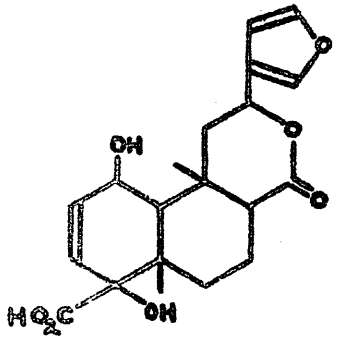




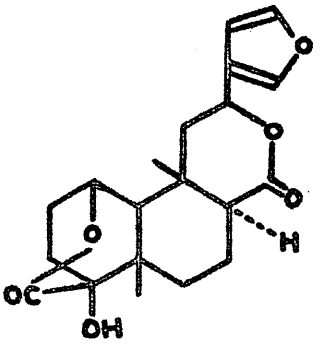
VII



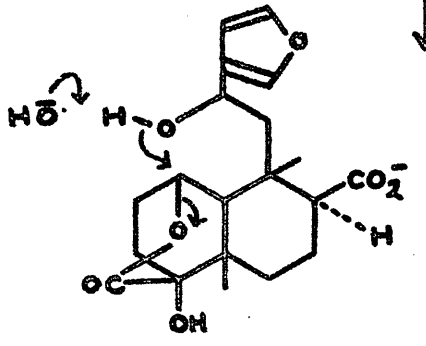
VIII



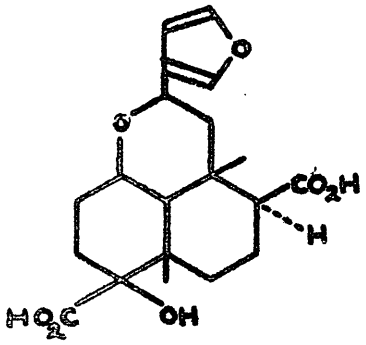
IX



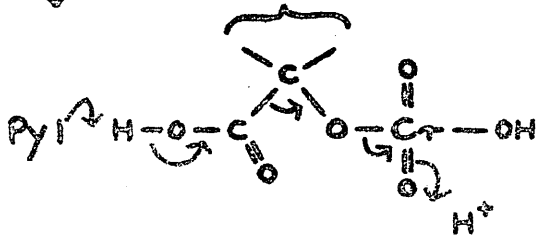
X

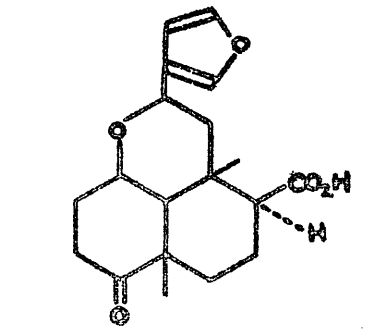


XI



XII





CO_2 $C{\equiv}O(OH)_2$

XIII

Since the structures of all the major bitter principles of the Colombo root have now been established and related to columbin (see page 20) the elucidation of the absolute stereochemistry of columbin is of importance in completing the chemistry of this unusual group of diterpenoids. Its solution in fact creates a problem of unusual biogenetic interest.

The problem presents itself in essentially two parts a) the nature of the A/B ring fusion, the stereochemistry of the angular methyl groups and the stereochemistry at $C_{(12)}$ b) the absolute stereochemistry of columbin and will be dealt with in that order.

Initial experiments to establish the nature of the A/B ring fusion were concerned with the ozonolysis of decarboxyacetyl<u>iso</u>columbin (VII) in an attempt to obtain the tricarboxylic acid (VIII). P K measurements on this acid[43,44] as well as thermal stability experiments[45] on the derived anhydride could be expected to indicate whether the 1, 2 dicarboxylic acid system is cis or trans. However various methods of ozonolysis, work-up and treatment of the product failed to yield the required acid. (See experimental page 80 .)

Efforts were then made to obtain a carbonyl function at $C_{(1)}$ since the stability of a 1 - Keto compound to base should throw light on the nature of the A/B ring fusion.

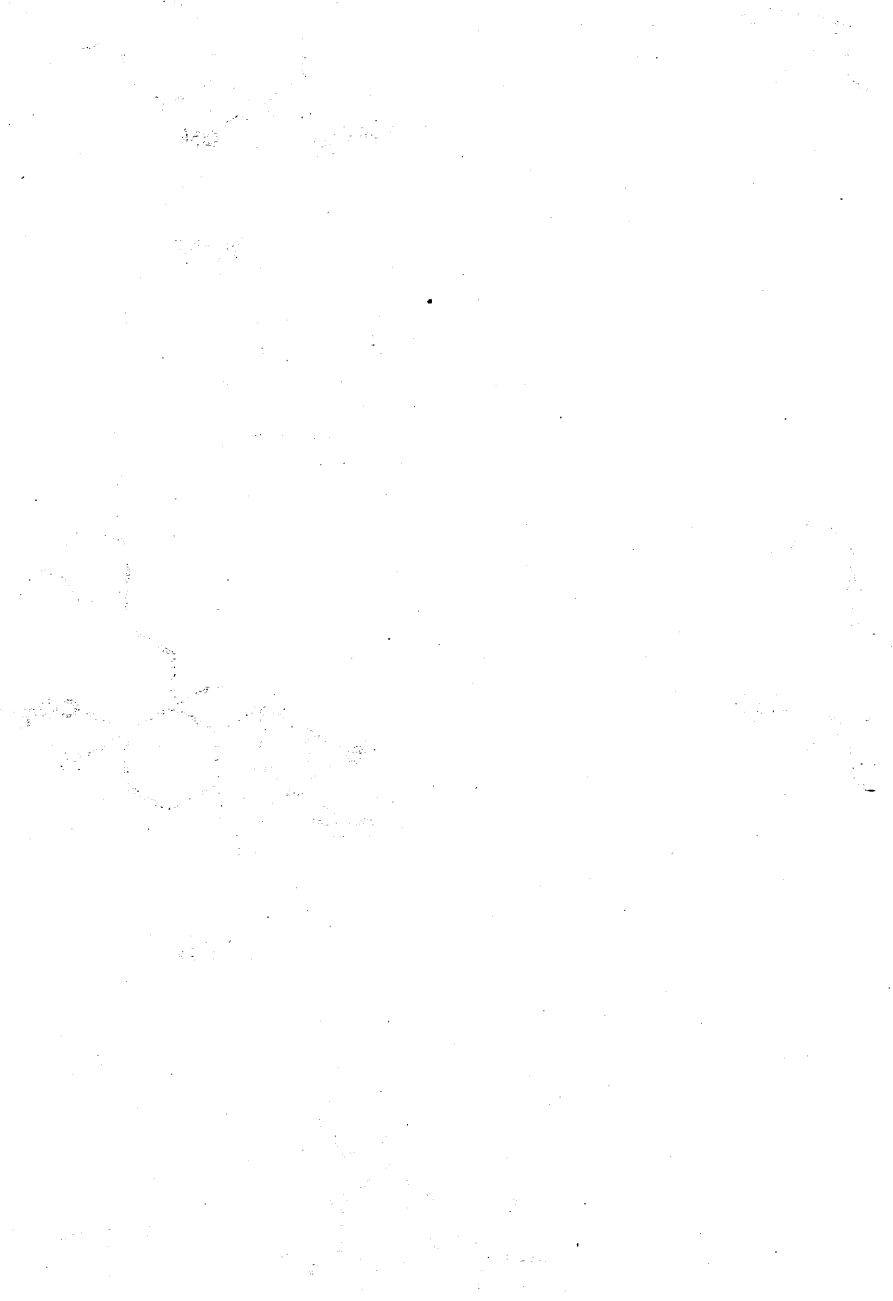
As already mentioned the lactone attached to ring A is fairly resistant to hydrolysis and one does not obtain a simple product of the type (IX). Instead dihydro<u>iso</u>columbin (X) consumed two moles of base on treatment with N sodium hydroxide to give dihydro<u>iso</u>columbinic acid $C_{20}H_{26}O_7$ (XII) m.p. 220 - 2°, $[\alpha]_D$ + 21° in which an ether bridge had been formed between $C_{(1)}$ and $C_{(12)}$[34]. A similar compound was obtained by Wessely[26] on hydrolysis of methyl<u>iso</u>columbin. The compound (XII), in agreement with the proposed structure, behaved as a diacid, had a tertiary hydroxyl group and an inert oxygen function. It was oxidised by chromium trioxide in pyridine[46], with loss of hydroxyl and one carbon atom, to the keto-acid $C_{19}H_{24}O_5$ (XIII) m.p. 204 - 7°, $[\alpha]_D$ + 16° presumably by a mechanism as shown. Since the two carboxyls/

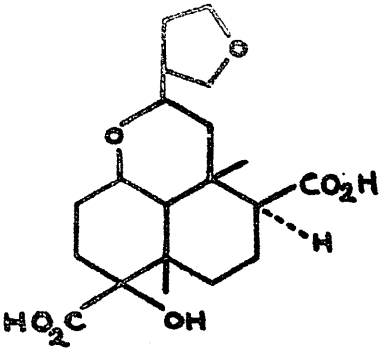
carboxyls in (XII) must have arisen from the lactone carbonyls in dihydroisocolumbin it would appear that the inert oxygen function must be related to the alkyl-oxygens of the original lactones and is best represented as an ether linkage between $C_{(1)}$ and $C_{(12)}$. Dihydroisocolumbinic acid (XII) can arise from dihydroisocolumbin (X) by a simple two stage mechanism. One mole of base is consumed and free rotation about the $C_{(9)} - C_{(11)}$ bond gives (XI). The $C_{(12)}$ hydroxyl is now in an ideal position for nucleophilic displacement, at $C_{(1)}$, of the alkyl-oxygen bond of the ring A lactone giving dihydroisocolumbinic acid (XII). (See page 73 for the bearing this reaction has on the stereochemistry of the $C_{(19)}$ methyl group.)

The first attempt to introduce a hydroxyl at $C_{(1)}$ utilised this compound. The ether function should undergo hydrogenolytic cleavage, because of its position with respect to the furan system, to give the compound (XIV). However neither dihydroisocolumbinic acid (XII) nor its methyl ester on hydrogenation with Adam's catalyst in acetic acid, or acetic acid with added hydrochloric acid, gave the required product. 3.3 moles of hydrogen were taken up readily, showing that hydrogenolysis had occurred but the products in all cases were oils. They all showed increased absorption in the 3500 cm^{-1} region of the infrared, indicating hydroxyl formation, but attempts to purify them via their acetates or benzoates were unsuccessful.

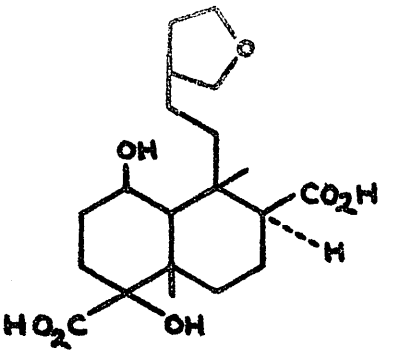
By analogy with lactone hydrogenolysis, as for example in the case of dihydrocolumbin, less than 3 moles of hydrogen should be taken up as some formation of a tetrahydro-compound (XV), not allowing hydrogenolysis to take place, would be expected. The fact that 3.3 moles of hydrogen were taken up then suggests that some cleavage of the furan ring had occurred. The hydrogenation of furan itself and 2 - methylfuran to give butanol and sec-amyl alcohol respectively provide analogies[47].

Hydrogenation of columbin derivatives results in the formation of the acidic and neutral compounds, formation of the acidic compound involving hydrogenolysis/

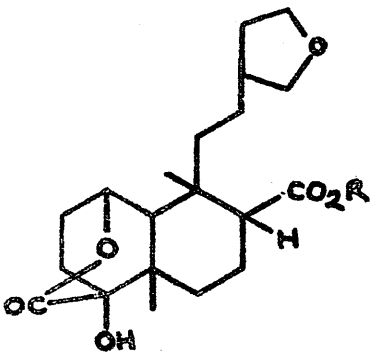




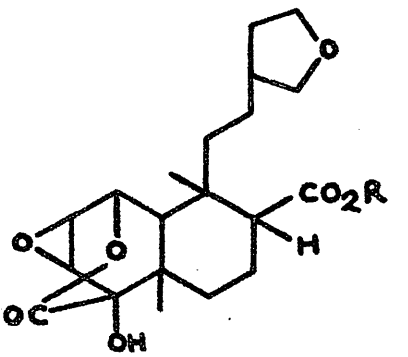
XV



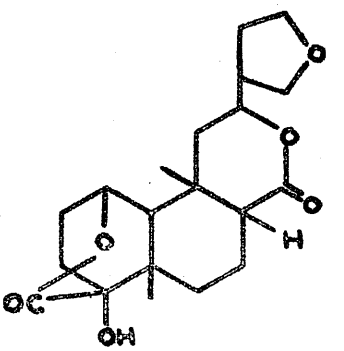
XIV



XVI



XVII



XVIII

hydrogenolysis of lactone C. The acidic hydrogenation products of columbin and isocolumbin were next considered as starting materials in obtaining a carbonyl function at $C_{(1)}$.

Wessely[25] and his colleagues describe the hydrogenation of columbin and isocolumbin with palladium black in methanol. In the case of columbin they reported the isolation of two esters (obtained from the acidic hydrogenation product after esterification and chromatography) (A) m.p. 124 - 131.5°, $[\alpha]_D$ - 18.6° and (B) m.p. < 80° $[\alpha]_D$ - 36°, and a neutral compound m.p. 240°. With isocolumbin they obtained an ester m.p. 120 - 138.5°, $[\alpha]_D$ + 48° but did not investigate the neutral fraction.

Cava[21] later reported obtaining, in good yield, a crystalline acid m.p. 158 - 60° by hydrogenation of columbin and dihydrocolumbin with 10% palladised charcoal in methanol. Under similar conditions he obtained from isocolumbin an acid m.p. 140 - 60°. In neither case were the neutral products characterised.

Columbin (I; R = H) m.p. 193 - 6° when hydrogenated in methanol with 10% palladised charcoal gave an oily acidic fraction which resisted all attempts at crystallisation in spite of careful chromatography over silica gel. The crude acid mixture was methylated and the oily product chromatographed on alumina (V) in benzene. Extensive fractional crystallisation of the solid material from chromatography finally resulted in the isolation of three methyl esters m.ps. (A) 93 - 6°, $[\alpha]_D$ - 25°; (B) 129 - 131.5°, $[\alpha]_D$ - 22° and (C) 176 - 7°, $[\alpha]_D$ - 18°. (A) and (B) analysed for the octahydrocolumbinic acid methyl esters $C_{21}H_{32}O_6$ (XVI: R = CH_3) and must be epimeric at $C_{(13)}$ (cf. Wessely)[25]. They both showed bands in the infrared at (CCl_4) 1759 (δ - lactone) and 1735 cm.$^{-1}$ (ester). The third compound had bands at (CCl_4) 1773 and 1733 cm.$^{-1}$ and analysed for hexahydrochasmanthinic acid methyl ester $C_{21}H_{30}O_7$ (XVII; R = CH_3) (Wessely[27] quotes m.p. 175 - 80°, $[\alpha]_D$ - 11.2° for this compound.) The isolation of a chasmanthin derivative from the hydrogenation/

hydrogenation of columbin is not surprising in view of the difficulty of separating chasmanthin and columbin by crystallisation. In fact mixtures of chasmanthin and columbin can only be successfully separated by crystallisation after the columbin has been decarboxylated (see page 20).

The neutral hydrogenation product appeared also to be a mixture (m.p. 245 - 56°) but was not investigated at this stage.

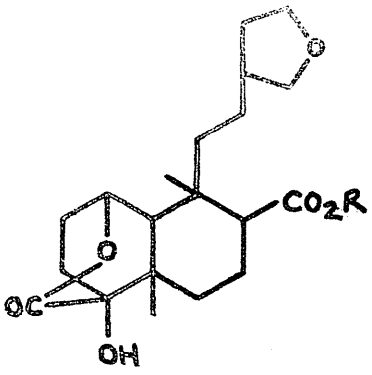
Hydrolysis of the two octahydro-esters gave two isomeric acids $C_{20}H_{30}O_6$ (XVI; R = H) (A) m.p. 173 - 5°, $[\alpha]_D$ - 32° (from ester m.p. 93 - 6°) and (B) m.p. 156 - 7°, $[\alpha]_D$ - 25° (from ester m.p. 129 - 131.5°)

Chromatography of dihydrocolumbin (X; $C_{(8)}$ β H) afforded (as previously described by Cava loc.cit.) pure dihydrocolumbin m.p. 238 - 40°, $[\alpha]_D$ + 6.7° and a second compound which, after isomerisation with N sodium hydroxide, was identical with palmarin (m.p. and infrared spectrum). The best m.p. of dihydrocolumbin, obtained by crystallisation (without chromatography),[20] was 232 - 3°; this must contain a substantial amount of chasmanthin.

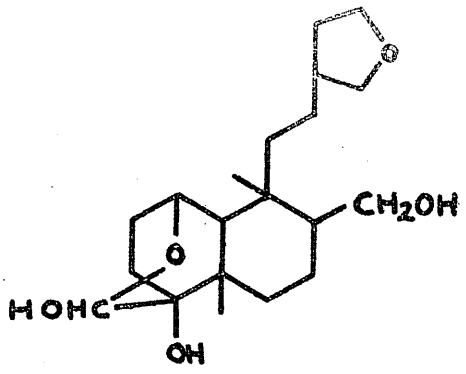
Hydrogenation of pure dihydrocolumbin (X; $C_{(8)}$ β H) m.p. 238 - 40° (under the same conditions as used for columbin) gave an acid m.p. 160 - 3° (cf. Cava)[21] which on methylation, followed by chromatography, furnished the two C_{21} esters already described, the third compound derived from chasmanthin being absent. The neutral hydrogenation product afforded hexahydrocolumbin $C_{20}H_{28}O_6$ (XVIII) m.p. 253 - 4°, $[\alpha]_D$ - 11° with no indication of a compound isomeric at $C_{(13)}$.

Hydrogenation of isocolumbin (I; R = H, $C_{(8)}$ α H), again under the same conditions, gave octahydroisocolumbinic acid (XVI; R = H, $C_{(8)}$ α H) $C_{20}H_{30}O_6$ m.p. 185 - 7°, $[\alpha]_D$ + 42° and small amounts of a second compound identified, by comparison with an authentic sample, as hexahydropalmarinic acid (XVII; R = H, $C_{(8)}$ α H).

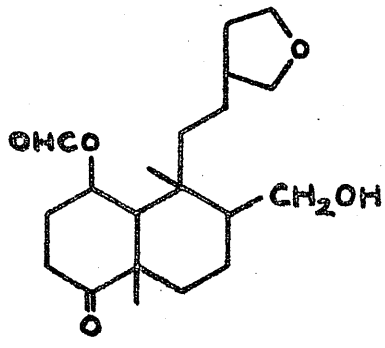
Hydrogenation of dihydroisocolumbin (X) m.p. 236 - 7°, $[\alpha]_D$ + 34° gave/



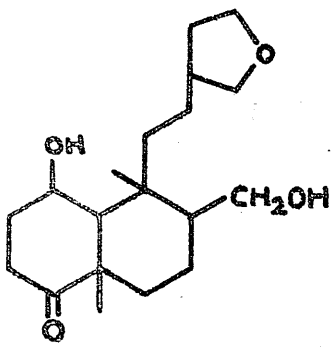
XIX



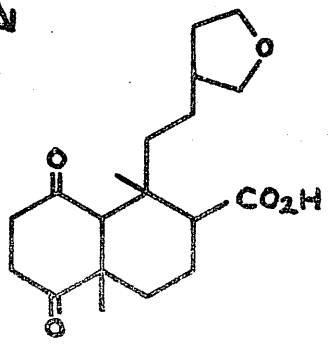
XX



XXI



XXII



XXIII

gave the acid m.p. 185 - 7° and no trace of the palmarin derivative. In both these experiments no indication of a $C_{(13)}$ epimer was found and no explanation can reasonably be given for the formation of only one acid in the 8 - iso series. The neutral hydrogenation compound gave hexahydroisocolumbin $C_{20}H_{28}O_6$ (XVIII; $C_{(8)}$ α H) m.p. 257 - 9°, $[\alpha]_D$ + 42.3° which was also a single substance as in the 8 - normal series.

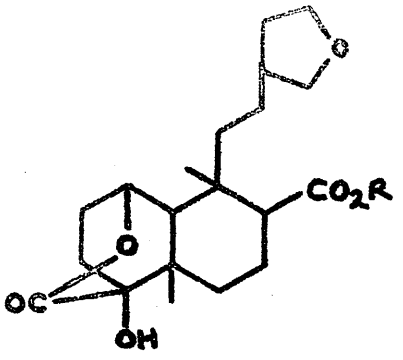
Hexahydrocolumbin (XVIII) on treatment with N sodium hydroxide furnished hexahydroisocolumbin (XVIII; $C_{(8)}$ α H) showing that these compounds have the same stereochemistry at $C_{(13)}$.

It has been shown that treatment of an axial carboxyl group with methoxide ion results in a change of configuration to the more stable equatorial carboxyl.[48,49,50] It was hoped that this experiment would convert one of the $C_{(13)}$ epimeric normal esters (XVI; R = CH_3) into the iso-ester (XVI; R = CH_3, $C_{(8)}$ α H) (or vice-versa) thereby establishing their relationship. However, the octahydrocolumbinic acid methyl esters (A) m.p. 93 - 6°, (B) m.p. 128 - 30° and the octahydroiso - ester m.p. 136 - 8° were recovered unchanged after 18 hours reflux with sodium methoxide (3%) in methanol.

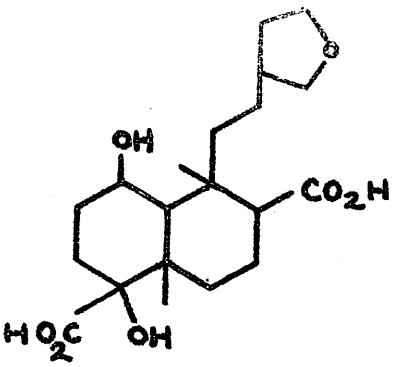
With the pure octahydro-acids in both the 8 - normal and 8 - iso series in hand, attempts were made to convert (XIX) into (XXIII). The two normal octahydro-esters, described above, and the ester m.p. 138 - 40°, $[\alpha]_D$ + 46°, derived from the octahydroiso - acid, were in turn treated with lithium aluminium hydride. The products obtained, in all cases, were oils showing little or no carbonyl absorption in the infrared and were formulated as (XX), by analogy with a similar reaction in the palmarin series (see page 20). Periodate oxidation[51] of the crude reduction products cleaved the α - glycol system giving a cyclohexanone at $C_{(4)}$ (XXI) as shown by a 1710 $cm.^{-1}$ band in the infrared. The oxidation products were again oils and attempts to characterise them via their p - toluene sulphonyl derivatives failed. Although the infrared spectra of all the above compounds indicated that reaction (XIX) to (XXI) had occurred this/

	ν max. cm^{-1} ($CHCl_3$)
Octahydroisocolumbinic acid methyl ester (XVI; R = CH_3, $C_{(8)}$ α H)	1750 (α - hydroxy - δ - lactone) 1734 (ester)
Dihydroisocolumbinic acid methyl ester (XII)	1724 (ester and hydroxy-ester)
Isolactone methyl ester (XXVI; R = CH_3)	1720 (δ - lactone and α - hydroxy-ester)
	ν max. $cm.^{-1}$ ($C Cl_4$)
Octahydroisocolumbinic acid methyl ester (XVI; R = CH_3, $C_{(8)}$ α H)	1759 (α - hydroxy - δ - lactone) 1741 (ester)
Dihydroisocolumbinic acid methyl ester (XII)	1737 (ester) 1721 (α - hydroxy - ester)
Isolactone methyl ester (XXVI; R = CH_3)	1745 (δ - lactone) 1723 (α - hydroxy - ester)

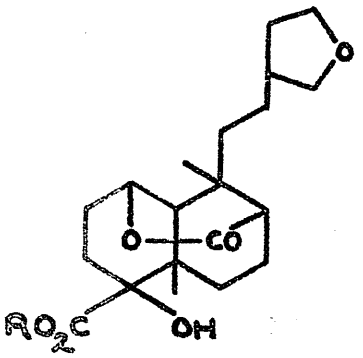
TABLE I.



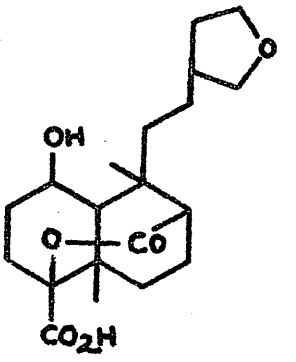
XIX



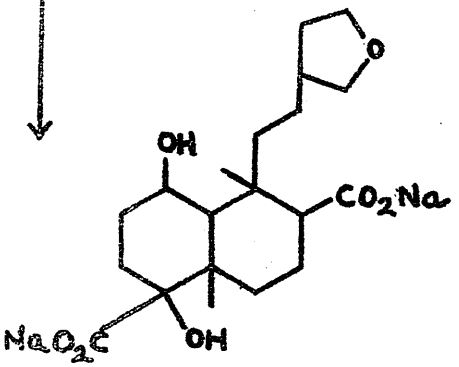
XXIV



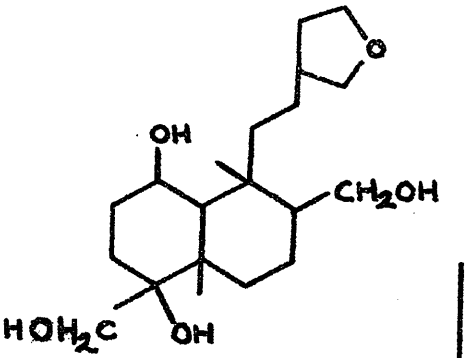
XXVI



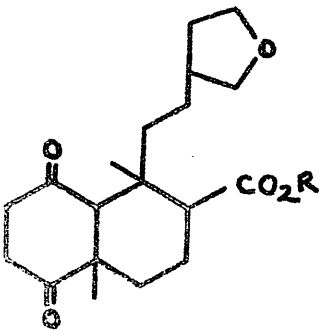
XXV



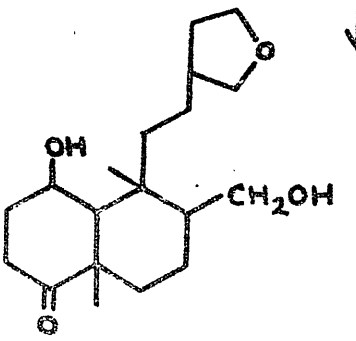
XXIX



XXVII



XXX



XXVIII

this approach was not pursued because characterisation of the intermediates had proved unsuccessful.

The approach summarised in expressions (XIX) to (XXIV) was next considered since, now that formation of a 1, 12 oxide is impossible, the lactone attached to ring A should hydrolyse under sufficiently vigorous conditions.

Octahydroisocolumbinic acid (XVI; R = H, $C_{(8)}$ α H) on treatment with N sodium hydroxide for 4 hours (the conditions which brought about the dihydroisocolumbin (X) to dihydroisocolumbinic acid (XII) change) consumed 1.2 moles of base indicating that partial hydrolysis had occurred. The product still had a 1760 cm^{-1} band in the infrared showing survival of the ring A δ - lactone system. Consequently to effect complete hydrolysis octahydroisocolumbinic acid was subjected to more vigorous alkaline conditions. With 4 N sodium hydroxide for 4 hours under nitrogen 2 moles of base were consumed and a crystalline compound was obtained in high yield. This compound m.p. 240 - 2°, $[\alpha]_D$ -47° (subsequently referred to as the 'iso-lactone') titrated in the cold as a mono-acid with an equivalent weight of 369 (calc.366), on heating as a di-acid and was found on analysis to be isomeric with the starting material $C_{20}H_{30}O_6$. Comparison of the infrared spectra of the two compounds (Table I) showed that the ring A δ - lactone system was absent in the product. The compound, however, cannot be the expected dihydroxy - di-acid (XXIV) but must contain lactone and carboxyl functions. Assuming that no deep-seated rearrangement of the carbon skeleton has occurred then either (XXV) or (XXVI; R = H) must represent the hydrolysis product. The hydroxyl function was stable to acetic anhydride in pyridine at 100° favouring structure (XXVI; R = H). In support of this a PK of 3.98 for the compound compares with PK 4.05 for dihydroisocolumbinic acid (XII) and differs from PK 5.1 for octahydroisocolumbinic acid (XVI; R = H, $C_{(8)}$ α H). Moreover the infrared spectrum of dihydroisocolumbinic acid methyl ester has carbonyl bands comparable with those of the iso-lactone methyl ester (Table I).

In/

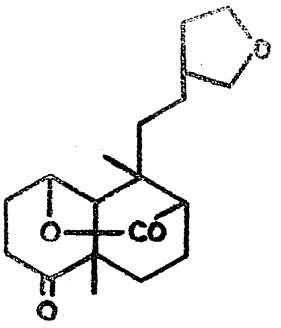
In view of the importance of this compound in elucidating the stereochemistry of columbin experiments were conducted further to support its structure.

The series (XXVI; R = H) to (XXX) was first attempted. The action of lithium aluminium hydride on the oily iso - lactone methyl ester (XXVI; R = CH_3) furnished an oily product (XXVII) whose carbonyl absorption in the infrared was very slight. The crude compound was treated with periodate[51] giving an oil (XXVIII) with a 1710 cm.$^{-1}$ (cyclohexanone) band but this could not be isolated in a pure state. The appearance of carbonyl absorption does however support the presence of an α - glycol in (XXVII) and hence an α - hydroxy acid in (XXVI; R = H).

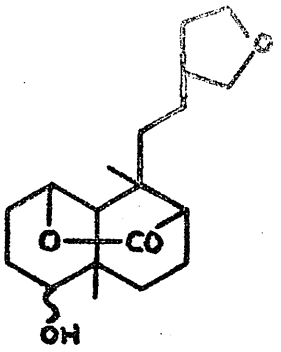
The sodium salt of the iso - lactone (XXIX) was obtained as a white amorphous solid showing a band in the infrared at 1580 cm.$^{-1}$ (carboxylate anion). The action of chromium trioxide on this compound suspended in pyridine gave a non-crystalline material with an intense 1710 cm.$^{-1}$ (cyclohexanone) band in the infrared. The infrared spectrum of the product on diazomethane treatment showed no hydroxyl absorption and therefore was probably (XXX; R = CH_3). Although again the spectral evidence indicated that the reaction sequences had taken place as expected none of the products could be satisfactorily characterised.

By analogy with dihydroisocolumbinic acid (XII) oxidation of the iso - lactone with chromium trioxide in pyridine gave the oily keto - lactone $C_{19}H_{28}O_4$ (XXXI) with bands in the infrared ($CH Cl_3$) at 1741 (δ - lactone) and 1712 cm.$^{-1}$ (cyclohexanone). The same compound was obtained with chromium trioxide in acetone - sulphuric acid[52] and with lead dioxide in acetic acid (at reflux)[53]. The latter was shown also to oxidise dihydroisocolumbinic acid smoothly to (XIII) in good yield and in the case of the iso - lactone was the most efficient of the methods tried. All attempts to obtain this ketone as a crystalline solid failed and it did not form an oxime, a 2:4 dinitrophenylhydrazone or condense with benzaldehyde under the usual conditions.[54]

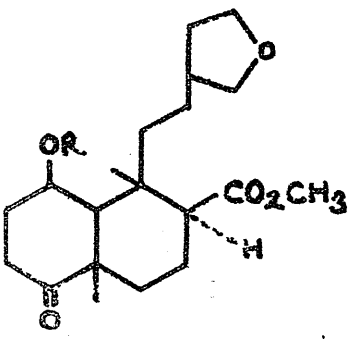
Reduction/



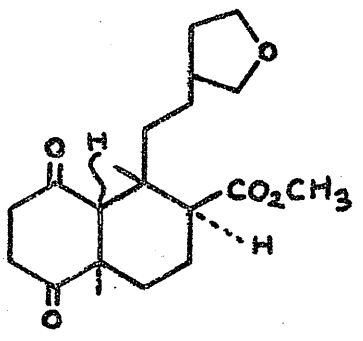
XXXI



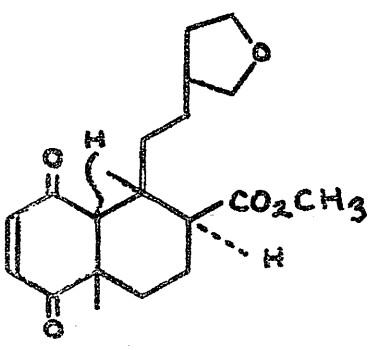
XXXII



XXXIII



XXXIV



XXXV

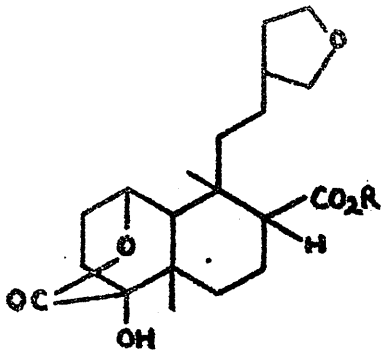
Reduction of the ketone $C_{19}H_{28}O_4$ (XXXI) with sodium borohydride in aqueous tetrahydrofuran[55] followed by chromatography afforded in about 10% yield the crystalline hydroxy - lactone $C_{19}H_{30}O_4$ (XXXII) m.p. 174 - 5°, $[\alpha]_D$ + 20° with infrared absorption at ($CH Cl_3$) 1727 $cm.^{-1}$ (δ - lactone). Some unchanged keto - lactone (XXXI) was obtained from the crude reaction product along with material in which absence of carbonyl absorption in the infrared suggested attack of borohydride on the lactone system. The method described in the experimental section represents the best of many attempts, using various amounts of borohydride and reaction times, to improve on the yield of crystalline reduction product (XXXII).

The hydroxy-lactone (XXXII) was reoxidised with chromium trioxide in acetic acid to give the keto - lactone (XXXI) (identical infrared spectrum).

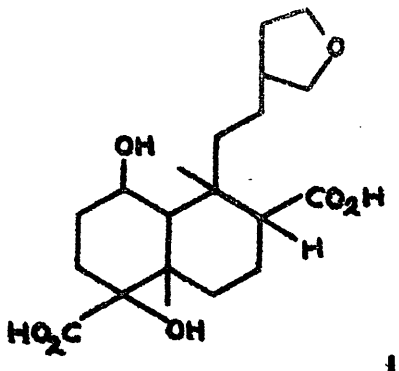
Hydrolysis of the keto - lactone with 1.5N sodium hydroxide occurred smoothly, 1 mole of base being consumed. After acidification, the hydroxy-acid was extracted quickly into ether and treated with ethereal diazomethane giving the hydroxy - ester (XXXIII; R = H) as an oil, characterised as the crystalline acetate (XXXIII; R = $COCH_3$) m.p. 130 - 3°, $[\alpha]_D$ + 74°.

Oxidation of the hydroxy - ester (XXXIII; R = H) with chromium trioxide in pyridine furnished the semi-crystalline diketone (XXXIV) which yielded, on crystallisation followed by chromatography of the mother liquors, two isomeric fractions $C_{20}H_{30}O_5$ (A) m.p. 130 - 140°, $[\alpha]_D$ - 64° (B) m.p. 116 - 29°, $[\alpha]_D$ - 49°. These fractions must presumably be different mixtures of the $C_{(10)}$ epimers of (XXXIV). The crude mixture was not converted to a single compound on treatment with base indicating that an equilibrium mixture of cis and trans isomers was formed. The two fractions had infrared absorption bands ($CHCl_3$) at 1740 (ester) and 1710cm^{-1} (cyclohexanone) and on dehydrogenation with selenium dioxide gave the ene-diones $C_{20}H_{28}O_5$ (XXXV) (A) m.p. ca.100°, $[\alpha]_D$ - 69°, λ max. 228 mμ , (ε 9,500) (from the dione mixture of m.p. ca. 135°) (B) an oil, λ max. 230 mμ (ε 9,000) (from the dione mixture of m.p. ca. 120°) which were again mixtures. (See page 56 for a discussion of the relative stabilities of cis and trans 10 - methyldecalones.)

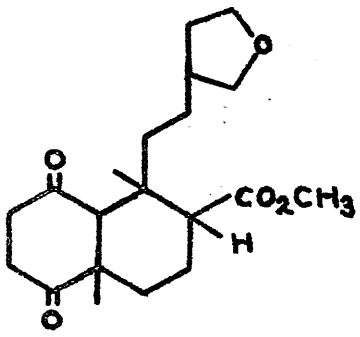
The/



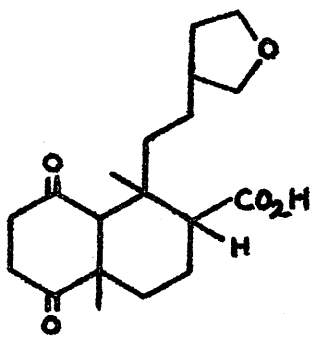
XVI



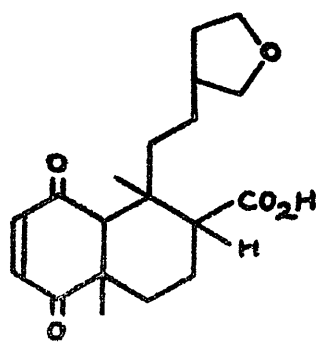
XXXVI



XXXVII



XXXVIII



XXXIX

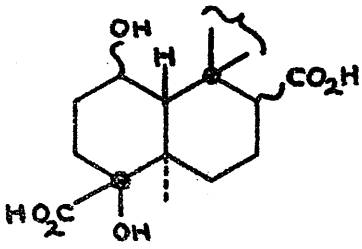
The results of this series of experiments is in accordance with the postulated structure (XXVI; R = H) for the iso-lactone and provide the necessary further evidence for establishing its structure.

Attention was then turned to the hydrolysis of octahydrocolumbinic acid methyl ester (XVI) under the same conditions which had yielded the iso - lactone. The octahydrocolumbinic acid methyl ester m.p. 128 - 30° on treatment with 4N sodium hydroxide for 4 hours under nitrogen gave the dihydroxy - diacid (XXXVI) $C_{20}H_{32}O_7$ m.p. 186 - 8°, $[\alpha]_D$ - 130°, equivalent weight 195 (calculated 192). This diacid was oxidised smoothly with chromium trioxide in acetone - sulphuric acid to the dione $C_{19}H_{28}O_5$ (XXXVIII) m.p. 218 - 20°, $[\alpha]_D$ + 80° which gave, with selenium dioxide, the ene - dione (XXXIX) as an oil λ max. 230m.μ (ε 9,200). Hydrolysis of the $C_{(13)}$ epimeric octahydroisocolumbinic acid methyl ester m.p. 95 - 6° did not give a single compound. The infrared spectrum of the product showed carbonyl absorption characteristic of ring A lactone indicating only partial hydrolysis and prolonged treatment with alkali did not afford the desired product. In view of the difficulties involved in preparing the pure octahydro - esters in the normal series this reaction was not pursued.

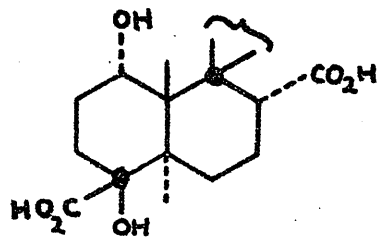
The stereochemical implications of all the above reactions will now be discussed.

The formation of the iso-lactone, in high yield, under conditions described makes it possible to reach a number of conclusions as to the stereochemistry of the columbin molecule.

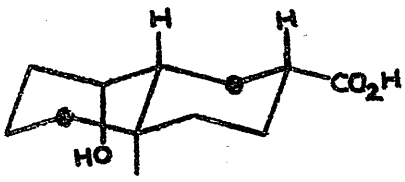
An examination of the model of a trans - fused decalin system (XL), with both rings in the stable chair conformation, shows that no intramolecular reaction could occur between a hydroxyl at $C_{(1)}$ and a carboxyl at $C_{(7)}$, irrespective of the relative stereochemistries of these groups. Allowing them for a conformational change to a boat form in either or both rings we find that lactonisation is again impossible; (XLI b) representing the situation with closest/



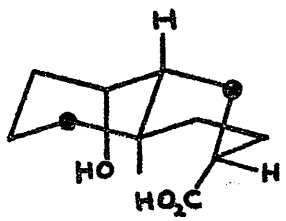
XL



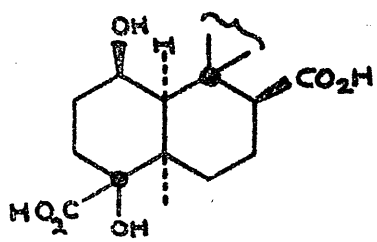
XLI



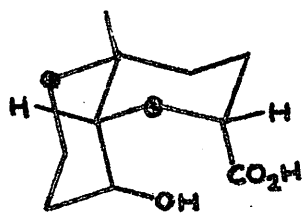
XLI a



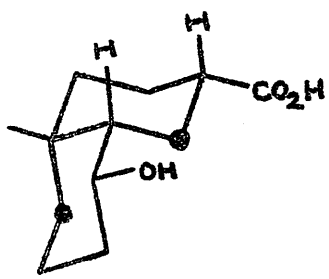
XLI b



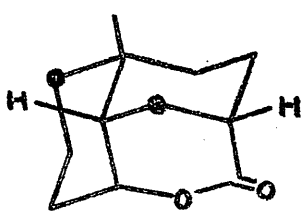
XLII



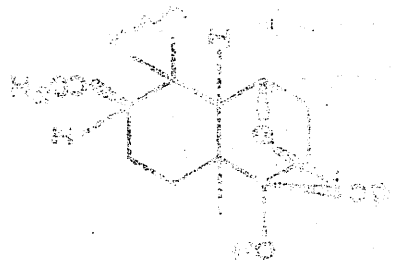
XLIII a

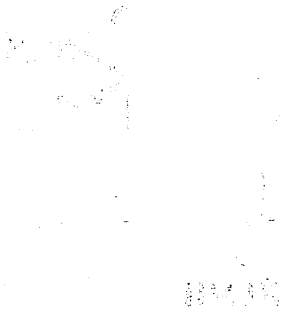


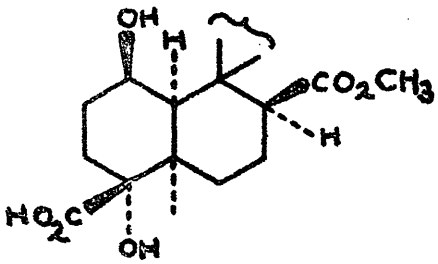
XLIII b



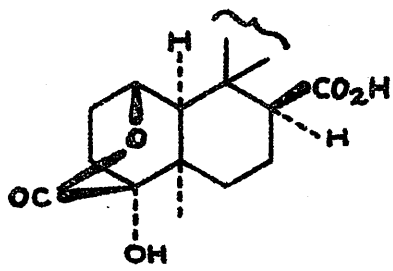
XLIV



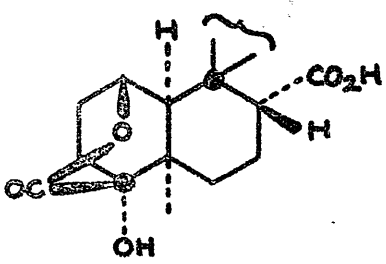




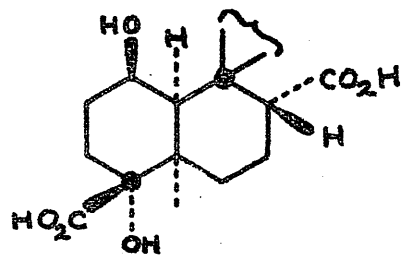
XLV



XLVI

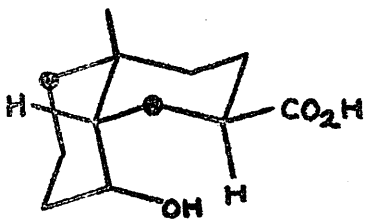


XLVII

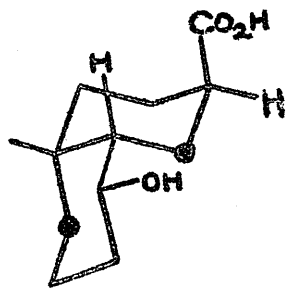


XLVIII

|||



OR



closest approach of hydroxyl to carboxyl shows serious methyl - carboxyl interactions. In any case there is no convincing reason for either of the rings adopting a boat conformation in the decalin system formed on hydrolysis of octahydroisocolumbinic acid.

With a cis fusion of rings A and B the situation is somewhat different, two stable conformations, (XLIII a) steroid type and (XLIII b) non-steroid type, are then possible. It can be readily seen that only with the steroid conformation of (XLII), with both hydroxyl and carboxyl β, is intramolecular formation of a δ - lactone (XLIV) possible. In view of the spontaneous formation of the iso - lactone, (XLV) must represent the compound formed by hydrolysis of the ring A lactone and (XLVI), or the enantiomer, the situation in octahydroisocolumbinic acid itself. It follows that in columbin the A/B ring fusion must be cis and the angular methyl group at $C_{(5)}$ must be on the opposite side of the molecule to the lactone bridge.

The fact that (XLVI) is a unique system which will give rise to the iso-lactone is confirmed by the hydrolysis of octahydrocolumbinic acid methyl ester (XLVII). The change in configuration of the carboxyl group attached to $C_{(8)}$ does not allow lactone formation to occur and the product, as expected, is the dihydroxy-diacid (XLVIII).

The reactions involving the iso - lactone discussed earlier will now be dealt with in the light of the above stereochemical observations.

Hydrolysis of octahydroisocolumbinic acid (XLVI) results in the opening of ring A lactone and is followed by a conformational change in both rings to give the situation (XLIII a) permitting easy lactonisation to the iso-lactone (XLIV). The α - hydroxy acid system formed in the hydrolysis must be responsible for the steroid conformation being adopted. This is shown by the fact that in the keto - lactone (XLIX) hydrolysis gives an acidic compound which does not immediately lactonise on standing in acid solution. (The acidic material/

(XLV) 1 : 3 (diaxial) non-bonded interactions.

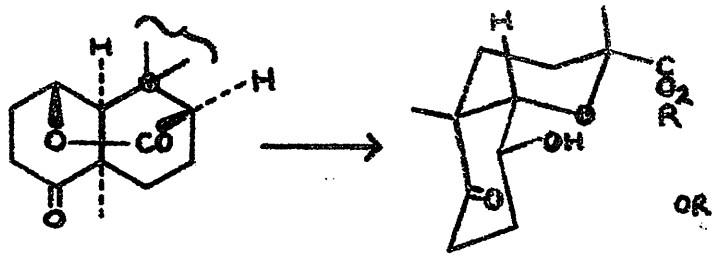
Steroid Conformation		Non-Steroid Conformation	
Number	Type	Number	Type
1	CH_3 - CH_3	2	CH_3 - CH_3
1	CH_3 - CO_2H	2	CH_3 - CO_2H
5	CH_3 - H	5	CH_3 - H
3	OH - H	1	OH - H
2	CO_2H - H	1	CO_2H - H
2	OH - CH_3	1	OH - CH_3
Difference			
1	CH_3 - OH	1	CH_3 - CH_3
2	OH - H	1	CH_3 - CO_2H
1	CO_2H - H		

TABLE XI.

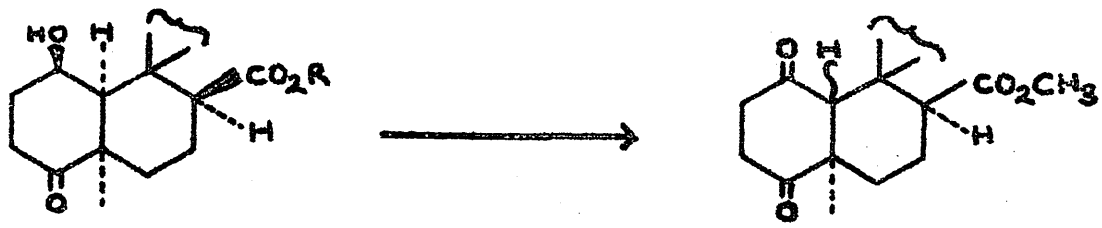
(L) and (LI) 1 : 3 diaxial non-bonded interactions.

Steroid Conformation (LI: R = H)		Non-Steroid Conformation (L; R = H)	
Number	Type	Number	Type
1	$CH_3 - CH_3$	2	$CH_3 - CH_3$
1	$CH_3 - CO_2H$		
2	$CH_3 - OH$	1	$CH_3 - OH$
5	$CH_3 - H$	5	$CH_3 - H$
1	$CO_2H - H$		
1	$OH - H$		
Difference			
1	$CH_3 - CO_2H$	1	$CH_3 - CH_3$
1	$CH_3 - OH$		
1	$CO_2H - H$		
1	$OH - H$		

TABLE III.



XLIX L LI



L LII

LIII

LIV

LV LVI

material on standing in tetrahydrofuran with dilute hydrochloric acid gave 1/3 unchanged hydroxy-acid after 6 hours.) In this case the non-steroid conformation (L; R = H) must be predominant.

The 1 : 3 diaxial non-bonded interactions occurring in the steroid and non-steroid conformations of (XLV), derived from octahydroisocolumbinic acid, are listed in Table (II). Consideration of these does suggest that the steroid conformation should be preferred. In Table (III) the interactions in (L) and (LI) are listed and in this case indicate that the non-steroid conformation (L) should be the more stable.

Oxidation of the hydrolysis product of the keto lactone, after methylation (L; R = CH_3) gives rise to a crystalline product (LII) which was found to be a mixture of $C_{(10)}$ epimers as already described.

It is known that with α decalone (LIII) the cis isomer is less stable than the trans by about 2.1 K.cals.[56] Equilibration of the cis isomer with base therefore results in quantitative conversion to the trans.[56] Studies by Sondheimer[57] in the 10 - methyl decalone series (LIV), a case more relevant to the present discussion, showed that at equilibrium (brought about by base treatment) a 40% cis to 60% trans mixture was obtained. The compound (LV) with a 1, 4 ene - dione system was found by Woodward[58] to give a mixture of $C_{(5)}$ epimers on treatment with sodium hydride in benzene but the compound (LVI) gave a quantitative yield of the trans isomer on treatment with sodium hydroxide in dioxan.[59]

Although a trans fusion of cyclohexane rings is the most stable in the case of the simple decalin, the relative stabilities of cis and trans decalones of different types must depend largely on non-bonded interactions and the results are less readily predictable. Since with cis α decalones two conformations are possible, the situation can arise where there is little difference in the relative stabilities of the trans and one of the cis conformers.

The/

(LII) 1 : 3 diaxial non-bonded interactions.

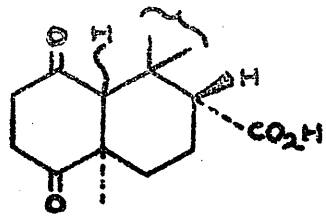
Trans Isomer		Cis Isomer (Steroid)		Cis Isomer (Non-Steroid)	
Number	Type	Number	Type	Number	Type
1	$CH_3 - CH_3$	1	$CH_3 - CH_3$	2	$CH_3 - CH_3$
3	$CH_3 - H$	4	$CH_3 - H$	4	$CH_3 - H$
2	$CO_2CH_3 - H$	1	$CO_2CH_3 - H$		
		1	$CO_2CH_3 - CH_3$		
Difference					
2	$COCH_3 - H$	1	$CH_3 - H$	1	$CH_3 - CH_3$
		1	$CO_2CH_3 - CH_3$	1	$CH_3 - H$
		1	$CO_2CH_3 - H$		

TABLE IV.

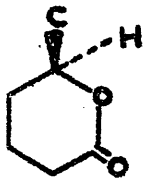
LVII 1 : 3 diaxial non-bonded interactions

Trans Isomer		Cis Isomer (Steroid)		Cis Isomer (Non-Steroid)	
Number	Type	Number	Type	Number	Type
1	$CH_3 - CH_3$	1	$CH_3 - CH_3$	2	$CH_3 - CH_3$
3	$CH_3 - H$	5	$CH_3 - H$	4	$CH_3 - H$
				2	$CO_2H - H$
Difference					
-	-	2	Me - H	1	$CH_3 - CH_3$
				1	$CH_3 - H$
				2	$CO_2H - H$

TABLE I.

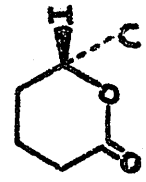


LVII



a

LVIII



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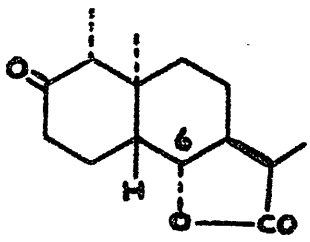
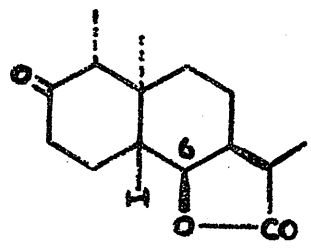


a

LIX



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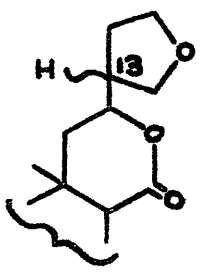
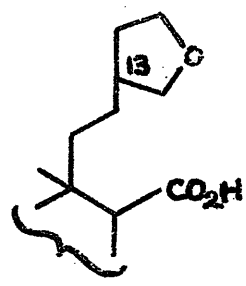


a

LX



b

The dione mixture (LII) obtained from the keto - lactone (XLIX) was not converted to a single compound on treatment with base showing that the energy differences between cis and trans forms must be small. Considerations of the 1 : 3 diaxial non-bonded interactions in the dione (LII), in the trans and two cis forms (TABLE IV), do suggest that the trans form is the more stable and it should predominate in an equilibrium mixture.

The situation in the case of the dione (LVII), obtained from the dihydroxy-diacid (XLVIII) in the normal series, is not clear. From its sharp melting point (see page 49) this compound appeared to be uniform and it is essentially unaffected by base treatment. The inference is that it exists predominantly as one isomer but consideration of the 1 : 3 interactions listed in Table (V) indicates that there should be little difference in stability between trans and cis (steroid conformation) types. Consequently this compound, in spite of its narrow melting range, may well be a mixture of isomers.

The stereochemistry of the rest of the columbin molecule will now be considered with particular reference to the B/C ring fusion and the configuration at $C_{(12)}$. That the B/C ring fusion in the iso-series is trans will be supported by the arguments which follow.

It was thought that molecular rotation measurements would help to establish the absolute configuration at $C_{(12)}$.

Klyne's[60] modification of the Hudson[61] lactone rule states that if $\Delta [M]_D$ for lactone minus corresponding acid (or corresponding deoxyacid) is positive the lactone is of the type (LVIII a), if negative of the enantiomeric type (LVIII b). It seemed reasonable that this rule should hold when measurements were made on a lactone in neutral and basic solutions. (This technique has been used by Barton[62] to predict the correct stereochemistry at $C_{(6)}$ in the compounds (LIX a) and (LIX b) derived from lumisantonin.) This method was applied to the columbin system since only the ring C lactone should be affected/

	$[M]_D^N$	$[M]_D^A$	$\Delta[M]_D$
Isocolumbin (I, R = H, $C_{(8)}$ H)	+ 160.5	+ 411.5	- 250.9
Dihydroisocolumbin (X)	+ 61.0	+ 935.8	- 874.4
Methyldihydroisocolumbin (X; OH = OCH_3)	+ 241.9	+ 257.4	- 15.5
Decarboxyacetylisocolumbin (VII)	-1170.0	- 581.3	- 765.7
Hexahydroisocolumbin (XVIII, $C_{(8)}$ H)	+ 120.6	+ 198	- 77.4
Hexahydrodecarboxyisocolumbin (LXXV)	+ 174	+ 280	- 106

N NEUTRAL SOLUTION
A ALKALINE SOLUTION.

TABLE VI.

	$[M]_D^N$	$[M]_D^A$	$\Delta[M]_D$
Palmarin	- 21.7	+ 121	- 142.7
Isojateorin	+ 23.9	+ 77.1	- 43.2
Methylpalmarin	+ 150	+ 237.5	- 87.5
Methylisojateorin	+ 166	+ 212	- 46.0
Tetrahydropalmarin	- 147	+ 79	- 226
Limonin	+ 358.1	+ 396	- 38
Guarigenyl - 3 β - iodoacetate	- 52.3	+ 78.4	- 130.7
Swietenine	+ 350	+ 358	- 8

N NEUTRAL SOLUTION
A ALKALINE SOLUTION.

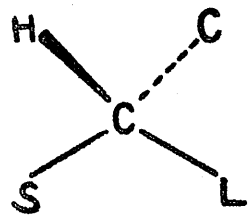
TABLE VII.

	$[M]_D$	$\Delta[M]_D$
Octahydroisocolumbinic acid (XVI; R = H, $C_{(8)} \alpha$ H)	+ 122	
		- 2
Hexahydroisocolumbin (XVIII; $C_{(8)} \alpha$ H)	+ 120	
Octahydrocolumbinic acids I and II (XVI; R = H)	- 91.5, 115.2	
		+ 49.8, + 73.5
Hexahydrocolumbin (XVIII)	- 41.7	
Hexahydropalmarinic acid (XVII ; $C_{(8)} \alpha$ H)*	+ 155.8	
		- 95.4
Tetrahydropalmarin	+ 60.4	
Hexahydroisojateorinic acid (XVII; $C_{(8)} \alpha$ H)*	+ 155.8	
		+ 3
Tetrahydroisojateorin	+ 158.8	

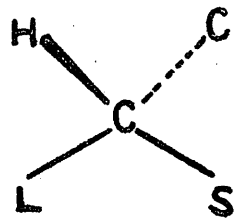
*(These are the same compound)

TABLE VIII.

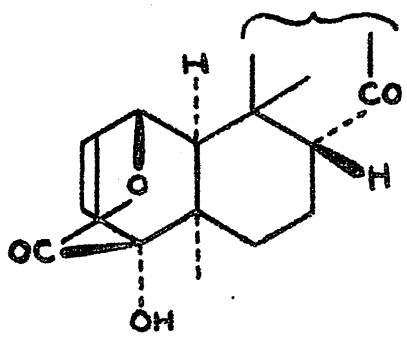
L Large group, S Small group.



LXI



LXII



LXIII

affected with weak base. It was found (Table VI) that all columbin derivatives gave a negative $\Delta[M]_D$ when their rotations were compared in neutral and basic media indicating a system of the type (LVIII b). However when this method was applied to palmarin (Table VII), which is known to have the enantiomeric stereochemistry at $C_{(12)}$ (see page 20), it was found that the $[M]_D$ change for neutral and alkaline solutions was still negative. This was also the case with isojateorin derivatives, limonin, guarigenyl - 3β - iodo-acetate and swietenine (Table VII). These results show that the procedure of comparing the $[M]_D$ of lactones in neutral and basic solutions can lead to erroneous conclusions and must be used with care (see Sykora)[63].

A more reliable application of the Hudson - Klyne lactone rule is to hand through use of the neutral and acidic hydrogenation products of columbin. This makes available pairs of compounds related as (LX a), (LX b) with the proviso that they may be epimeric at $C_{(13)}$.

The $[M]_D$ values of the acidic and neutral hydrogenation products of columbin, isocolumbin palmarin and isojateorin are listed in Table (VIII). The $\Delta[M]_D$ values for the columbin and palmarin derivatives have opposite signs indicating, as shown by direct interrelation, opposite stereochemistries at $C_{(12)}$. In this case use was made of the Hudson - Klyne rule to assign absolute configurations at $C_{(12)}$ in palmarin and columbin. The positive $\Delta[M]_D$ in the case of columbin shows that lactone C is of the type (LVIII a); the negative $\Delta[M]_D$ in the case of palmarin shows that lactone C is of the enantiomeric type (LVIII b). The sign of the $\Delta[M]_D$ values in the case of the isocolumbin and isojateorin derivatives is not really certain as the small difference in $[M]_D$ values between the acidic and neutral hydrogenation products is within the limits of experimental error.

Bose,[64] by considering the various rotation rules of Hudson,[61] Klyne and Stokes[65] and Mills,[66] has proposed that with the systems (LXI) and/

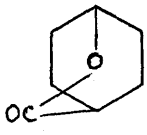
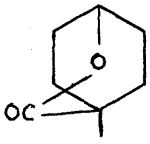
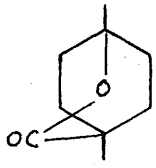
	$[\alpha]_D$
Palmarin	+ 12
Isojateorin	+ 30
Methylpalmarin	+ 42
Methylisojateorin	+ 58
Tetrahydropalmarin	+ 16
Tetrahydroisojateorin	+ 39

TABLE IX.

			ν max. cm.$^{-1}$	
			$CHCl_3$	CCl_4
Columbin		A	1753	1762
		C	1731	1743
Isocolumbin		A + C	1755	1765
Dihydrocolumbin		A	1752	—
		C	1731	—
Dihydroisocolumbin		A + C	1754	—
Decarboxycolumbin		C	1733	1741
		D	1713	1717
Decarboxyisocolumbin		C	1748	1761
		D	1712	1717
Octahydroisocolumbinic acid methyl ester		A	1750	1759
		ester	1734	1741
Octahydrocolumbinic acid methyl ester	I	A	—	1759
		ester	—	1735
	II	A	—	1759
		ester	—	1735
Dihydrodecarboxyisocolumbin		C	1748	1763
		D	1704	1711

(A = ring A δ - lactone, C = ring C δ - lactone, D = cyclohexanone)

TABLE X.

		ν max. cm.$^{-1}$	
		$CHCl_3$	CCl_4
a 		—	1768
b 		—	1765
c 		—	1759
Methylpalmarin	A	1778	-
	C	1741	-
Acetylpalmarin	A	1791	-
	C	1756	-
Hexahydropalmarinic acid methyl ester	A	1776	-
	ester	1732	-
Tetrahydromethylpalmarin	A	1778	-
	C	1743	-

(A = ring A - lactone, and C = ring C - lactone)

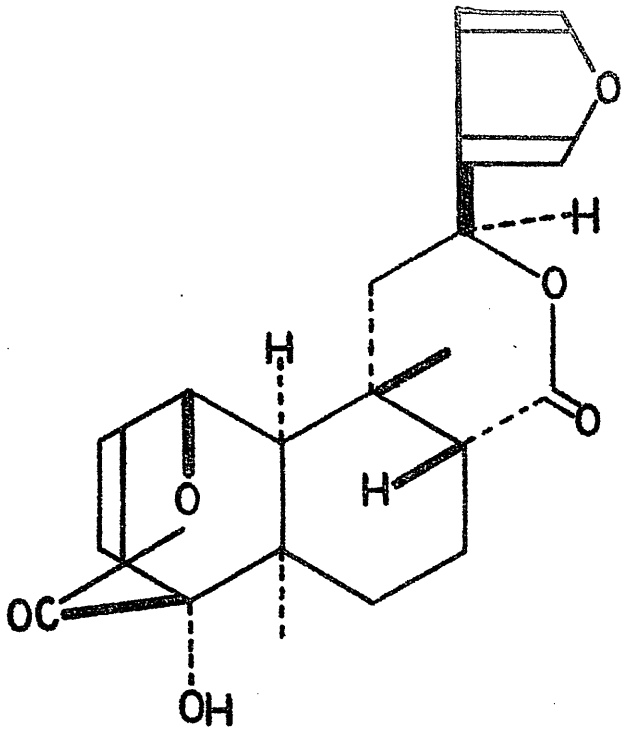
TABLE XI.

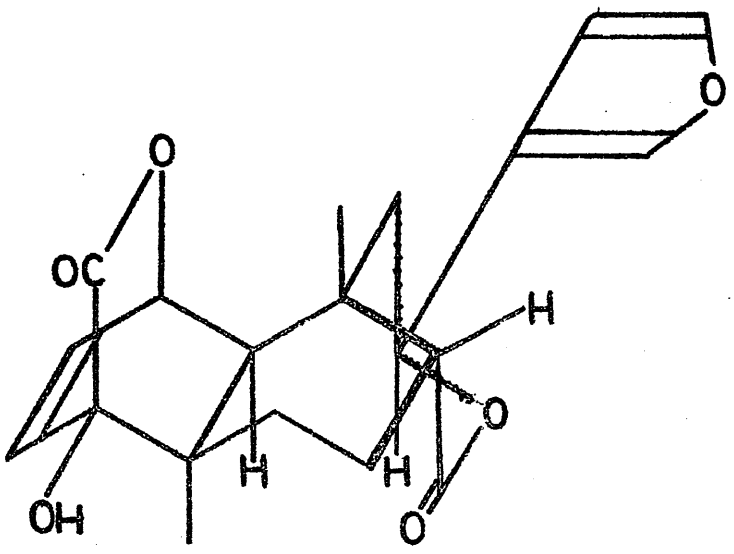
and (LXII) the former will be more dextrarotatory. He found that this rule was of general application in the sugar and steroid field and used it successfully to predict the absolute configuration of a number of naturally occurring compounds.

Application of the Bose rule to palmarin and isojateorin (Table IX) derivatives lead to the same conclusions as above namely a lactone of the type (LVIII a) in columbin and isojateorin and of the type (LVIII b) in palmarin.

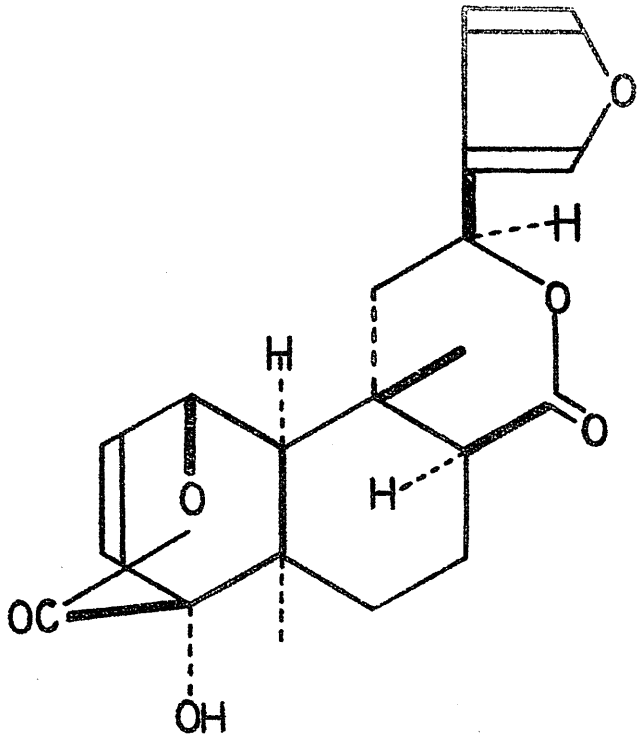
In Table (X) the carbonyl frequencies in the infrared spectra of various columbin and isocolumbin derivatives are listed. It can be seen that in all cases a change from the normal to the iso-series is accompanied by an upwards shift in frequency of the band associated with the ring C lactone. This is most noticeable in the decarboxy compounds and the shift is usually of the order of 15 - 20 wave numbers. The carbonyl frequencies of the ring A α - hydroxy boat δ - lactones in columbin derivatives are abnormally high for δ-lactones (Table X) presumably because of the strained nature of the lactone system. In the palmarin series the additional strain in ring A, induced by the presence of the 2, 3 - epoxide, is probably responsible for this abnormally high frequency being exaggerated (Table XI) even further. The compounds (a), (b) and (c) in Table (XI) also show abnormally high carbonyl frequencies for a δ- lactone and this could be due to the fact that the lactone is fixed in a boat conformation. The high frequency bands in columbin derivatives could also be explained by the fixed boat conformation of the ring A lactone and it seems reasonable to state that the columbin — isocolumbin change involves change from a normal chair δ - lactone (1731 cm.$^{-1}$ ($CH\ Cl_3$), 1743 cm.$^{-1}$ ($C\ Cl_4$)) to a boat δ - lactone (1755 cm.$^{-1}$ ($CH\ Cl_3$), 1765 cm.$^{-1}$ ($C\ Cl_4$)).

The partial stereochemistry (LXIII), or the enantiomer, has been deduced for columbin from the chemistry of the iso - lactone (XXVI; R = H). In/





LXIV


O
H
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H
O
O
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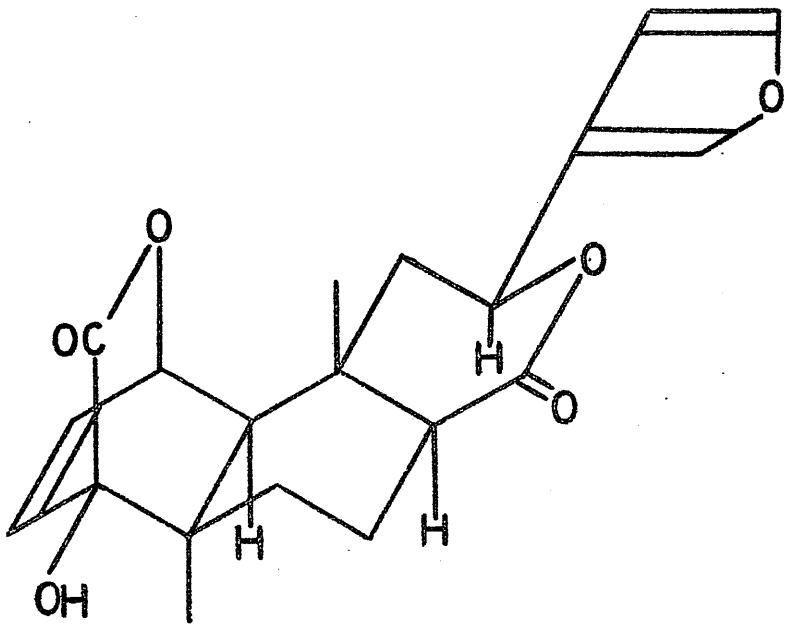
OC
OH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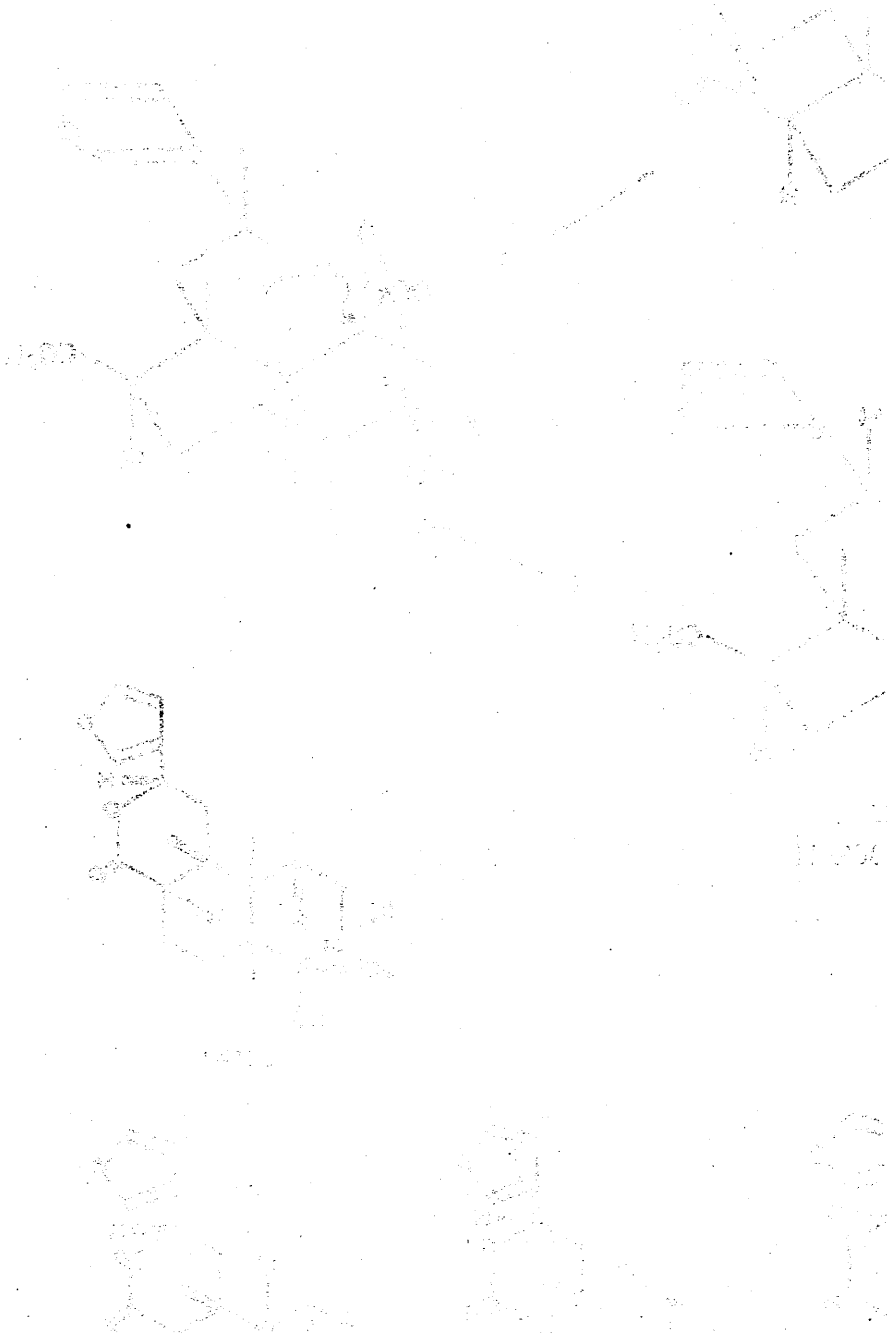
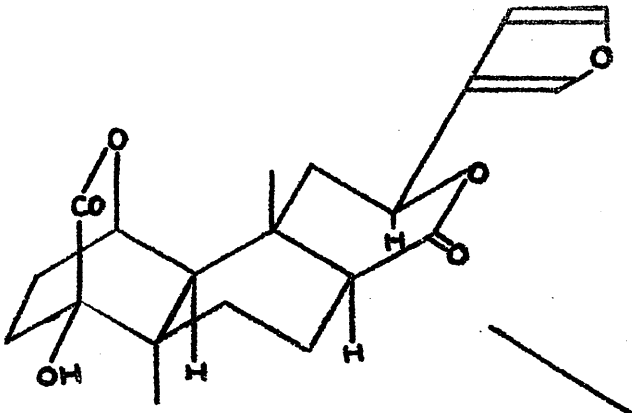
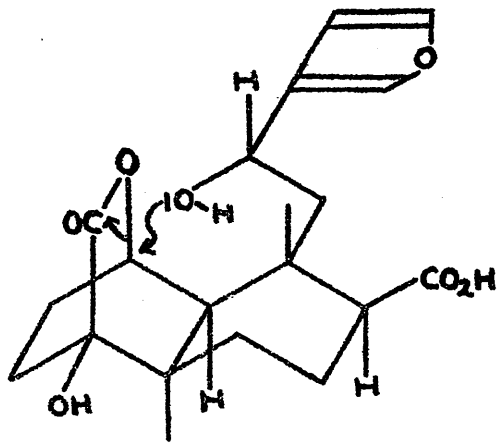
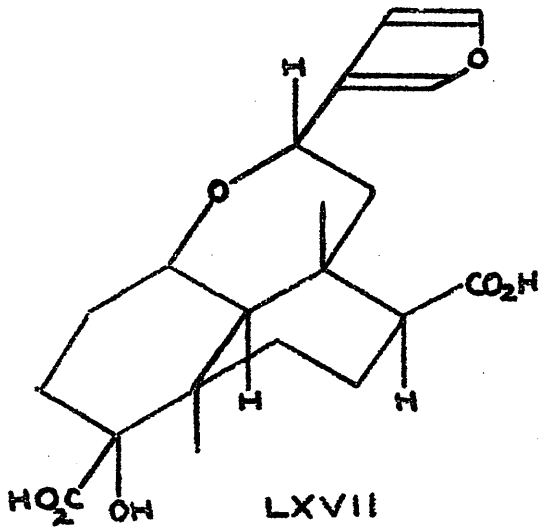


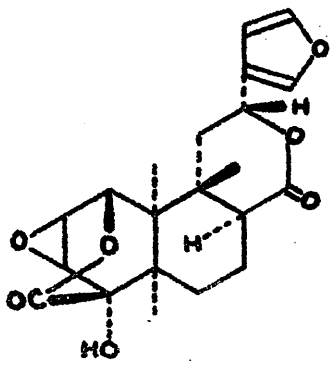


LXVI

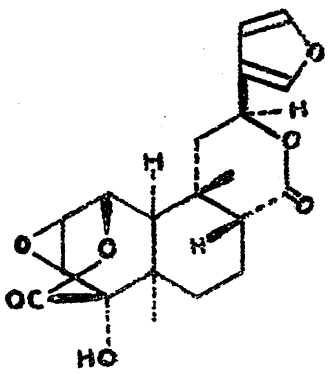




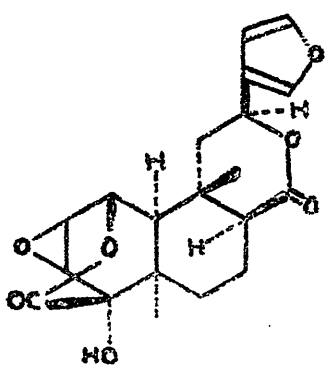
LXVII



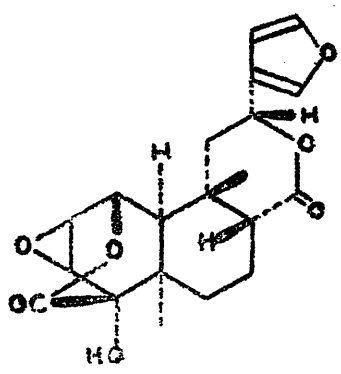
LXXI



LXVIII



LXIX

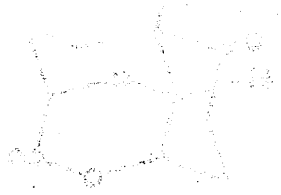


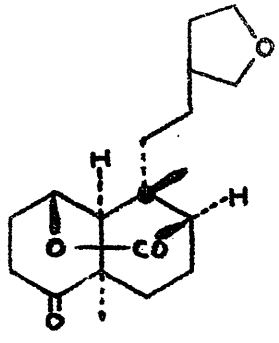
LXX

In order to accommodate what has been deduced concerning the stereochemistry at $C_{(12)}$ and the nature of the columbin - isocolumbin change, the argument which follows will show that columbin must have the absolute stereochemistry (LXIV) and isocolumbin the absolute stereochemistry (LXV).

The 19 - methyl group is placed on the side of the molecule opposite the 18 - methyl group since this readily permits the nucleophilic displacement at $C_{(1)}$ occurring in the change from dihydroisocolumbin (X) to dihydroisocolumbinic acid (XII), represented in three dimensions by (LXVI) to (LXVII). The columbin - isocolumbin change then becomes a change from a cis - to a trans - fused lactone, since the $C_{(8)}$ hydrogen atom in isocolumbin has been shown to have the same configuration as the 18 - methyl group. In order that the columbin - isocolumbin change will also involve change from a chair to a boat δ-lactone the $C_{(12)}$ hydrogen atom must be on the side of the molecule opposite the 19-methyl group. (Since the driving force in this change is the necessity for the bulky furan substituent adopting the equatorial configuration in both cases,) From these considerations (LXIV) or the enantiomer must represent the columbin molecule. The absolute stereochemistry at $C_{(12)}$ has been shown to be (LVIII a) and it follows that the columbin molecule is (LXIV). Bearing in mind the relationships of columbin to the other Colombo root bitter principles (see page 20), jateorin, isojateorin, chasmanthin and palmarin are shown as (LXVIII), (LXIX), (LXX) and (LXXI) respectively. The oxide ring in these compounds if situated on the same side of the molecule as the ring A lactone would best explain the resistance of the epoxide to normal cleaving reagents (see page 20).

The work of Djerassi,[67] Klyne[68] and their colleagues have shown that optical rotatory dispersion curves can be used to assign the absolute stereochemistry of a ketone, provided that a reference compound of known absolute stereochemistry, having its carbonyl group in a similar stereochemical environment is available. It was decided to use this approach to support the conclusions/





LXXII

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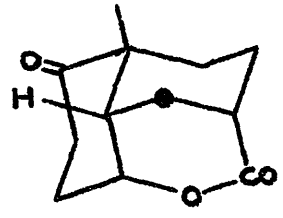
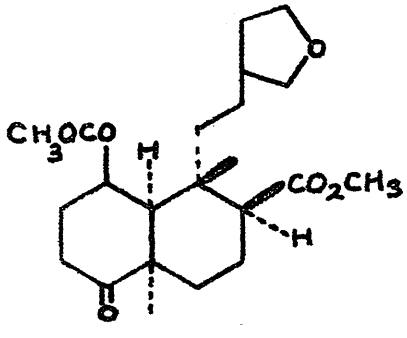
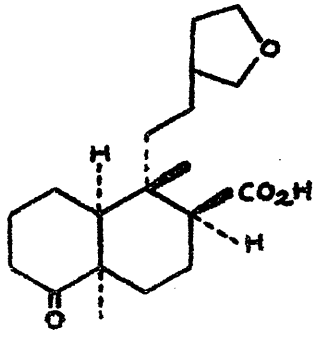


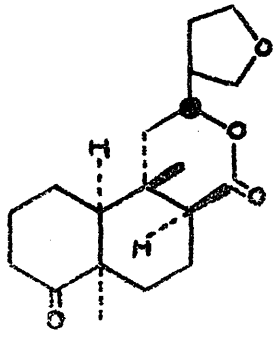
STEROID TYPE



LXXIII

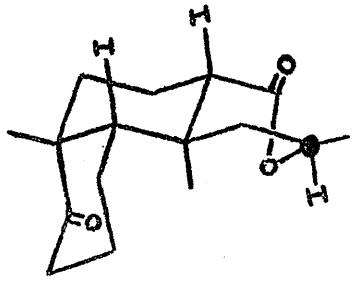


LXXIV

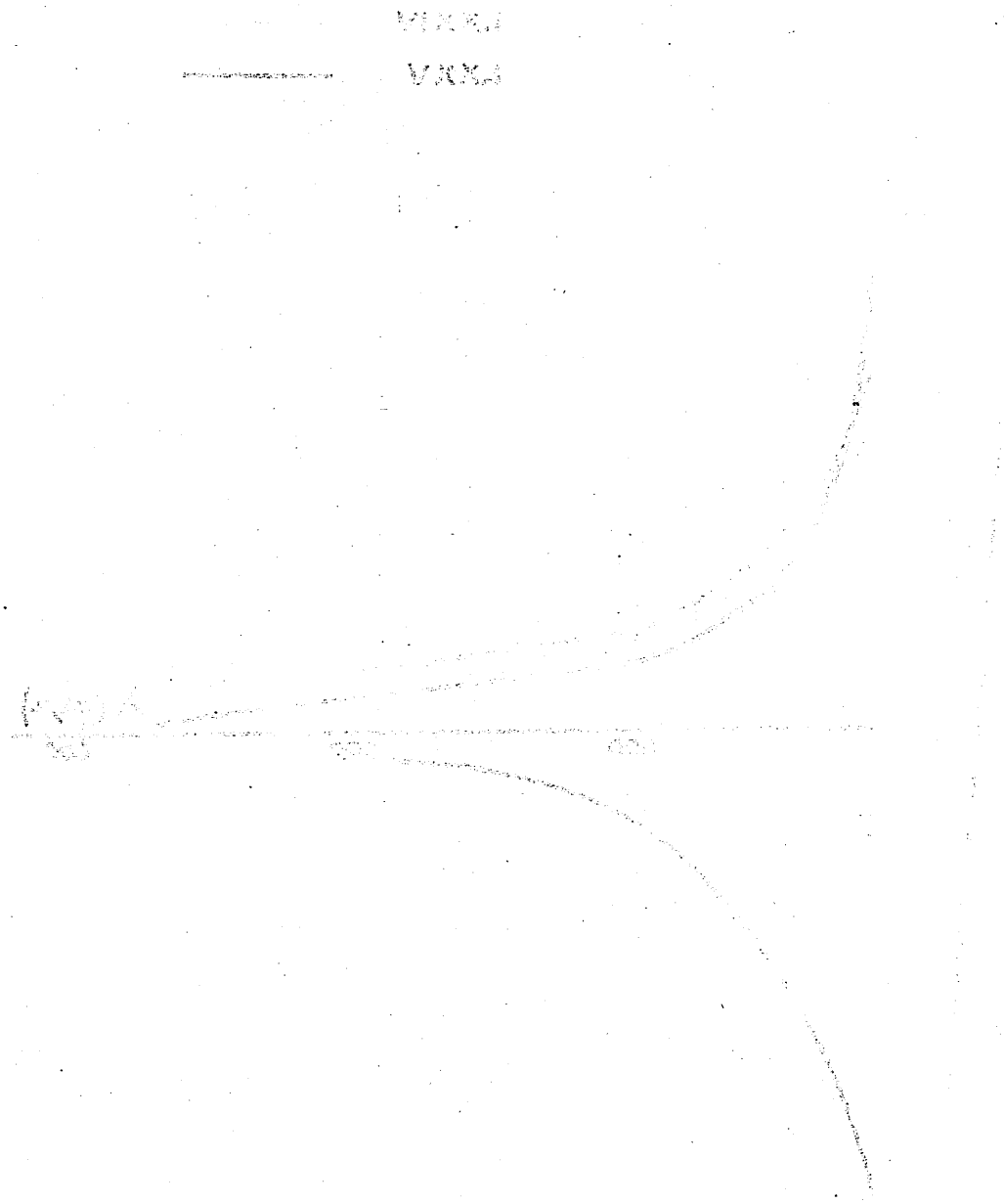


LXXV

≡



NON-STEROID TYPE



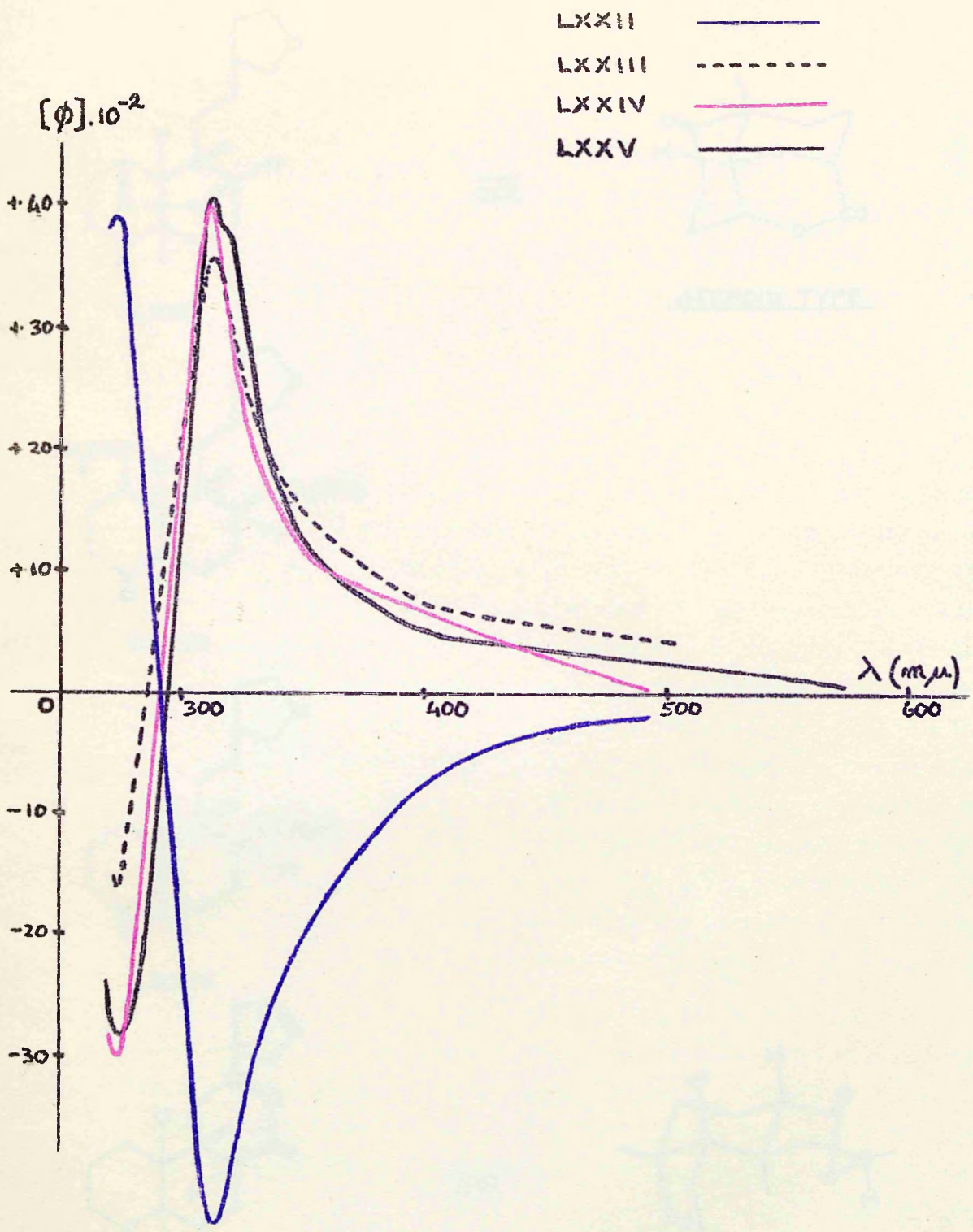
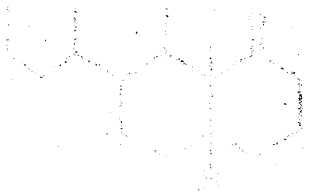

LXXII
LXXIII
LXXIV
LXXV
[φ].10^-2
+40
+30
+20
+10
0
-10
-20
-30
300
400
500
600
λ(mμ)

FIG. I



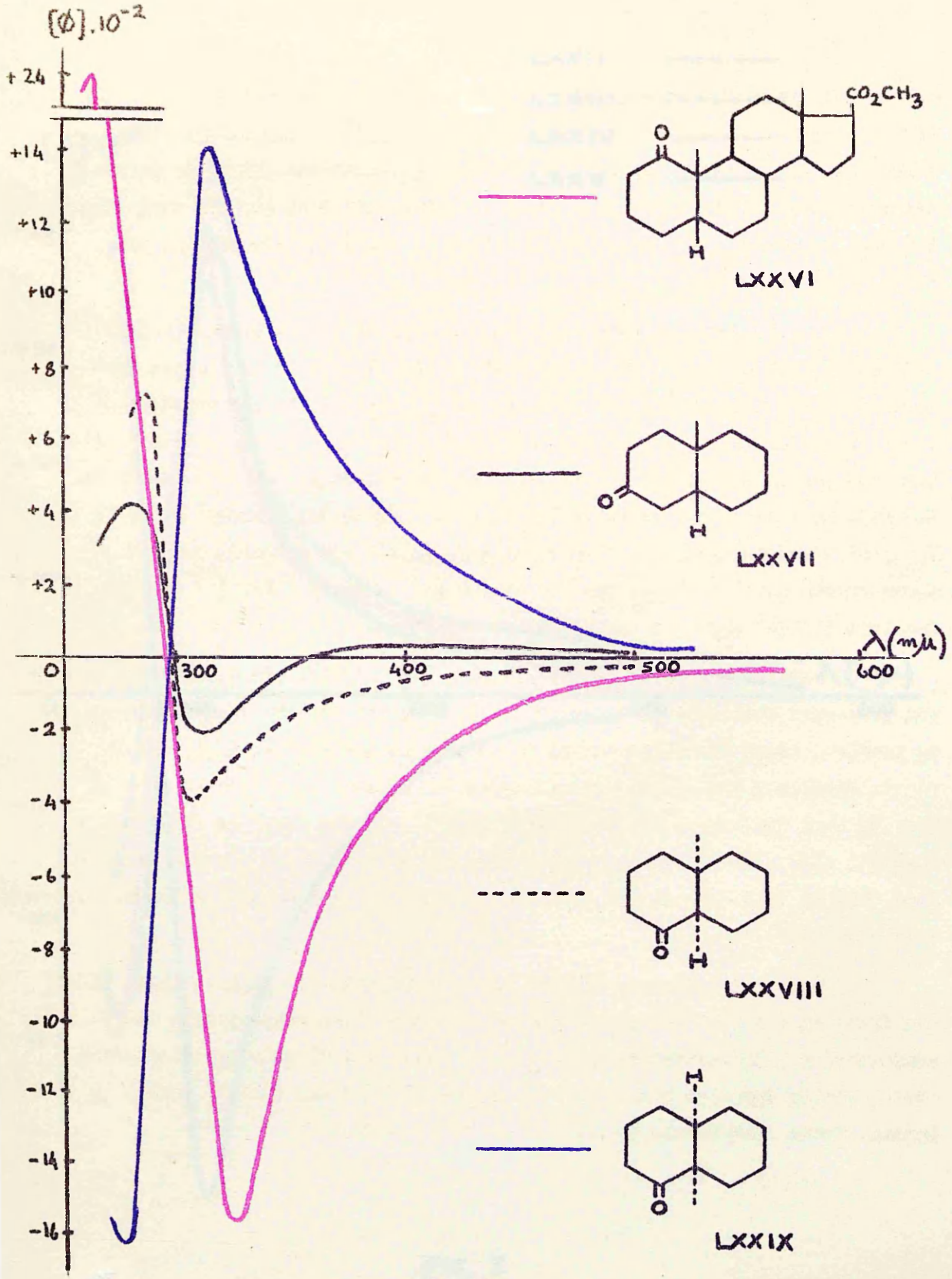

[Φ].10⁻²
+24
+14
+12
+10
+8
+6
+4
+2
0
-2
-4
-6
-8
-10
-12
-14
-16
300
400
500
600
λ(mμ)
CO₂CH₃
O
H
LXXVI
O
H
LXXVII
O
H
LXXVIII
H
O
LXXIX

Fig. II.

conclusions presented above. The optical rotatory dispersion curves of the keto - lactone (LXXII), the derived acetate (LXXIII), octahydrodecarboxy*iso*-columbinic acid (LXXIV) and hexahydrodecarboxy*iso*columbin (LXXV) in methanol are reproduced in Fig.I. Since these compounds all have cis A/B ring fusions the best reference compounds would be found in the 5 β - steroid and the cis - decalone series.

The optical rotatory dispersion curves of the decalones (LXXVII),[69] (LXXVIII)[70] and (LXXIX)[71,70] are reproduced in Fig. II and it is obvious from these that the position of the carbonyl group, relative to the angular methyl group, has a marked effect on the nature of the curve. It is essential then that the carbonyl - methyl relationship in the reference compound should be the same as that in the columbin derivative. Since the carbonyl group is at $C_{(4)}$, adjacent to the angular methyl group, in all the columbin compounds whose curves were measured, the 1 - keto - 5 β-steroid (LXXVI)[72] and the decalone (LXXIX) were used as reference compounds.

Another factor of importance when dealing with cis - fused systems is the preferred conformation of the molecule. In the steroid series this presents no problem, since the molecule can only exist in one conformation, but in simple decalones two stable conformations are possible. It can be seen in Fig. II that the curves of the steroid (LXXVI) and the decalone (LXXIX) have opposite sign although the compounds have the same absolute stereochemistry. From this it must be inferred that the decalone (LXXIX) exists in the non-steroidal conformation.

The keto - lactone (LXXII) and hexahydrodecarboxy*iso*columbin (LXXV) are fixed in the steroid and non-steroid conformations respectively and this conformational difference is reflected in their optical rotatory dispersion curves having opposite sign. The compounds (LXXIII) and (LXXIV) judged by their Cotton curves must belong to the non-steroid series.

Since/

Since the keto-lactone (LXXII) has a Cotton curve similar in shape and sign to that of the steroid model (LXXVI) this indicates the same absolute stereochemistry for these compounds. This is supported by the fact that the curves of the non-steroid compounds (LXXIII), (LXXIV) and (LXXV) are similar in shape and sign to that of the non-steroid model (LXXIX). It follows therefore that in these columbin derivatives the $C_{(18)}$ methyl group must be α. The evidence derived from two distinct approaches then leads to the absolute stereochemistry of columbin as depicted in (LXIV).

(b) Experimental.

M.p.s. were determined on the Kofler block and analysis was carried out on specimens dried *in vacuo* at room temperature overnight. Observed and calculated values are expressed to the nearest 0.05%.

Infrared spectra were taken with the Unicam S.P.100 double beam spectrophotometer in chloroform or carbon tetrachloride solutions unless otherwise stated. Nujol mull spectra were taken with the Infracord spectrophotometer.

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

Chromatographic alumina was prepared and standardised according to Brockmann[76] and solvents used were thoroughly dried. Light petroleum of B.p. 60 - 80° was used in all procedures.

Separation into acidic and neutral fractions was effected by dissolving the material in ether or methylene chloride and extracting with aqueous sodium bicarbonate.

$[\alpha]_D$ measurements were made in pyridine solution at room temperature using the sodium D line unless otherwise stated.

$[M]_D$ measurements in Table (VI) were determined in acetone - ethanol (V : V; 1 : 1) solutions and in 1% sodium hydroxide solutions of the same solvent at room temperature.

$[M]_D$ measurements in table (VII) were determined in dioxan-water (V : V; 70 : 30) solutions and in 1% potassium hydroxide solutions of the same solvent at room temperature. The sodium D line was used in all cases.

P.K. measurements were done in aqueous tetrahydrofuran solutions.

Isocolumbin (I; R = H, $C_{(8)}$ α H)[22]

Columbin (10 gms., m.p. 185° dec.), in ethanol (100 ml.) and N sodium hydroxide (70 ml.), was heated on the steam bath until solution was complete (ca 10 mins.). The hot alkaline solution was acidified (N hydrochloric acid) and, after cooling gave material (8.3 gms.) m.p. 176 - 84° dec. Recrystallisation from aqueous ethanol afforded isocolumbin (I; R = H, $C_{(8)}$ α H) (6.4 gms.) as needles m.p. 185 - 7° dec., $[\alpha]_D$ + 77° (C, 1.6).

Acetylisocolumbin (I; R = $COCH_3$, $C_{(8)}$ α H)[22].

Isocolumbin (6 gms., m.p. 185 - 7° dec.), acetic anhydride (280 ml.) and sodium acetate (23.5 gms.) were heated on an oil bath at 145° for 2.1/4 hours. On cooling the excess sodium acetate was removed by filtration and the mother liquors poured into ice water (1400 ml.). The precipitate was filtered off and extracted with a little ethanol. The residue was crystallised from acetone - alcohol giving acetylisocolumbin (I; R = $COCH_3$, $C_{(8)}$ α H) (4.7 gms.) as needles mp. 228 - 9° dec., $[\alpha]_D$ + 25°.

Decarboxyacetylisocolumbin (VII)[22]

Acetylisocolumbin (4.6 gms., m.p. 228 - 9° dec.), was heated at 225 - 30° in a nitrogen atmosphere until gas evolution ceased (ca 20 minutes). The resulting glassy solid was extracted with cold benzene giving, after evaporation and crystallisation from aqueous ethanol, decarboxyacetylisocolumbin (VII) (3.6 gms.) as prisms m.p. 165 - 6°, $[\alpha]_D$ - 320°, λ max. 272mμ (ε 7,010).

Ozonolysis of Decarboxyacetylisocolumbin (VII)

Decarboxyacetylisocolumbin (500 mg) in methylene chloride (150 ml.) was treated with ozone at - 60° (acetone - solid carbon dioxide) until the solution was transparent in the ultraviolet. The solution was concentrated in vacuo (ca 10 ml.), water (5 ml.) added and the mixture warmed on the steam bath. The aqueous solution on/

on extraction with ethyl acetate gave an amorphous solid residue (340 mg.) which could not be crystallised.

Ozonolysis was repeated in (a) ethyl acetate at -60° and the ozonide decomposed with 30% hydrogen peroxide, (b) ethyl acetate - acetic acid at -60°, decomposition with 30% hydrogen peroxide, (C) ethylacetate - acetic acid at 0°, decomposition with 90% formic acid - 30% hydrogen peroxide and (d) ethyl acetate - acetic acid at -60°, decomposition with 90% formic acid - 30% hydrogen peroxide. In all cases solid material was obtained but this was not obtained crystalline despite chromatography on silica gel.

Dihydroisocolumbin (X)[20].

Isocolumbin (6 gms., m.p. 185 - 7° dec.) in ethyl acetate (250 ml.) was hydrogenated over freshly prepared 1% palladium - calcium carbonate catalyst[73] (1.5 gms.) until 1 mole of hydrogen had been absorbed (ca 30 mins.). The catalyst was removed by filtration through kieselguhr and the solvent evaporated in vacuo. Recrystallisation of the residue from aqueous ethanol gave dihydroisocolumbin (X) (4.7 gms.) as large needles m.p. 234 - 6°, $[\alpha]_D$ + 34° (C, 1.8). Three further crystallisations raised the m.p. to 236 - 7°.

Dihydroisocolumbinic Acid (XII)[34].

Dihydroisocolumbin (2 gms., m.p. 234 - 6°) was suspended in N sodium hydroxide (60 ml.) and heated at 95° (steam bath) for 4 hours, under a vigorous nitrogen stream. Solution was complete after about 10 minutes. Acidification and ether extraction followed by separation of the product into acidic and neutral fractions, gave in the latter unchanged dihydroisocolumbin (ca 70 mg.). The acidic fraction dihydroisocolumbinic acid (XII) crystallised from acetone-benzene as stout prisms (740 mg.) m.p. 219 - 22°, $[\alpha]_D$ + 23° (C, 2.1), PK 4.05. Further crystallisation gave a m.p. of 222 - 3°.

Methylation/

Methylation of Dihydroisocolumbinic Acid (XII)

Dihydroisocolumbinic acid (690 mg.) in ether (240 ml.) was treated at room temperature for 4 hours with an excess of ethereal diazomethane.[74] The solvent was removed *in vacuo* and the residue, crystallised from acetone - petrol, gave the methyl ester as prisms m.p. 119 - 21°, $[\alpha]_D$ + 46° (C, 2.4) (Found : C, 65.2; C, 7.5. $C_{22}H_{30}O_7$ requires C, 65.0; H, 7.5%) ν max. (nujol) 1750 (ester), 1725(α-hydroxy-ester) and 3550 cm.$^{-1}$ (tertiary hydroxyl), ν max ($CHCl_3$) 1724 cm.$^{-1}$ (ester and α-hydroxy-ester) and ν max. (CCl_4) 1737 (ester), 1721 cm.$^{-1}$ (α-hydroxy-ester).

Hydrogenation of Dihydroisocolumbinic Acid (XII)

a) Dihydroisocolumbinic acid (25 mg.) in "Analar" acetic acid (5 ml.) was hydrogenated over presaturated Adam's catalyst (20 mg.), 2.9 moles of hydrogen being taken up in 35 minutes. The catalyst was removed by filtration and the acetic acid evaporated *in vacuo* giving a glassy residue (25 mg.). The crude material showed strong hydroxyl absorption (3500 cm.$^{-1}$) in the infrared but could not be crystallised.

b) Dihydroisocolumbinic acid (25 mg.) in "Analar" acetic acid (5 ml.) with 0.5% 10 N hydrochloric acid was hydrogenated as above, but the reaction stopped after 1 mole of hydrogen had been taken up. The oily product showed no increase in hydroxyl absorption and could not be crystallised.

c) As in (b) above but with complete hydrogenation (3.3 moles of hydrogen taken up). The oily product had strong hydroxyl (3500 cm.$^{-1}$) absorption in the infrared. Attempts to purify this material by acetylation (acetyl chloride in pyridine at room temperature overnight) or benzoylation (benzoyl chloride in pyridine at room temperature overnight) failed to produce crystalline material despite chromatography.

Hydrogenation of Dihydroisocolumbinic Acid Methyl Ester.

The methyl ester (225 mg.) in "Analar" acetic acid (40 ml.) with 1% 10 N hydrochloric acid was hydrogenated over presaturated Adam's catalyst, 3.3 moles of hydrogen

hydrogen being absorbed. The product (209 mg.) could not be crystallised and attempted purification by acetylation and benzoylation (as above) failed.

Dihydrocolumbin (X; $C_{(8)}$ β H)[20]

a) Columbin (2.4 gms; m.p. 193 - 6° dec., $[\alpha]_D$ + 38°) was hydrogenated by the method described for isocolumbin. Recrystallisation of the crude product from aqueous ethanol gave dihydrocolumbin (X; $C_{(8)}$ β H). (1.6 gms.) as large needles m.p. 232 - 4°, $[\alpha]_D$ + 4.3° (C, 3.7).

b) Columbin (1.3 gms; m.p. 189 - 92° dec., $[\alpha]_D$ + 36°) was hydrogenated as above. The crude product, in methylene chloride, was chromatographed on grade III alumina when most of the material was recovered on elution with methylene chloride - ether (7 : 3). Recrystallisation from methylene chloride - petrol gave dihydrocolumbin (X; $C_{(8)}$ β H) (750 mg.) as needles mp. 238 - 40°, $[\alpha]_D$ + 6.7° (C, 3.8).

The remainder of the material (103 mg.) was eluted with methanol and after isomerisation (see isocolumbin) with N sodium hydroxide gave, from methylene chloride - petrol, palmarin (40 mg.) as prisms m.p. 253 - 5° with an infrared spectrum identical to that of an authentic sample.

Hydrogenation of Columbin (I; R = H)[21]

Columbin (2.1 gms., m.p. 193 - 6° dec., $[\alpha]_D$ + 38°) in methanol (180 ml.) was hydrogenated over 10% palladised charcoal (1.2 gms.). After 1 hour hydrogen uptake ceased, 3.8 moles having been consumed. The solution was filtered through kieselguhr, evaporated to small volume and the residue separated into acidic and neutral fractions.

The acidic fraction (1.6 gms.) was a straw coloured glass and could not be crystallised despite chromatography on silica gel. This material (750 mg.) in methanol (10 ml), was treated with an excess of ethereal diazomethane giving an oily methyl ester. The ester, in benzene, was chromatographed on grade V alumina and/

and fractional crystallisation, using ether - petrol, of the resulting solid material gave (a) two octahydrocolumbinic acid methyl esters (XVI; R = CH_3) (i) 60 mg. as rods and prisms m.p. 93 - 6°, $[\alpha]_D$ - 25° (C, 1.0 $CHCl_3$), ν max. (CCl_4) 1759 (δ-lactone) and 1735 $cm.^{-1}$ (ester) (Found: C,66.45; H, 8.75. $C_{21}H_{32}O_6$ requires C, 66.4; H, 8.5%). (ii) 190 mg. as plates m.p. 129 - 131.5°, $[\alpha]_D$ - 22° (C, 1.0 $CHCl_3$), ν Max. (CCl_4) 1759 (δ-lactone) and 1735 $cm.^{-1}$ (ester) (Found: C, 66.2; H, 8.65. $C_{21}H_{32}O_6$ requires C, 66.4; H, 8.5%) (b) hexahydrochasmanthinic acid methyl ester (XVII; R = CH_3) (60 mg.) as needles m.p. 176 - 7°, $[\alpha]_D$ - 18° (C, 1.4), ν max. (CCl_4) 1773 (δ-lactone), 1733 $cm.^{-1}$ (ester) (Found: C, 64.1; H, 7.71. $C_{21}H_{30}O_7$ requires C, 63.95; H, 7.65%) (Wessely[27] quotes m.p. 175 - 80°, $[\alpha]_D$ - 11.2° for this compound)

The neutral fraction (22- mg.) had m.p. 245 - 56° but was not investigated further.

Hydrogenation of Dihydrocolumbin (X; $C_{(8)}$ β H)[21]

Dihydrocolumbin (750 mg., m.p. 238 - 40°, $[\alpha]_D$ + 6.7°) in methanol (100 ml) was hydrogenated over 10% palladised charcoal (400 mg.) until hydrogen uptake had ceased (35 mins. ca 2.8 moles consumed). The solution was treated as above giving acidic (680 mg.) and neutral (42 mg.) material.

The acidic material on crystallisation from ethyl acetate-petrol gave an acid m.p. 161-3°(cf. Cava). This material on treatment with ethereal diazomethane followed by chromatography on grade V alumina in benzene afforded the two octahydrocolumbinic acid methyl esters m.ps. 93 - 6° and 129 - 131.5° described above. The mother liquors from crystallisation of the acid m.p. 161 - 3° were combined and treated with ethereal diazomethane. Chromatography again furnished the two octahydro - esters and no trace of the chasmanthin derivative.

The neutral material (42 mg.) on crystallisation from methylene chloride - petrol afforded hexahydrocolumbin (XVIII) (20 mg.) as needles m.p. 253 - 4°, $[\alpha]_D$ - 11.4° (C, 0.6 $CHCl_3$) ν max. (nujol) 1760 (boat δ-lactone), 1745 $cm.^{-1}$ (δ-lactone)/

(δ-lactone) (Found: C, 65.65; H, 7.55. $C_{20}H_{28}O_6$ requires C, 65.9; H, 7.75%.)

Hydrolysis of the Octahydrocolumbinic Acid Methyl Esters m.ps. 129 - 131° and 93 - 6° (XVI; R = CH_3)

a) The ester (50 mg., m.p. 129 - 131°) was treated under nitrogen with N sodium hydroxide (5 ml.) on the steam bath for 1.1/2 hours. The residue, after acidification, gave on crystallisation from ethyl acetate - petrol octahydrocolumbinic acid I (XVI; R = H) (25 mg) as prisms m.p. 156 - 7°, $[\alpha]_D$ - 25° (C, 0.9 $CHCl_3$) ν max. (nujol) 1760 (δ-lactone), 1730 cm.$^{-1}$ (carboxyl) (Found: C, 65.55; H, 8.15. $C_{20}H_{30}O_6$ requires C, 65.55; H, 8.25%)

b) The ester (50 mg. m.p. 93 - 6°) was treated as above giving, from ethyl acetate - petrol, octahydrocolumbinic acid II (XVI; R = H) (23 mg.) as prisms m.p. 173 - 5°, $[\alpha]_D$ - 31.5° (C, 0.9 $CHCl_3$) ν max. (nujol) 1760 (δ-lactone), 1730 cm.$^{-1}$ (carboxyl) (Found: C, 65.45; H, 8.1. $C_{20}H_{30}O_6$ requires C, 65.55; H, 8.25%)

Hydrogenation of Isocolumbin (I; R = H $C_{(8)}$ α H)[21]

Isocolumbin (2 gms., m.p. 185 - 7° dec., $[\alpha]_D$ + 77°) in methanol (180 ml) was hydrogenated under the identical conditions used for columbin. Working up in the same way gave acidic (1.7 gms.) and neutral (200 mg.) material.

The acidic material on fractional crystallisation from ethyl acetate - petrol afforded octahydroisocolumbinic acid (XVI; R = H, $C_{(8)}$ α H) (500 mg.) as rods m.p. 186 - 8°, $[\alpha]_D$ + 42°(C, 1.0 $CHCl_3$), PK 5.1, ν max. (nujol) 1758 (δ-lactone) 1730 cm.$^{-1}$ (carboxyl) (Found: C, 65.75; H, 8.4. $C_{20}H_{30}O_6$ requires C, 65.55; H, 8.25%) and hexahydropalmarinic acid (XVII; R = H, $C_{(8)}$ α H) (20 mg.) as rods m.p. 234 - 6°, $[\alpha]_D$ + 43° (C, 1.3) (Found: C, 62.95; H, 7.3. $C_{20}H_{28}O_7$ requires C, 63.15; H, 7.4%). The infrared (nujol) spectrum of the latter was identical with that of an authentic sample.

Treatment/

Treatment of octahydroisocolumbinic acid with ethereal diazomethane furnished the methyl ester as needles m.p. 138 - 40°, $[\alpha]_D$ + 45° (C, 1.4 $CHCl_3$), ν max. ($CHCl_3$) 1750 (δ-lactone), 1734 (ester) and C Cl_4) 1759 (δ-lactone), 1741 cm.$^{-1}$ (ester) (Found: C, 66.5; H, 8.2. $C_{21}H_{32}O_6$ requires C, 66.4; H, 8.5%).

The netral product m.p. 245 - 52° was not investigated at this stage.

Hydrogenation of Dihydroisocolumbin (X)[21]

Dihydroisocolumbin (3 gms., m.p. 236 - 7°) in methanol (300 ml.) was hydrogenated as above affording acidic (2.6 gms.) and neutral (210 mg.) material.

Recrystallisation of the acidic fraction from ethyl acetate-petrol gave octahydroisocolumbinic acid (1.7 gms) m.p. 186 - 8° and no indication of the palmarin derivative.

The neutral material on crystallisation from ethyl acetate - petrol yielded hexahydroisocolumbin (XVIII; $C_{(8)}$ α H) (60 mg.) as needles m.p. 257 - 9°, $[\alpha]_D$ + 42.5° (C, 1.1 $CHCl_3$), ν max. (nujol) 1760 (boat δ-lactone), 1740 cm.$^{-1}$ (δ-lactone) (Found: C, 65.55; H, 7.9. $C_{20}H_{28}O_6$ requires C, 65.9; H, 7.75%)

Epimerisation of Hexahydrocolumbin (XVIII)

Hexahydrocolumbin (20 mg. m.p. 253 - 4°) on treatment with N sodium hydroxide (2 ml.) on the steam bath for 5 minutes afforded hexahydroisocolumbin (XVIII; $C_{(8)}$ α H) (15 mg.) identical in every respect to the material obtained above from the hydrogenation of dihydroisocolumbin.

Attempted Epimerisation of the Octahydrocolumbinic Acid Methyl Esters m.ps. 128 - 30° and 93 - 6° (XVI; R = CH_3)[50]

a) Octahydrocolumbinic acid methyl ester (200 mg., m.p. 128 - 30°) was added to a solution of sodium (25 mg.) in absolute methanol (20 ml.) and the mixture refluxed for 18 hours.[50] Hydrochloric acid (50 ml.) was added and extraction with chloroform gave a solid residue (196 mg.). This material (m.p. 126 - 9°) had an infrared spectrum/

spectrum identical with the starting ester and after recrystallisation from ethyl acetate - petrol yielded octahydrocolumbinic acid methyl ester (100 mg.) m.p. 129 - 30°.

b) Octahydrocolumbinic acid methyl ester (100 mg. m.p. 93 - 6°) was also recovered unchanged on treatment as above.

Attempted Epimerisation of Octahydroisocolumbinic Acid Methyl Ester m.p. 136 - 8° (XVI; R = CH_3, $C_{(8)}$ α H)[50].

Octahydroisocolumbinic acid methyl ester (90 mg., m.p. 136 - 8°) with the above treatment gave a good recovery (80 mg.) of the octahydroiso - ester.

Lithium Aluminium Hydride Reduction of the Octahydrocolumbinic Acid Methyl Esters m.ps.,126 - 9° and 93 - 6° (XVI; R = CH_3)

a) Octahydrocolumbinic acid methyl ester (90 mg., m.p. 126 - 9°) in tetrahydrofuran (5 ml.) was added to a stirred suspension of lithium aluminium hydride (300 mg.) in refluxing tetrahydrofuran (10 ml.) over a period of 1 hour. When addition was complete the mixture was refluxed for a further hour. The excess lithium aluminium hydride was destroyed with ethyl acetate and saturated aqueous ammonium sulphate (ca 10 ml.) added. The organic layer was separated by decantation and the grey residue washed with further amounts of ethyl acetate (3 x 10 ml.). The combined organic layers (after drying over magnesium sulphate) afforded, on evaporation, a gummy residue (85 mg.) which could not be crystallised. The infrared spectrum of this compound (XX; $C_{(8)}$ β H) showed an intense 3500 $cm.^{-1}$ (Hydroxyl) band and virtually no carbonyl absorption.

The crude hydroxy compound (64 mg.) in aqueous methanol (3.5 ml., V : V, 2.5 : 1) was treated overnight at room temperature with sodium periodate[51] (64 mg.) in water (1 ml.). The aqueous solution was saturated with ammonium sulphate and extracted with ether. The ether extract afforded an oily product (49 mg.) (XXI; $C_{(8)}$ β H) whose infrared spectrum had a strong 1700 $cm.^{-1}$ (cyclohexanone) band/

band. This material could not be crystallised and did not give a crystalline product on treatment with toluene - p - sulphonyl chloride in pyridine at room temperature overnight.

b) Octahydrocolumbinic acid methyl ester (90 mg. m.p. 93 - 6°) was treated with lithium aluminium hydride as above giving an oily residue (78 mg.). This material was not obtained crystalline and treatment with sodium periodate (as above) gave a non-crystalline residue showing a 1710 cm.$^{-1}$ (cyclohexanone) band in the infrared.

Lithium Aluminium Hydride Reduction of Octahydroisocolumbinic Acid Methyl Ester (XVI; R = CH_3, $C_{(8)}$ α H)

Octahydroisocolumbinic acid methyl ester (200 mg. m.p. 136 - 8°) was treated with lithium aluminium hydride (600 mg.) as above giving an oily residue (180 mg.). The infrared spectrum of this material showed a strong 3,500 cm.$^{-1}$ (hydroxyl) band and no carbonyl absorption.

The hydroxylic material (XX; $C_{(8)}$ α H) (110 mg.) in aqueous methanol (6 ml.) was treated, as above, with a solution of sodium periodate (120 mg.) in water (2 ml.). The recovered material (XXI; $C_{(8)}$ α H) was an oil showing 1710 cm.$^{-1}$ (cyclohexanone) absorption in the infrared.

Hydrolysis of Octahydroisocolumbinic Acid (XVI; R = H, $C_{(8)}$ α H)

a) Octahydroisocolumbinic acid (50 mg., m.p. 180-3°) was treated under nitrogen with N sodium hydroxide on the steam bath for 4 hours. 1.2 moles of base were consumed (by back titration) and the product (recovered after acidification) showed a 1759 cm.$^{-1}$ (boat δ-lactone) band in the infrared indicating incomplete hydrolysis.

b) Octahydroisocolumbinic acid (650 mg., m.p. 182-5°) in methanol (8 ml.) was treated under nitrogen with 4 N potassium hydroxide (22 ml.) on the steam bath for 4 hours. 2 moles of base were consumed and the product (400 mg.) (recovered after acidification) had m.p. 230-5°. Crystallisation from ethyl acetate - petrol gave the iso-lactone (XXVI; R = H) (361 mg.) as needles m.p. 239 - 41°. Further crystallisation gave m.p. 240-2°, $[\alpha]_D$ - 46.5° (C, 1.0 $CHCl_3$), PK 3.98, equivalent/

equivalent weight 369 (calculated 366) (Found: C, 65.45; H, 8.4. $C_{20}H_{30}O_6$ requires C, 65.55; H, 8.25%)

Treatment of the iso - lactone with ethereal diazomethane gave the methyl ester (XXVI; R = CH_3) as an oil, ν max. ($CHCl_3$) 1720(α - hydroxy ester and ester) and (CCl_4) 1745 (ester), 1723 cm.$^{-1}$ (α- hydroxy ester). This material was not obtained crystalline despite careful chromatography.

Lithium Aluminium Hydride Reduction of the Iso - lactone Methyl Ester (XXVI; R = CH_3)

The iso - lactone methyl ester (from 150 mg. iso - lactone) in tetrahydrofuran (3 ml.) was added to a stirred suspension of lithium aluminium hydride (800 mg.) in refluxing tetrahydrofuran (10 ml.) over 1 hour. The mixture was refluxed for a further 4 hours and working up as previously described (page 87) gave an oily residue (100 mg.) whose infrared spectrum showed 3500 cm.$^{-1}$ (hydroxyl) and no carbonyl absorption.

The hydroxylic compound (XXVII) (90 mg.) in methanol (10 ml.) was treated at room temperature overnight with sodium periodate (130 mg.) in water (5 ml.). Evaporation of the aqueous solution in vacuo at room temperature followed by extraction with ethyl acetate afforded an oil (50 mg.) (XXVIII). The infrared spectrum showed 1710 cm.$^{-1}$ (cyclohexanone) absorption but the material could not be crystallised.

Sodium Salt of the Iso- lactone (XXIX)

The iso - lactone (130 mg., m.p. 240 - 2°) in methanol (2 ml.) was titrated with 0.5 N sodium hydroxide until 1 mole of base had been consumed. A further mole of alkali was added and the solution heated on the steam bath for 10 minutes. Evaporation of the solution to dryness afforded the sodium salt (XXIX) as a white amorphous solid ν max. (nujol) 1580 cm.$^{-1}$ (carboxylate anion).

Oxidation/

Oxidation of the Sodium Salt of the Iso-lactone (XXIX)[46]

To the sodium salt (150 mg.) suspended in pyridine (5 ml.) was added chromium trioxide (200 mg.) and the mixture stood at room temperature overnight. The solution was acidified and ethyl acetate extraction yielded an oil (140 mg.) which could not be crystallised. The infrared spectrum of this compound (XXX) showed 1710 (cyclohexanone) and 3400 $cm.^{-1}$ (hydroxyl) absorption. The latter disappeared when the crude material was treated with ethereal diazomethane.

Oxidation of the Iso - lactone (XXVI)

a) Lead dioxide[53]

The iso - lactone (1.5 gms., m.p. 240 - 2°) in acetic acid (25 ml.) was refluxed for 4 hours with lead dioxide (2 gms.). The acetic acid was removed *in vacuo* and the residue extracted with benzene. The benzene solution was washed with aqueous sodium bicarbonate and boiled with charcoal. Evaporation of the benzene gave the keto-lactone (XXXI) (1.3 gms) as an oil, $[\alpha]_D$ - 50° (C, 0.9 C_2H_5OH) ν max. (CCl_4) 1745 (δ - lactone), 1711 $cm.^{-1}$ (cyclohexanone). (Found: (for material distilled at 0.005 m.m., 90°) C, 69.7; H, 8.75. C, 70.15; H, 8.7. C, 70.0; H, 8.75. $C_{19}H_{28}O_4$ requires C, 71.2; H, 8.8%).

b) Chromium trioxide - pyridine[46]

The iso-lactone (40 mg., m.p. 240 - 2°) in pyridine (1 ml.) was added to a mixture of chromium trioxide (20 mg.) in pyridine (1 ml.) and allowed to stand at room temperature overnight. Acidification of the solution followed by ether extraction afforded the keto-lactone (XXXI) (23 mg.) identical in very respect with the material obtained in (a).

c) Chromium trioxide in acetone - sulphuric acid[52]

The iso - lactone (100 mg., m.p. 240 - 2°) in acetone (3 ml) was treated with chromium trioxide reagent* (0.45 ml.) and allowed to stand at room temperature overnight. Removal of the acetone *in vacuo* followed by ethyl acetate extraction of/

of the residue afforded the keto-lactone (XXXI)(68 mg.) identical in every respect with the material obtained in (a).

* (2.67 gms. chromium trioxide + 2.3 ml. concentrated sulphuric acid made up to 100 ml. with water).

The keto - lactone could not be obtained crystalline despite chromatography and did not form an oxime, a 2 : 4 - dinitrophenylhydrazone or condense with benzaldehyde.

Sodium Borohydride Reduction of the keto - lactone (XXXI)[55]

The following method represents the best of many attempts (using varying amounts of sodium borohydride and reaction time) to improve the yield of hydroxy - lactone (XXXII).

The keto - lactone (150 mg.) in tetrahydrofuran (25 ml.) was treated with sodium borohydride (700 mg.) in water (5 ml.). Further amounts of water were added until the solution was homogeneous and the mixture was allowed to stand at room temperature for 6 hours. The excess sodium borohydride was destroyed with acetic acid and extraction with ethyl acetate (followed by sodium bicarbonate washing) yielded an oil (100 mg.). The oil was chromatographed on grade V alumina in benzene giving the hydroxy - lactone (XXXII) (20 mg.) as stout prisms m.p. 172 - 4°, oily material with no carbonyl absorption in the infrared and some unchanged keto-lactone. Further crystallisation of the hydroxy - lactone, from chloroform - petrol, gave m.p. 173 - 4.5°, $[\alpha]_D$ + 20° (C, 0.9 $CHCl_3$), ν max. (CCl_4) 1727 $cm.^{-1}$ (δ - lactone) (Found: C, 70.8; H, 9.4. $C_{19}H_{30}O_4$ requires C, 70.8; H, 9.4%).

Oxidation of the Hydroxy - lactone (XXXII)

The hydroxy - lactone (25 mg., m.p. 173 - 4.5°) was shaken overnight with chromium trioxide (1.3 moles) in acetic acid (5 ml. with a few drops of water added.) Removal of the acetic acid in vacuo followed by chloroform extraction gave/

gave the keto - lactone (XXXI) (20 mg.) with identical infrared spectrum to the material obtained by oxidation of the iso - lactone.

Hydrolysis of the keto - lactone (XXXI)

The keto - lactone (520 mg.) in tetrahydrofuran (4 ml.) was treated with 1.5 N potassium hydroxide (10 ml.) on the steam bath for 2.1/2 hours. The alkaline solution was acidified and quickly extracted into ether. The ether solution was treated with ethereal diazomethane giving the hydroxy - ketoester (XXXIII; R = H) (500 mg.) as an oil (Found: (after distillation at 0.005 m.m., 110°) C, 68.1; H, 8.95. $C_{20}H_{32}O_5$ requires C, 68.15; H, 9.15%)

The hydroxy - ketoester (48 mg.) was treated on the steam bath for 1.1/2 hours with acetic anhydride (0.1 ml.) in pyridine (0.5 ml.). Evaporation to dryness followed by crystallisation of the residue from carbon tetrachloride - petrol gave the acetate (XXXIII; R = $COCH_3$) (18 mg.) as stout prisms m.p. 130 - 3°, $[\alpha]_D$ + 74° (C, 1.0 $CHCl_3$) (Found: C, 67.5; H,8.45. $C_{22}H_{34}O_6$ requires C, 67.3; H, 8.2%).

The acidic hydrolysis product of the keto - lactone (40 mg.) in tetrahydrofuran (5 ml.) with dilute hydrochloric acid (0.5 ml.) gave the keto-lactone (26 mg.) after 6 hours standing. The remainder of the material was unchanged hydrolysis product as judged by its infrared spectrum.

Oxidation of the Hydroxy-ketoester (XXXIII; R = H)[46]

The ester (300 mg.) in pyridine (2 ml.) was added to a mixture of chromium trioxide (150 mg.) in pyridine (2 ml.) and allowed to stand at room temperature overnight. The excess oxidising agent was destroyed with methanol and the mixture taken to dryness at as low a temperature as possible. Dilute hydrochloric acid was added and the mixture extracted in turn with benzene, ethyl acetate and ether. The combined extracts on evaporation yielded a semi-crystalline residue (220 mg.) m.p. 120 - 40°. Crystallisation from carbon tetrachloride - petrol afforded the dione/

dione mixture I (XXXIV) (50 mg.) as needles m.p. 130 - 40°, $[\alpha]_D$ - 64° (C, 1.2 $CHCl_3$) ν max. (C Cl_4 Infracord) 1700 (cyclohexanone), 1740 cm.$^{-1}$ (ester) (Found: C, 68.6; H, 8.8. $C_{20}H_{30}O_5$ requires C, 68.55; H, 8.6%). Chromatography of the residues from crystallisation on grade V alumina in petrol - benzene (6 : 4) afforded the dione mixture II (XXXIV) (32 mg.) as needles m.p. 116 - 29°, $[\alpha]_D$ - 49° (C, 1.0 $CHCl_3$) ν max. (C Cl_4 Infracord) 1700 (cyclohexanone), 1740 cm.$^{-1}$ (ester) (Found: C, 68.8; H, 8.2. $C_{20}H_{30}O_5$ requires C, 68.55; H, 8.6%)

Selenium Dioxide Oxidation of the Diones I and II (XXXIV)[9]

a) The dione (40 mg. m.p. ca 135°) in acetic acid (5 ml.) was heated on the steam bath with selenium dioxide (160 mg.) for 2 hours. The acetic acid was removed in vacuo and the residue sublimed giving the ene -dione (XXXV) (35 mg.) m.p. 90 - 100°, λ max. 228 mμ (ε 9,500), $[\alpha]_D$ - 69° (C, 0.4 $CHCl_3$) (Found: C, 68.6; H, 7.8. $C_{20}H_{28}O_5$ requires C, 68.9; H, 8.1%)

b) The dione (10 mg., m.p. ca 120°) in acetic acid (1.5 ml.) was treated as above giving the ene - dione as an oil λ max. 230 mμ (ε 9,000).

Attempted Epimerisation of the Crude Dione Mixture (XXXIV)[75]

a) The crude oxidation product of the methyl ester (see above) (50 mg. m.p. 120 - 40°) in methanol (3.5 ml.) with 6% potassium hydroxide was allowed to stand at room temperature overnight. Acidification followed by ethyl acetate extraction gave a residue (40 mg.) m.p. 115 - 30° with an infrared spectrum practically identical with the original material.

b) As above but with further 2 hours heating on the steam bath gave a mixture m.p. ca 120 - 40° with an infrared spectrum practically identical with the original material.

Hydrolysis of Octahydrocolumbinic Acid Methyl Ester m.p.128 - 30° (XVI; R = CH_3)

The octahydrocolumbinic acid methyl ester (650 mg., m.p. 128 - 30°) was treated under nitrogen with 4 N potassium hydroxide (24 ml.) on the steam bath for 4 hours. Acidification/

Acidification, followed by ethyl - acetate extraction gave an oil (570 mg.). This material on crystallisation from ethyl acetate gave the dihydroxy - diacid (XXXVI) (280 mg.) as fine needles m.p. 186 - 8°. Further crystallisation from ethyl acetate gave m.p. 187 - 8°, $[\alpha]_D$ - 130° (C, 0.5 $CHCl_3$), equivalent weight 195 (calculated 192), (Found: C, 62.25; H, 8.4. $C_{20}H_{22}O_7$ requires C, 62.5; H, 8.4%).

Oxidation of the Dihydroxy-diacid (XXXVI)[46]

The dihydroxy-diacid (100 mg., m.p. 187 - 8°) in acetone (3 ml.) was treated with chromium trioxide reagent (see page 91) (1 ml.) at room temperature overnight. The excess reagent was destroyed with methanol and the solvents removed *in vacuo* at room temperature. Ethyl acetate extraction of the residue gave solid material (80 mg.) which on crystallisation from ethyl acetate - petrol, followed by sublimation, afforded the dione (XXXVIII) (30 mg.) as needles m.p. 218 - 20°, $[\alpha]_D$ + 80° (C, 1.4 $CHCl_3$), ν max. (on oily methyl ester XXXVII) ($CHCl_3$ Infracord) 1710 (cyclohexanone), 1735 $cm.^{-1}$ (ester), (Found: C, 67.35; H, 8.6. $C_{19}H_{28}O_5$ requires C, 67.8; H, 8.4%) (λ max. 235 - 40 mμ (ε 800) suggest some ene-dione in product). Sublimation of the residues from crystallisation gave further amounts of the dione m.p. 216 - 20°.

The dione (10 mg.) was treated with selenium dioxide (40 mg.)(as for the ene-dione (XXXV) above) giving the ene-dione (XXXIX) as an oil λ max. 230mμ, (ε 9,200).

Attempted Epimerisation of the Dione m.p. 218-20°(XXXVIII)[75]

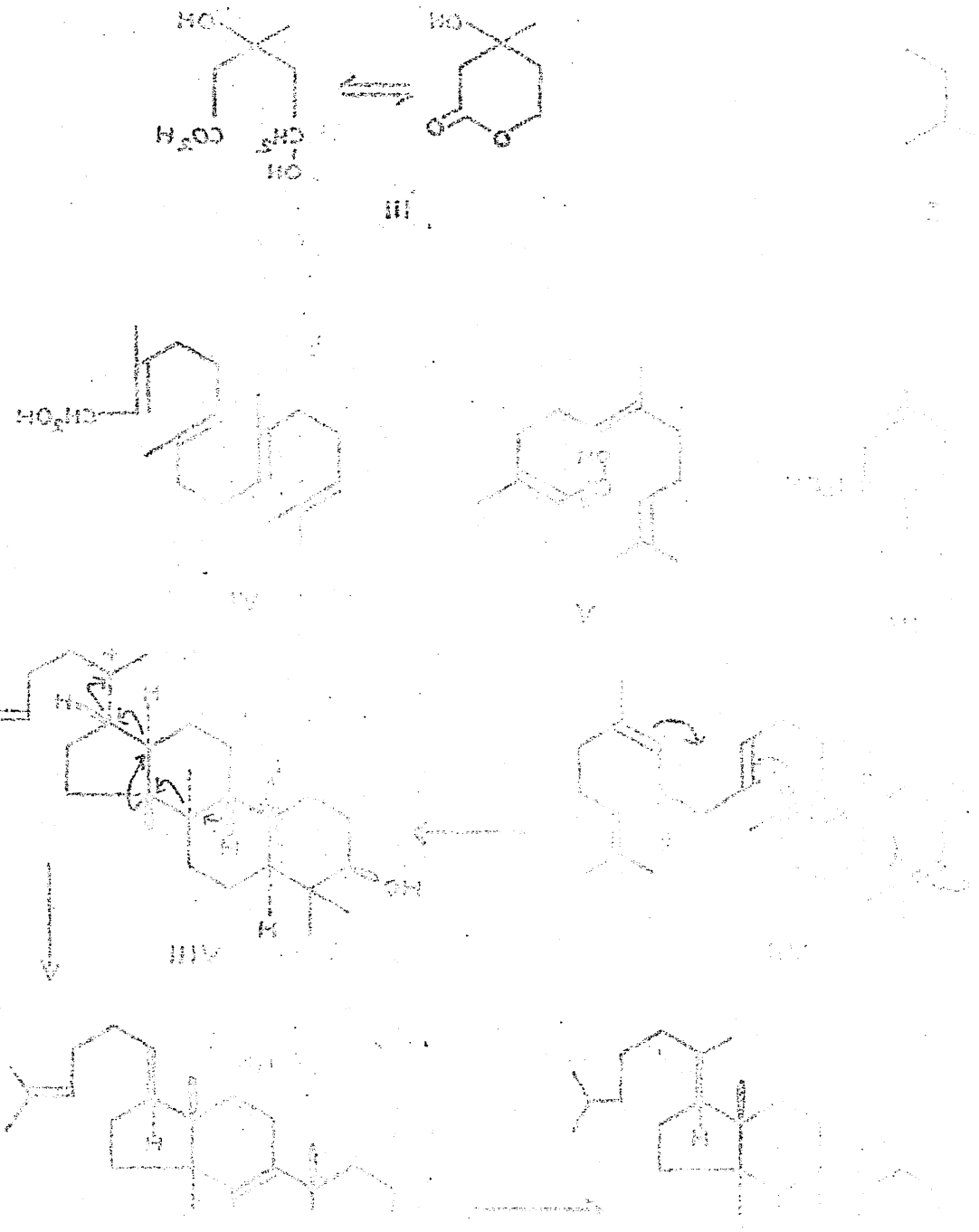
The dione (50 mg. m.p. 218-20°) in methanol (3 ml.) with 6% potassium hydroxide was allowed to stand at room temperature overnight. Acidification followed by ethyl acetate extraction gave material (45 mg.) which on sublimation afforded the dione m.p. 215 - 19° with identical infrared spectrum to the starting material (λ max. 235mμ (ε 1,250) suggested further oxidation to the ene-dione).

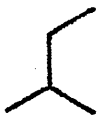
Hydrolysis/

Hydrolysis of Octahydrocolumbinic Acid Methyl Ester m.p. 93 - 6°
(XVI: R = CH_3)

The octahydrocolumbinic acid methyl ester (300 mg., m.p. 93 - 6°) in methanol (4 ml.) was treated under nitrogen with 4 N potassium hydroxide (15 ml.) on the steam bath for 4 hours. Acidification followed by ethyl acetate extraction gave an oily residue (280 mg.). This material could not be crystallised and showed a 1760 $cm.^{-1}$ (boat δ-lactone) band in the infrared. It was treated with alkali as above for a further 4 hours giving material (200 mg.) (no 1760 $cm.^{-1}$ band surviving) which could not be crystallised, despite chromatography either before or after methylation.

III BIOGENESIS OF DITERPENES.





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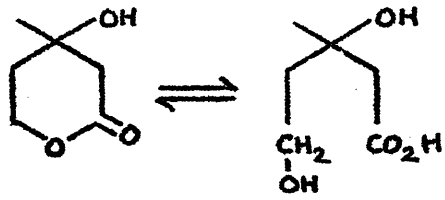

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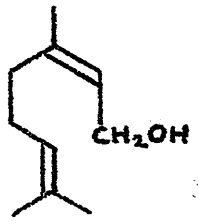

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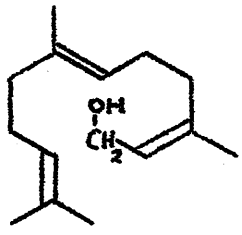

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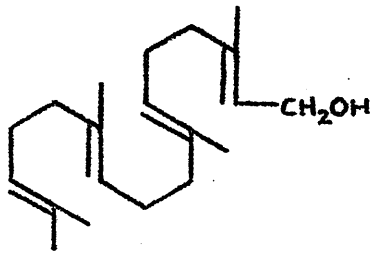

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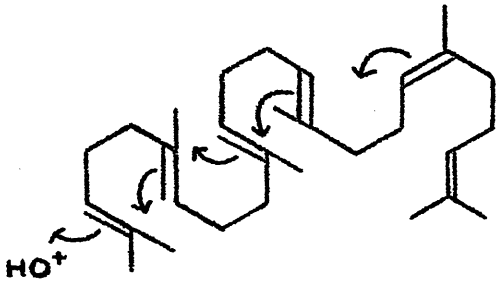

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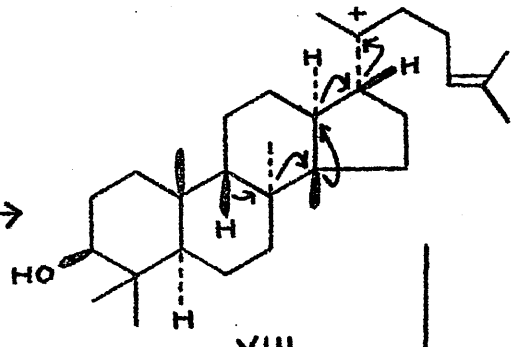

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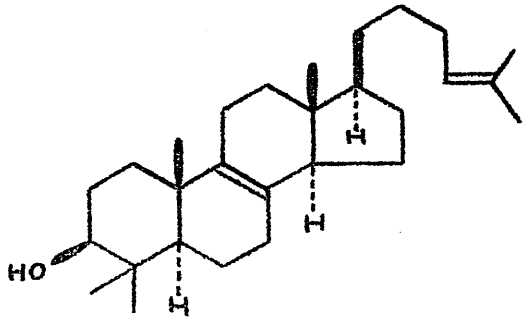

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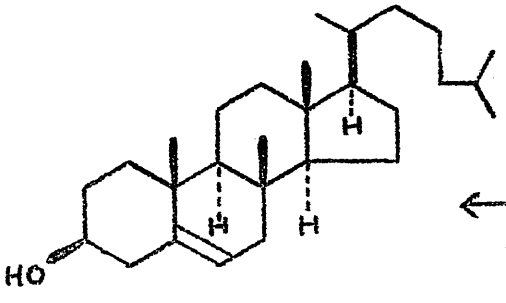


II

Biogenesis of Diterpenes.

a) General.

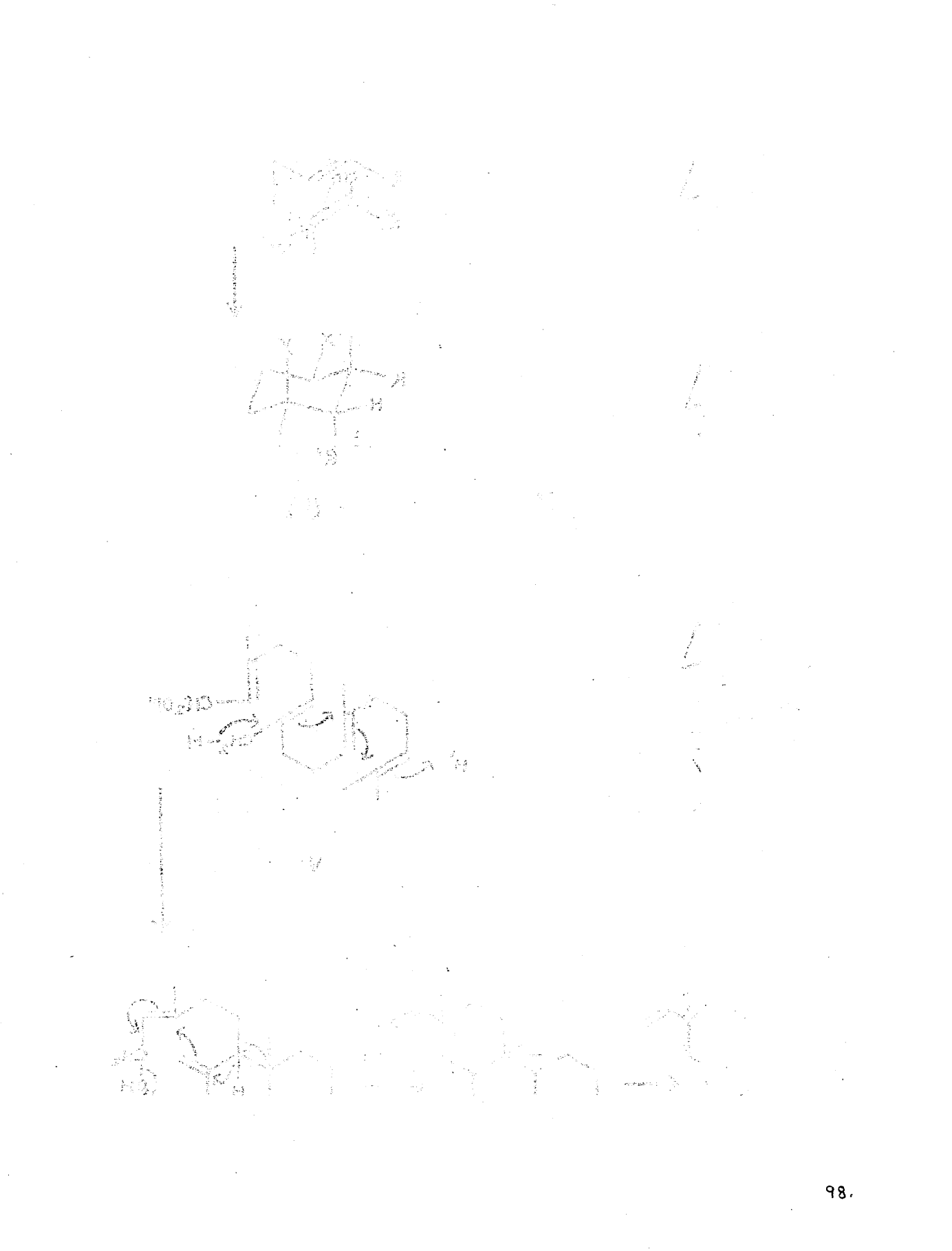
The nature of terpene biogenesis has been extensively reviewed in the literature[77,78] and only a brief outline of the main points will be considered here.

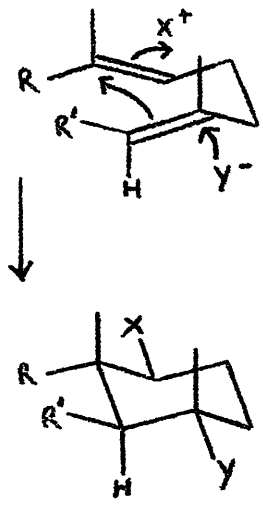
The present theories of terpene and steroid biogenesis are based on the Isoprene rule which states that the carbon skeletons of these natural products are built up by condensation of two or more isopentane (I) units. That this five carbon unit has its origin in a two carbon precursor is supported by the biosynthesis of labelled cholesterol (II) from labelled acetic acid.[79] The nature of the isopentane unit, derived from acetate, and its mode of synthesis was established by the isolation of β - hydroxy - β - methyl - δ - valerolactone (M.V.A.) (III).[80] This compound, which can decarboxylate to give a five carbon unit, is converted virtually quantitatively into cholesterol[80] and is more probably the direct precursor in biosynthesis. Further it has been shown that the labelling pattern in a molecule synthesised from labelled acetic acid or M.V.A. is consistent with a derivation based on linkage of isoprenoid units.[83]

The Isoprene rule has been extended by Ruzicka[81,82] and it is now considered that initial condensation of the five carbon unit, derived from M.V.A., into geraniol (IV) farnesol (V), geranylgeraniol (VI) and squalene (VII) provide the biogentic intermediates in the formation of mono - , sesqui - , di - , and triterpenes (and steroids) respectively.

In the case of the triterpenes and steroids this is supported by considerable experimental evidence, e.g. the transformation of acetic acid into cholesterol (II) via squalene (VII) and lanosterol (IX).[84,85,86]

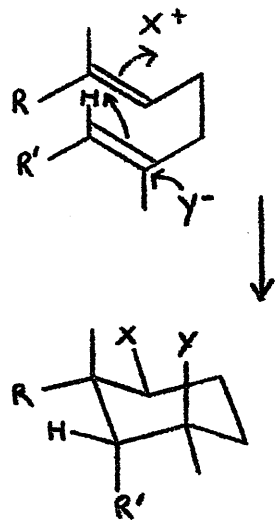
By analogy with the cyclisations, occurring by a concerted anti-planar mechanism, of the polyisoprenoid (X) folded in (a) the chair and (b) the boat conformation cyclisation of squalene (VII), folded in the chair - boat - chair - boat conformation/



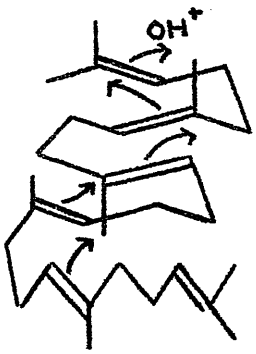


(a)

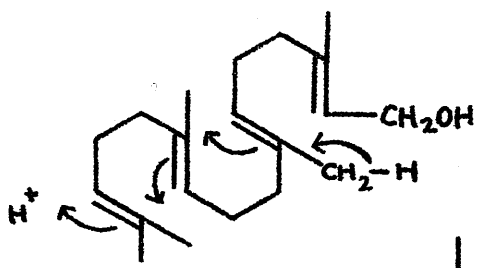
X



(b)



VII a



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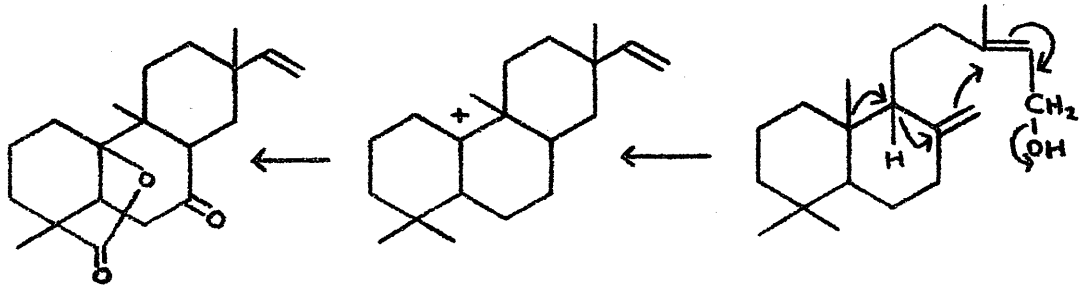
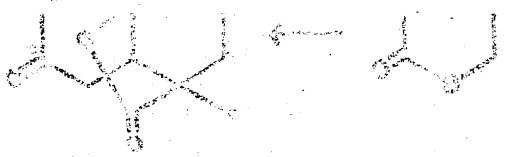
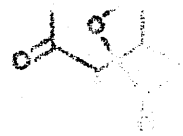


XI



XIIIX



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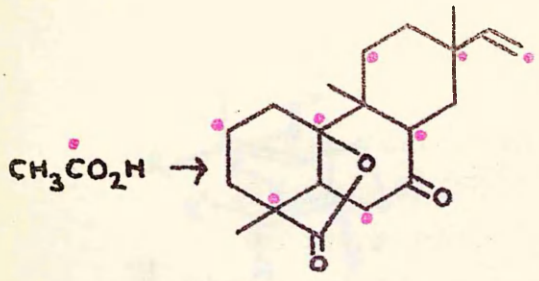

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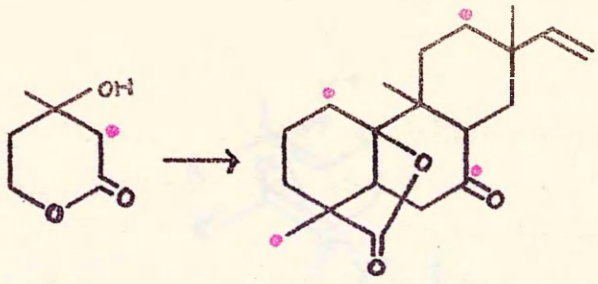

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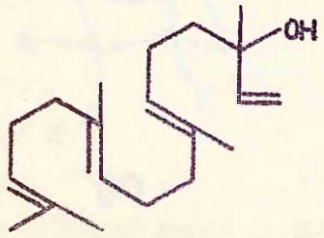

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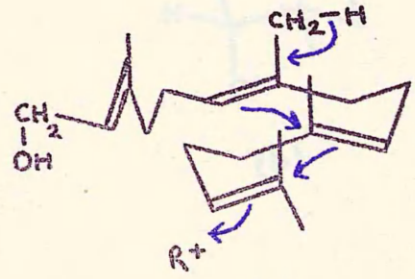

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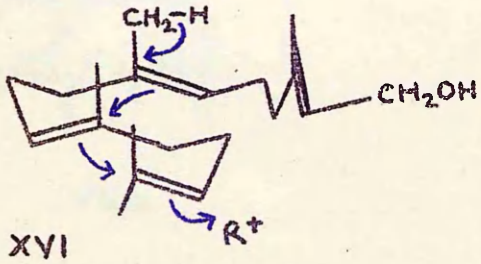

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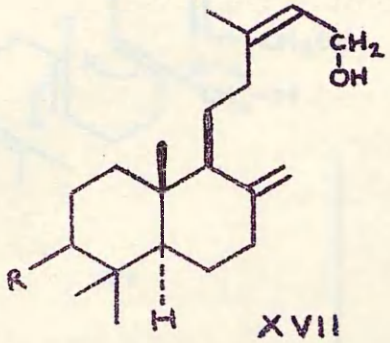

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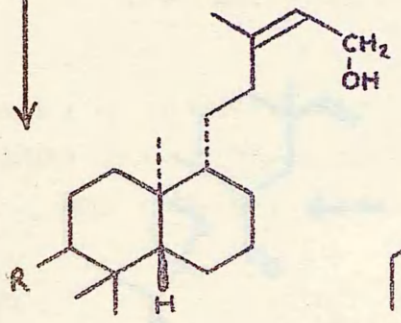

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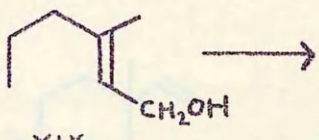

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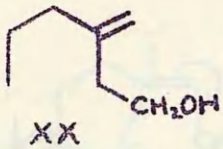

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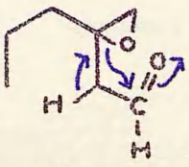
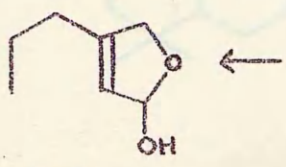
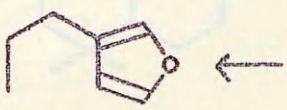


XXI



XXII



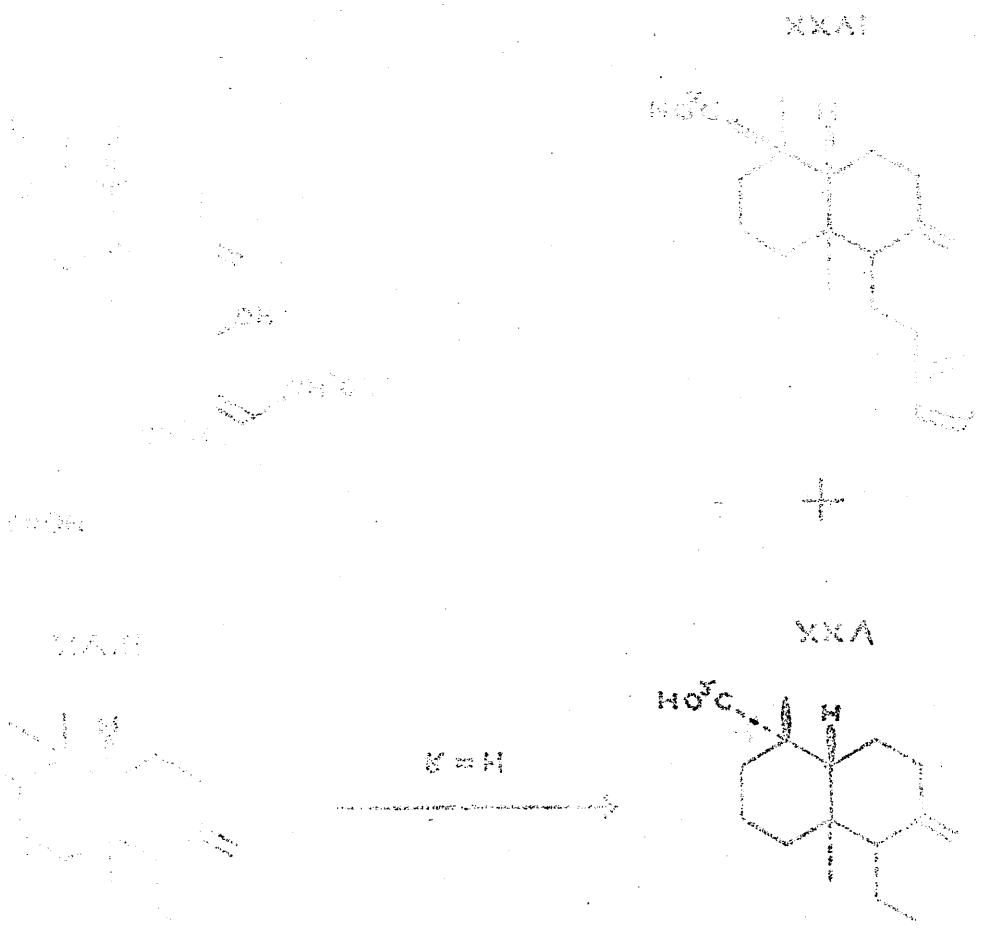
XXIII

conformation (VIIa), to (VIII) is assumed to occur by a trans - planar mechanism. The derivation of lanosterol (IX) from (VIII) is assumed to occur by trans - planar 1 : 2 shifts of hydrogen and methyl groups the overall process being concerted with no intermediate carbonium ion formation.[82] The conversion of lanosterol (IX) to cholesterol (II) occurs with oxidative loss of three carbon atoms.[86]

Experimental evidence for the derivation of diterpenes from the geranylgeraniol (VI) system is not so abundant but what has been determined is in accord with this biogenetic isoprene rule. Rosenonolactone (XI) is considered to be derived from geranylgeraniol (VI) by the scheme represented as (VI) to (XI). This sequence predicts that rosenonolactone derived from 2 - ^{14}C - M.V.A. and 1 - ^{14}C - acetic acid should have an isotope distribution as shown in (XII) and (XIII) respectively. These predictions have been confirmed by degradation studies on labelled rosenonolactone.[87]

The biogenesis of the labdane group of furanoid diterpene bitter principles reviewed earlier will now be dealt with. These compounds are derived from geranylgeraniol (VI) or a related system such as geranyllinalool (XIV). Cyclisation of the geranylgeraniol system (cyclisation and subsequent rearrangement are assumed to follow the scheme outlined for squalene) can occur in two ways as shown in (XV) and (XVI) to give the systems (XVII) and (XVIII) the former having a β $C_{(10)}$ methyl group ('natural' stereochemistry) the latter an α $C_{(10)}$ methyl group ('unnatural' stereochemistry). These systems (XVII) and (XVIII) are the biogenetic precursors of the compounds in the labdane group, the one utilised depending on the absolute stereochemistry of the molecule under consideration.

Before dealing with any compound in particular the derivation of a furan ring will be mentioned. It has been suggested [88] that the allylic alcohol (XIX) on rearrangement to (XX) followed by epoxidation and oxidation to (XXI) with further/



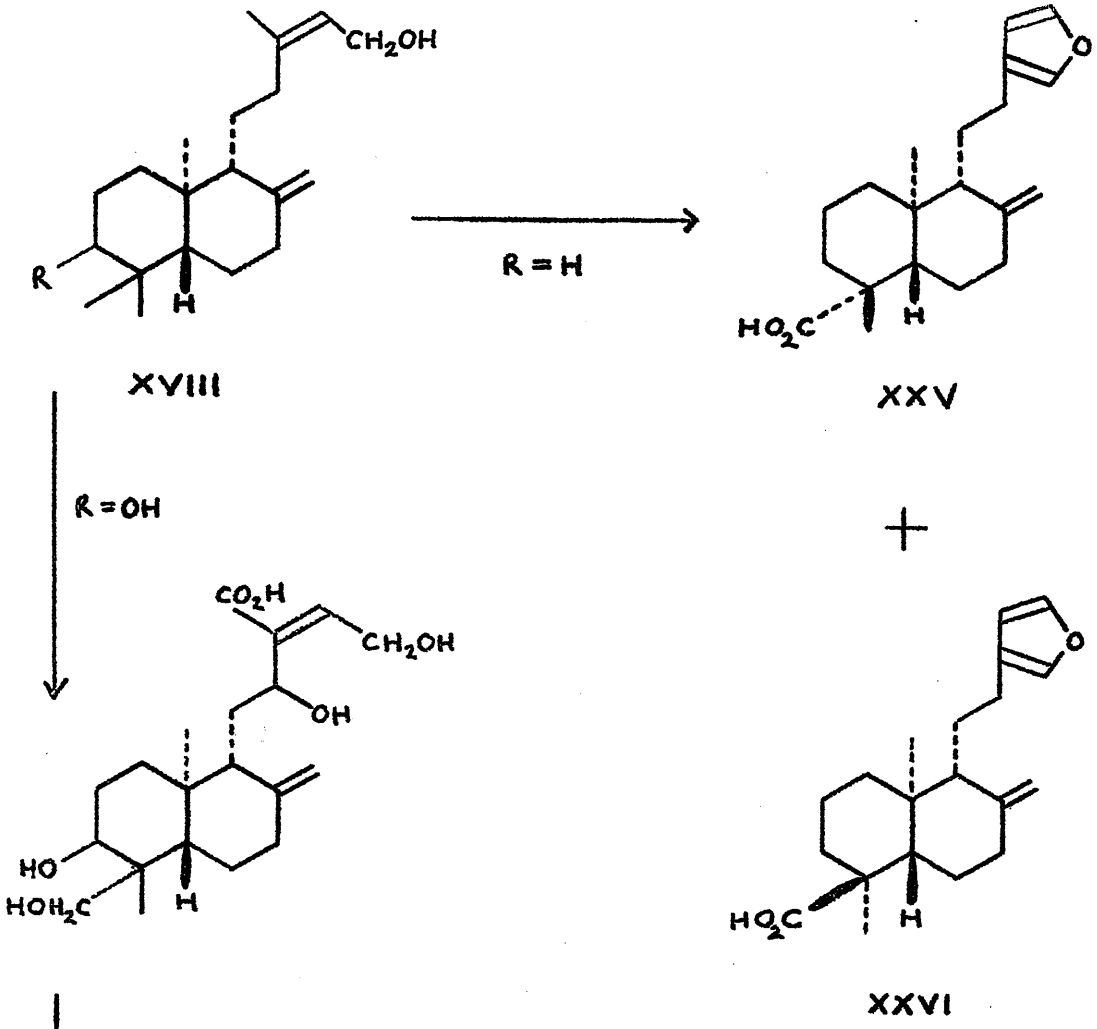
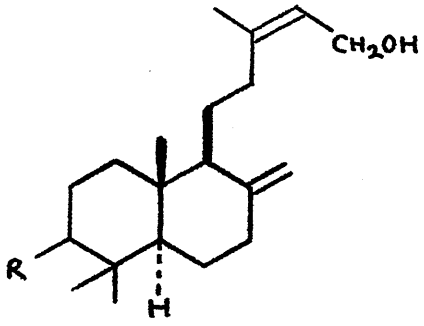
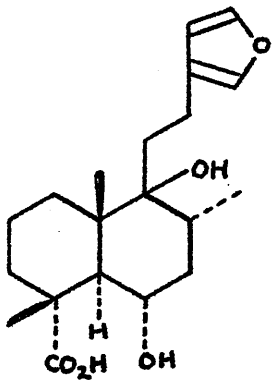

CH2OH
R
H
R=H
O
HO2C
H
XVIII
XXV
R=OH
+
CO2H
CH2OH
OH
HO
HOH2C
H
HO2C
H
O
XXVI
O
O
OH
HO
HOH2C
H
XXIV

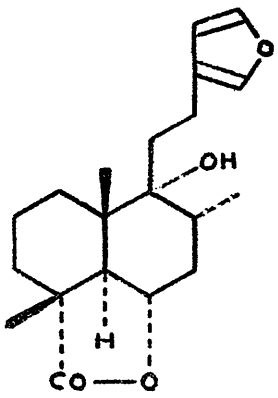
Fig. 1.



XVII

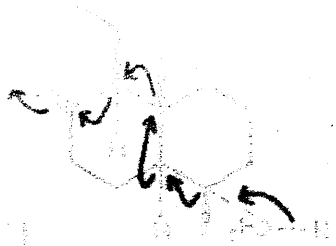
R=H





XXVII

FIG. 2.



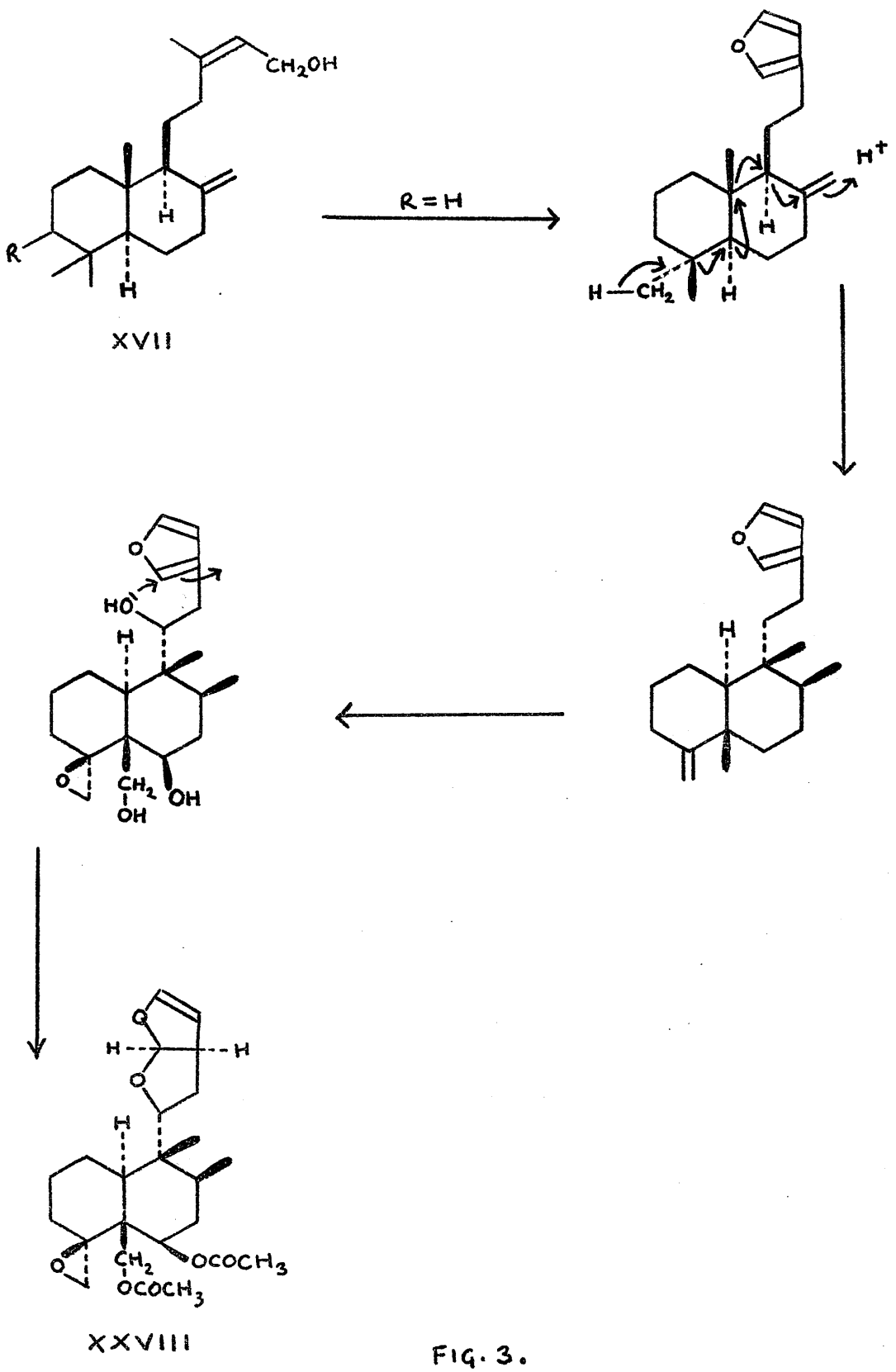
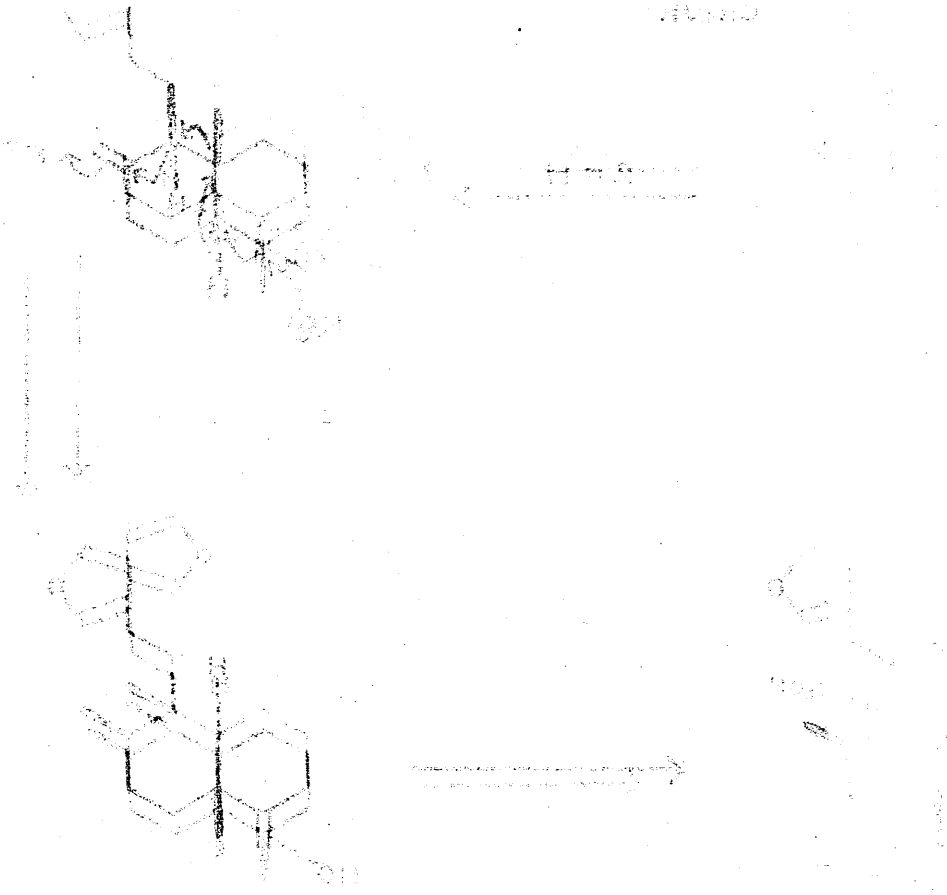

CH2OH
R
H
H
XVII
R=H
O
H+
H
H—CH2
H
O
H
HO
H
O
CH2
OH
OH
O
H
H
O
O
CH2
OCOCH3
OCOCH3
XXVIII

FIG. 3.



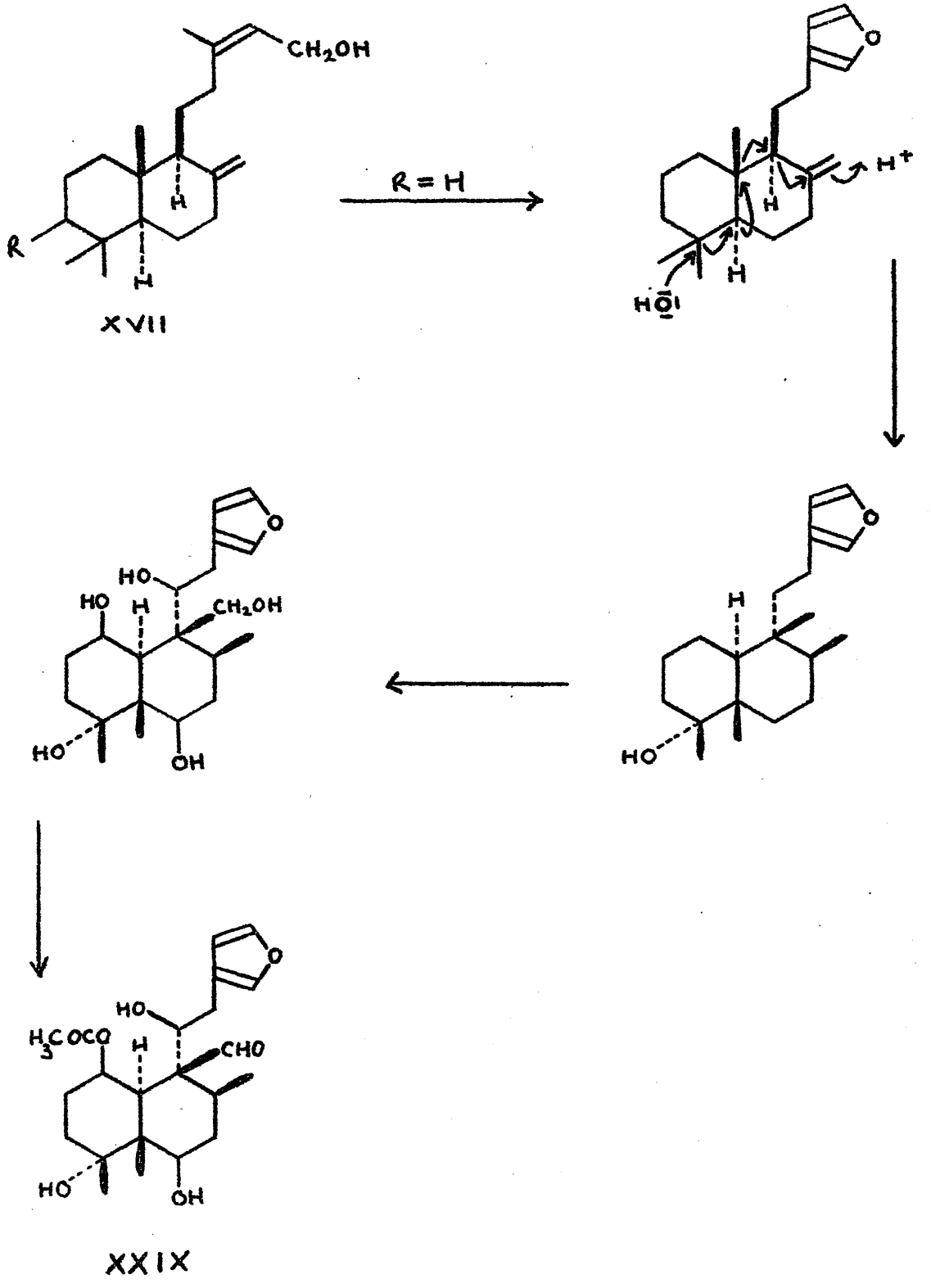
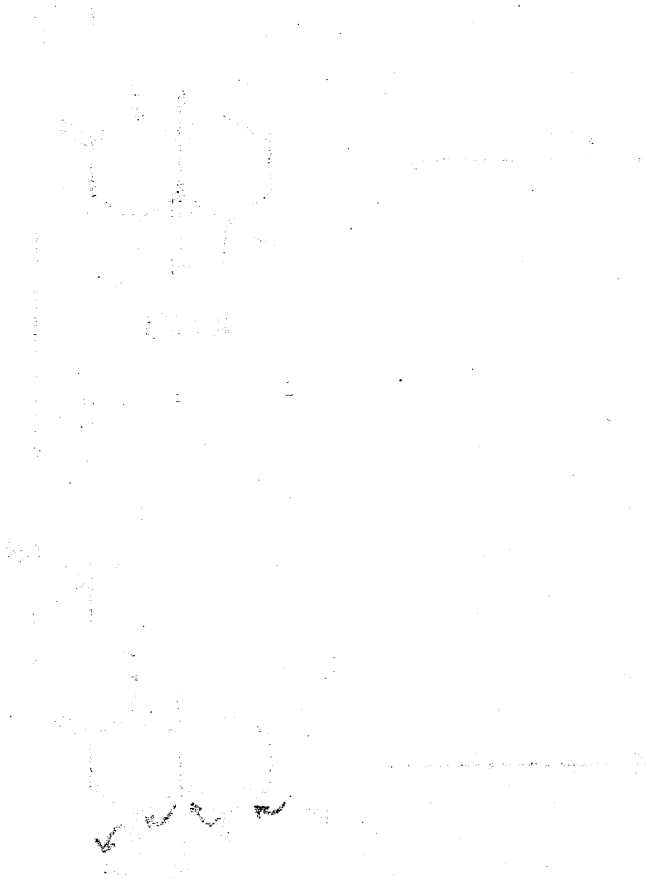

CH2OH
R
H
H
XVII
R = H
O
H+
H
H
HO
HO
HO
H
CH2OH
HO
OH
HO
H3COCO
H
CHO
HO
OH
XXIX

FIG. 4.



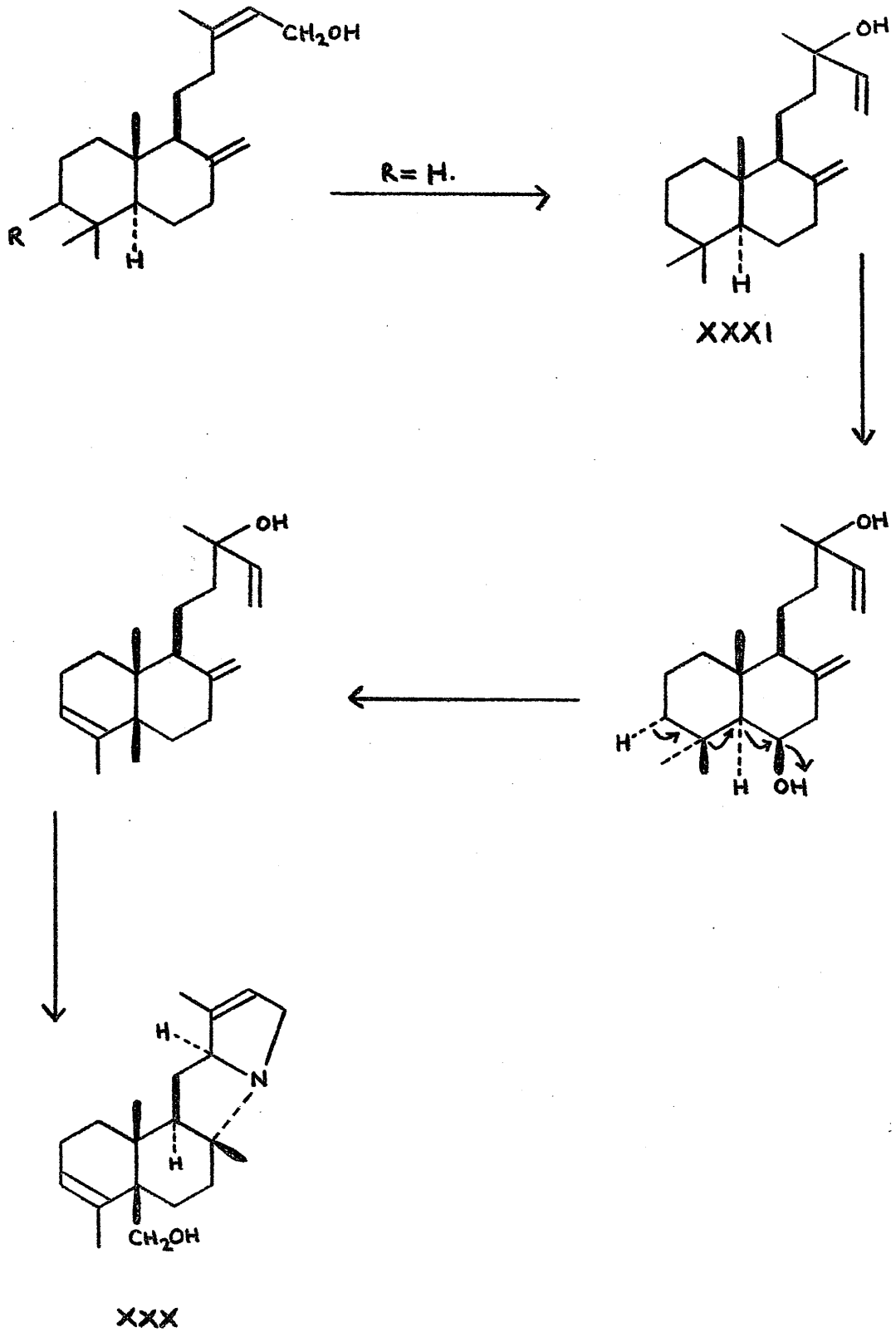


FIG. 5.

further rearrangement to (XXII) gives a furan ring (XXIII). This mechanism, when applied to the side chain of geranylgeraniol (VI), indicates that natural products derived from this system should all have a β - substituted furan ring if it is present in the molecule.

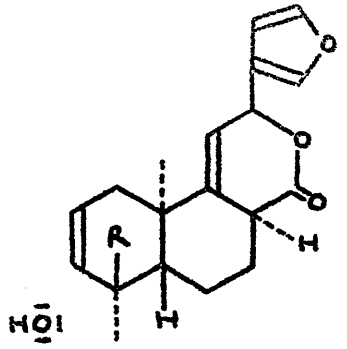
The biogenesis of andrographolide (XXIV), daniellic acid (XXV) and polyalthic acid (XXVI), with α $C_{(10)}$ methyl groups, can be represented as shown in Fig.(1), the lactone system in andrographolide being formed by oxidation of the side chain methyl group to carboxyl.

The derivation of marrubiin (XXVII) can be represented as shown in Fig(2) the precursor having 'natural' stereochemistry at $C_{(10)}$.

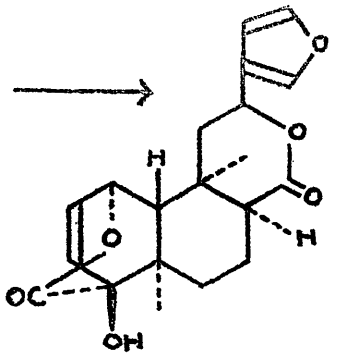
The biogenesis of clerodin (XXVIII), shown in Fig(3), involves rearrangement of the carbon skeleton of a precursor having 'natural' stereochemistry at $C_{(10)}$.

In the case of cascarillin (XXIX) the absolute stereochemistry is not known but it could be derived from a 'natural' precursor as shown in Fig.(4).

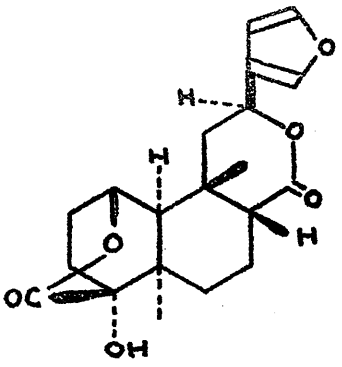
The compound thelepogine (XXX), though not a terpene, is closely related to the labdane group of diterpenes. It can be considered as derived from the manool system (XXXI) as shown in Fig.(5).



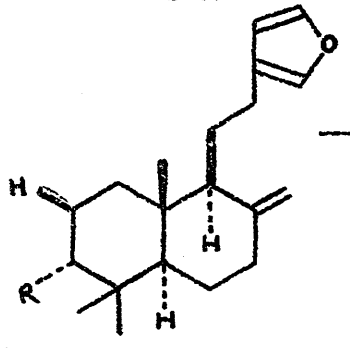
XXXII



XXXIII

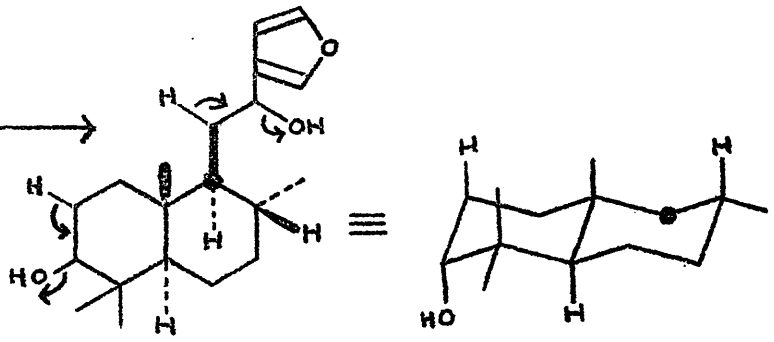


XXXIV

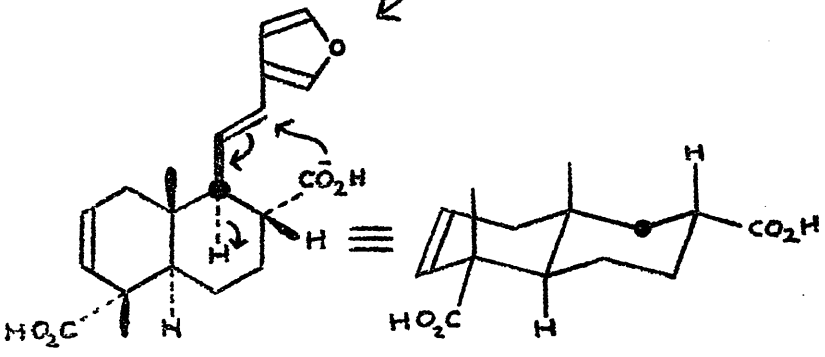


R=OH

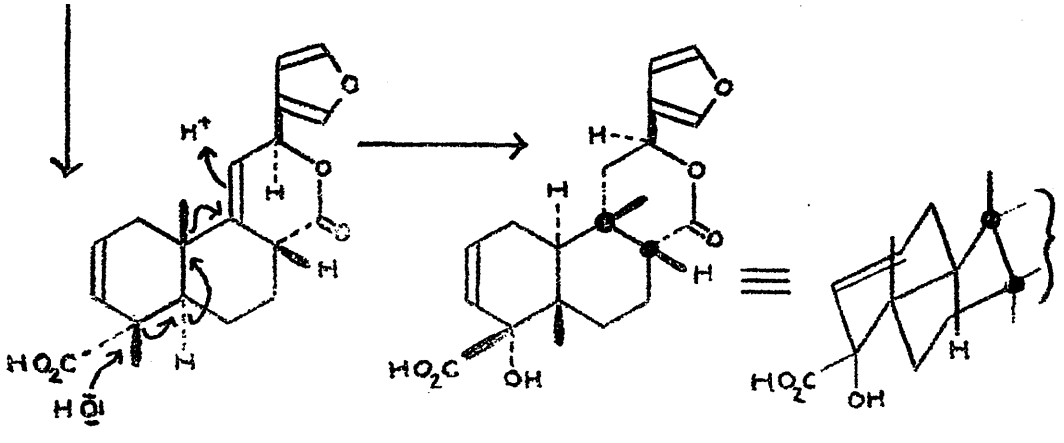
XXXV



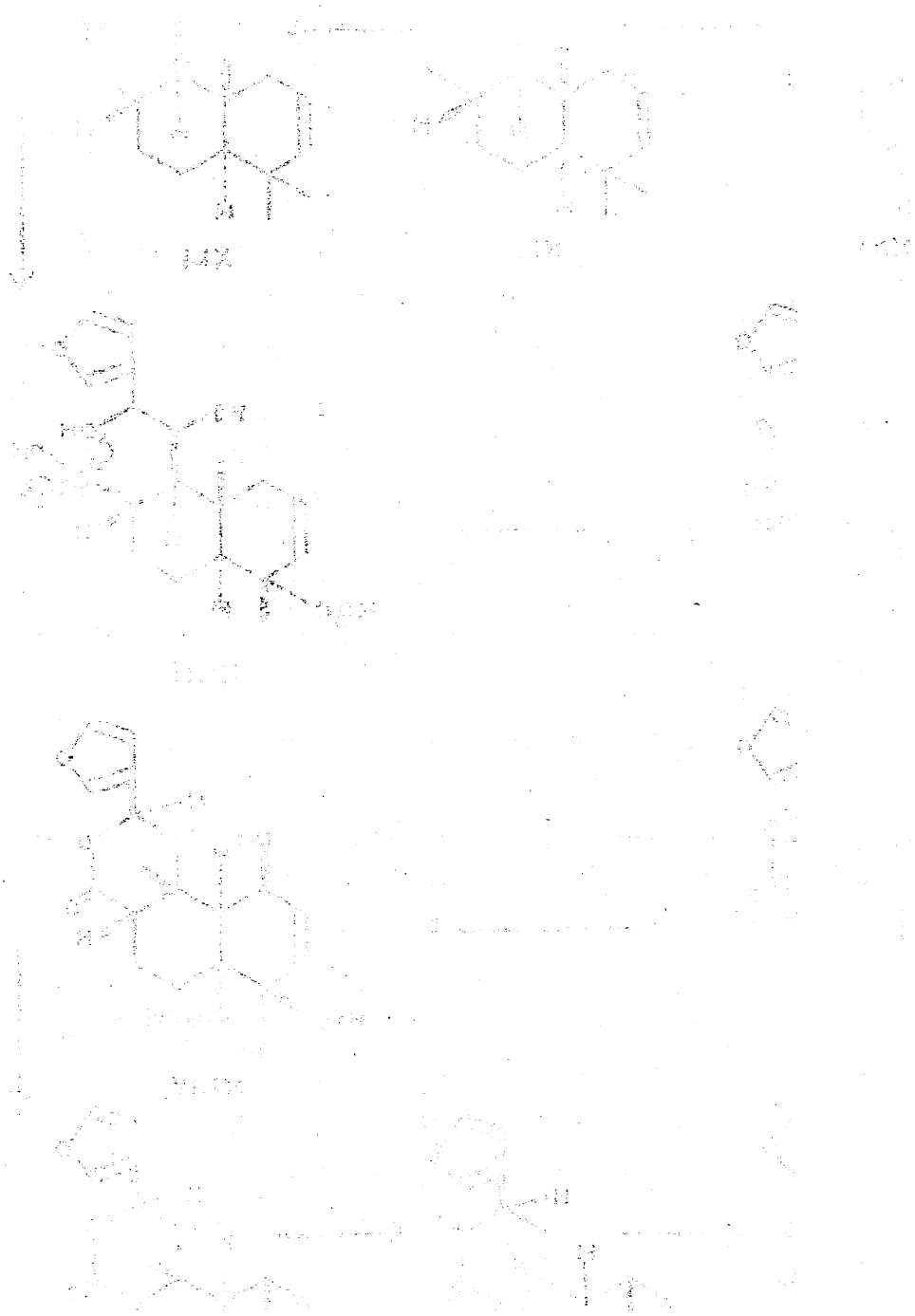
XXXVI

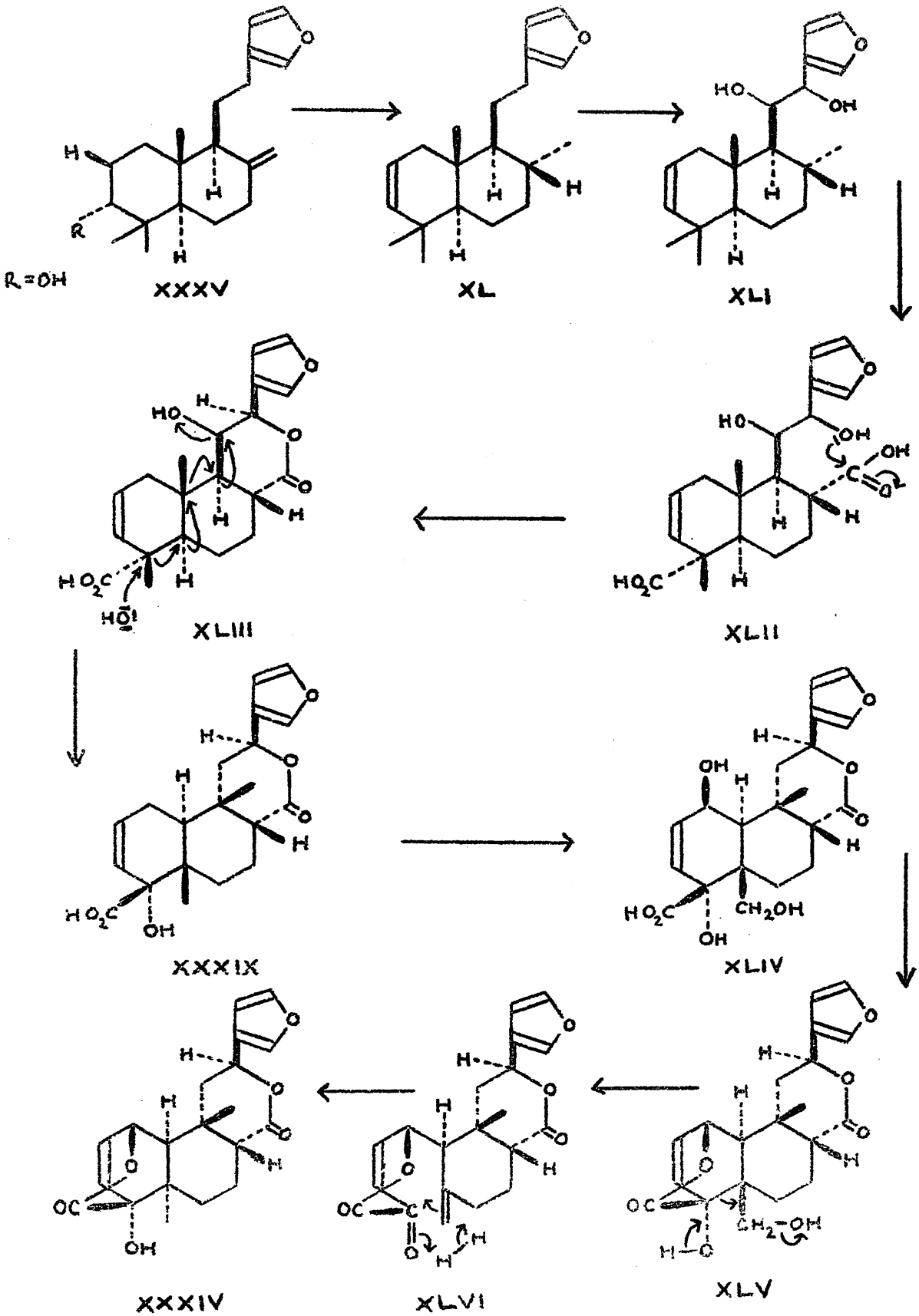


XXXVII



XXXVIII XXXIX




R=OH
XXXV
XL
XLI
XLII
XLIII
XXXIX
XLIV
XLV
XLVI
XXXIV

b) The Biogenesis of Columbin.

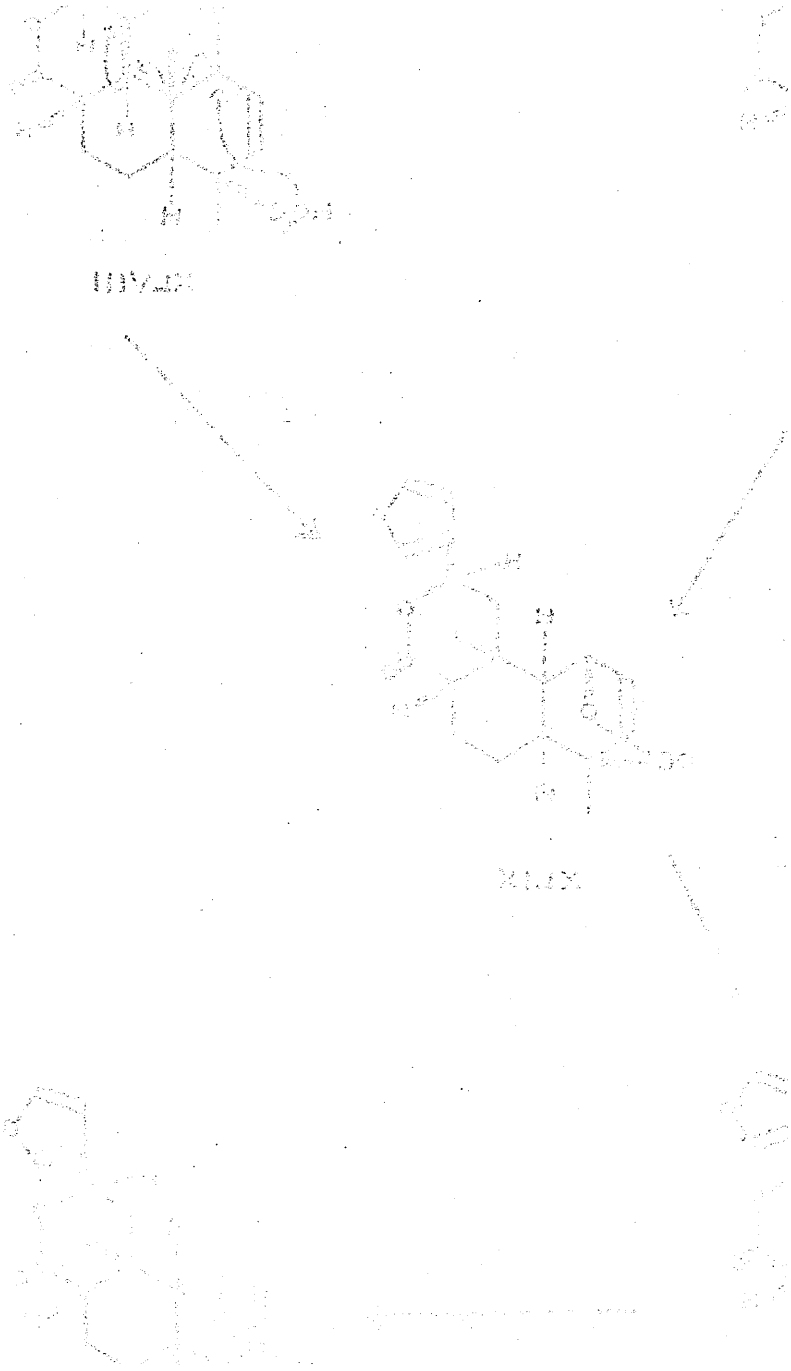
A previously published proposal concerning the stereochemistry[42] of columbin suggested a biogenetic derivation as shown in (XXXII) to (XXXIII). The precursor (XXXII) was drawn with the 'wrong' absolute stereochemistry and gave the situation, with an A/B trans fusion, as shown in (XXXIII).

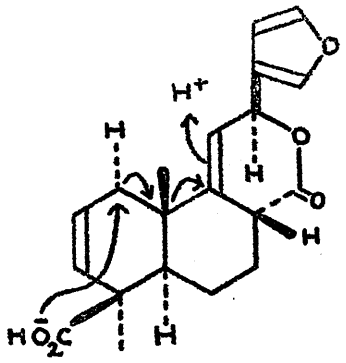
The stereochemistry of columbin has been shown, as a result of the work described in this thesis, to be as in (XXXIV) with A/B and B/C cis ring fusions. Further the $C_{(10)}$ hydrogen has been shown to be α indicating derivation from a precursor with 'natural' stereochemistry at $C_{(10)}$.

A possible biogenetic sequence starting with (XXXV), derived by normal means from geranylgeraniol (VI) could be as follows. Protonation on the β face of the molecule gives an equatorial (α) methyl group at $C_{(8)}$, oxidation then giving (XXXVI). Dehydration of (XXXVI) followed by oxidation gives (XXXVII) and attack by the carboxylate anion at $C_{(8)}$ on the $C_{(9)} - C_{(12)}$ double bond furnishes the lactone (XXXVIII). Trans anti-planar methyl and hydride shifts give (XXXIX). An alternative derivation of (XXXIX) from (XXXV) could be as follows. Formation of an equatorial methyl group at $C_{(8)}$ and dehydration gives (XL) which furnishes (XLI) on oxidation. Further oxidation gives (XLII) which lactonises to (XLIII) and methyl shifts, as shown, give (XXXIX).

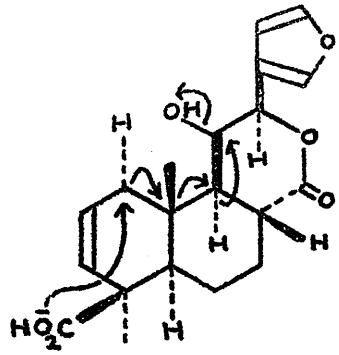
Oxidation of (XXXIX) gives (XLIV) with a primary hydroxyl attached to $C_{(5)}$, a situation similar to that in clerodin. Lactonisation then affords (XLV) with a β 1:4 lactone bridge. It is now postulated that, because of the highly strained nature of (XLV), the system undergoes a reverse aldol reaction to give (XLVI) which is cyclised reductively to columbin (XXXIV), with the very much less strained cis fusion of rings A and B.

As/

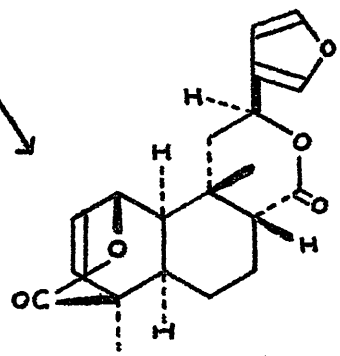




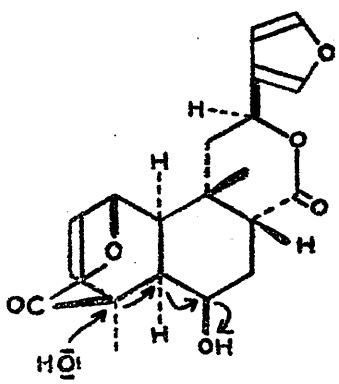
XLVII



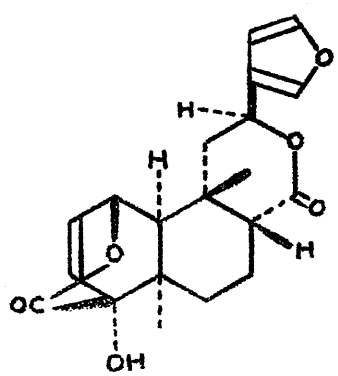
XLVIII



XLIX



L

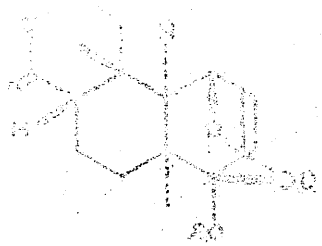


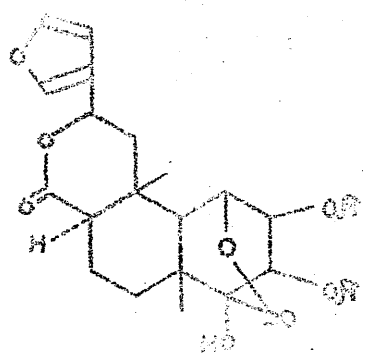
XXXIV

As an alternative the scheme (XLVII) or (XLVIII) to (XXIV) can be considered. (XLVII) and (XLVIII) can be derived by the schemes which give (XXXVIII) and (XLIII) respectively, the only difference being oxidation to give a β carboxyl group at $C_{(4)}$. Attack by carboxylate anion, as shown, with accompanying methyl and hydride shifts, gives (XLIX). Oxidation to (L) then methyl migration gives columbin (XXXIV).

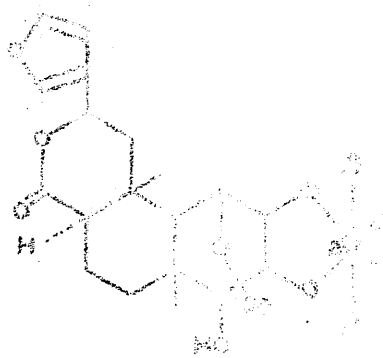
It will be interesting to examine these proposals by biosynthetic experiments, using labelled precursors, once the rather unusual stereochemistry of columbin has been confirmed by X - ray crystallographic studies on a suitable derivative.

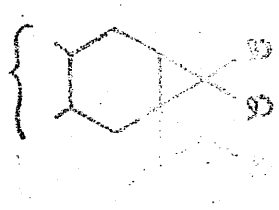
IV. A DERIVATIVE OF COLUMBIN SUITABLE FOR X-RAY CRYSTALLOGRAPHIC STUDIES.

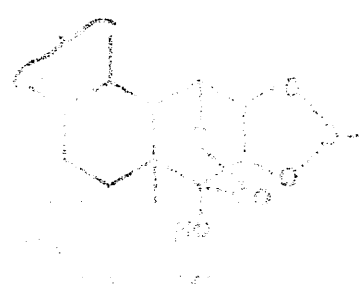


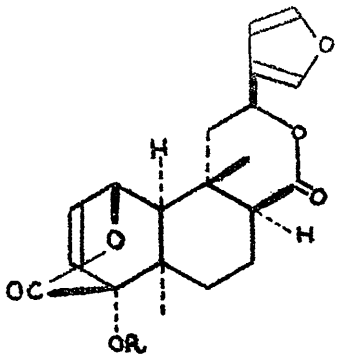


III

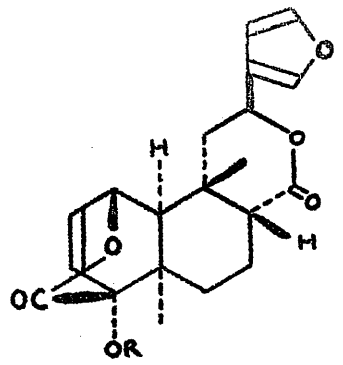




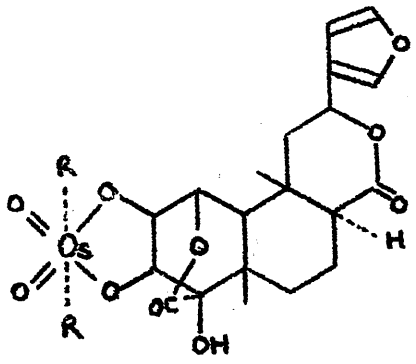




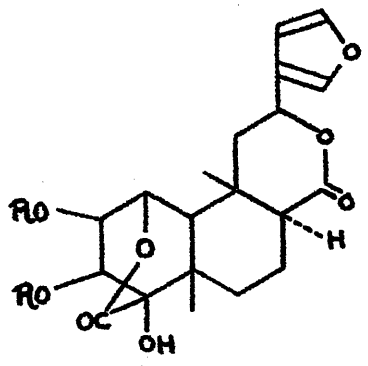
I



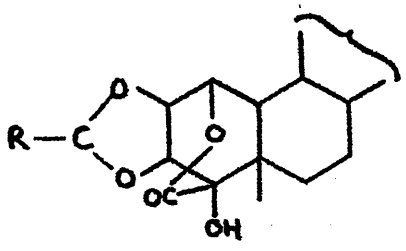
I a



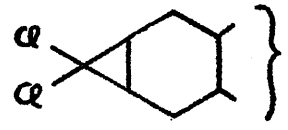
II



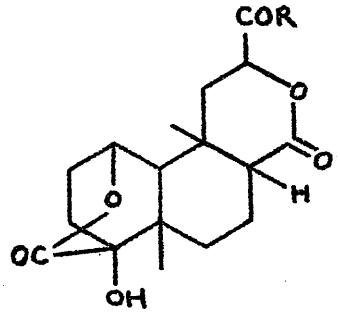
III



IV



V



VI

Attempts to Prepare a Columbin Derivative Suitable for Examination by X-Ray Crystallography.

a) The absolute stereochemistry of columbin has been shown to be (Ia; R = H) utilising purely chemical methods and the techniques of molecular rotation differences, infrared spectroscopy and optical rotatory dispersion measurements. In the three dimensional representation of the molecule we have rings A and B in the boat conformation and ring C in the chair conformation. In isocolumbin (I; R = H) all three rings will have the boat conformation.

Perhaps the most interesting features of the columbin molecule are the cis nature of the A/B and B/C ring fusions. There is no doubt about the nature of the A/B fusion and the arguments, put forward in assigning a cis B/C ring fusion and a β $C_{(9)}$ methyl group seem sound. However a direct check on the stereochemistry of columbin, in view of its many abnormal features, was thought to be necessary.

Providing a suitable derivative of columbin, containing a heavy atom such as bromine or iodine, was prepared the technique of X - ray crystallography could be used to give the complete relative stereochemistry of the molecule (and possibly in favourable circumstances the absolute stereochemistry also).

With columbin the 'lightest' heavy atom which could be successfully used was thought to be bromine and attempts were made to obtain a derivative, in the form of single crystals, containing bromine or a heavier element.

Initial experiments were conducted using the available functional groups (a tertiary hydroxyl, a double bond and a furan ring) in the molecule as it was desired to preserve as much of the original structure of columbin as possible.

Under a variety of conditions, using acetyl chloride, it was found that isocolumbin (I; R = H) could be acetylated with great ease. When the same methods/

methods were used (see experimental) with chloroacetyl chloride no ester was isolated. Most of the reactions yielded unchanged isocolumbin and an unidentified oil.

On treatment with β - bromopropionyl chloride[89] isocolumbin again gave no crystalline product.

Reaction of the double bond in the molecule was then considered. Isocolumbin (I; R = H) on treatment with osmium tetroxide in dioxan, then hydrogen sulphide, furnished the cis diol (III; R = OH).[20] A method of isolating the intermediate (II; R = an organic base) is described in the literature[90] and experiments were conducted in order to obtain a series of these compounds. Reaction of isocolumbin with osmium tetroxide in dry tetrahydrofuran in the presence of, in turn, α -, β -, γ - picoline, pyridine, 2 : 4 : 6 - collidine, quinoline and isoquinoline gave in only two cases (α - and β - picoline) crystalline solids.

The compound (II; R = α - picoline) derived from α - picoline was unstable at room temperature, losing solvent of crystallisation with breakdown of the crystal structure, and was for this reason unsuitable as an X - ray derivative. The compound (II; R = β - picoline) derived from β - picoline had m.p. ca 230° and did not lose solvent of crystallisation till ca 180°. However lengthy attempts to obtain this compound in the form of single crystals in a size which would permit manual manipulation failed.

The double bond in columbin reacts readily with bromine but the compound appeared to decompose rapidly on standing at room temperature and no crystalline product was obtained.

Attempts were then made to obtain a cyclic acetal of the form (IV) from the triol (III; R = OH)[91]. Reaction of the triol with a) O - bromo benzaldehyde b) benzaldehyde c) p - bromo acetophenone in the presence of toluene - p - sulphonic acid or with toluene - p - sulphonic acid alone furnished small amounts of a compound (the same in all cases) m.p. 299 - 300°. This material had no aromatic/

aromatic absorption bands in the infrared, gave a negative Beilstein test, and, because the present work was concerned with obtaining a heavy atom derivative, was not investigated further.

Reaction of the triol (III; R = OH) with chloroacetyl chloride and pyridine failed to give a crystalline product despite chromatography.

Formation of a bromohydrin by the reaction of N - bromosuccinimide on a double bond has been described in the literature[92] but when isocolumbin was reacted with 1 mole of N - bromosuccinimide the product (a white amorphous solid) showed no furan absorption but strong hydroxyl (3500 $cm.^{-1}$) absorption in the infrared, gave a faint positive Beilstein test and decomposed on warming with solvent. Reaction of dihydroisocolumbin in a similar manner gave an amorphous solid, with hydroxyl and no furan absorption in the infrared, which gave a faint positive Beilstein test. Attempted crystallisation of this compound gave a brown gum which showed acidic hydroxyl and double bond absorption in the infrared and appeared not to contain bromine (negative Beilstein test).

Attempted substitution of mercury into the furan ring by the action of mercuric chloride[93] on isocolumbin gave recovery of isocolumbin.

Reaction of dichlorocarbene[94,95] on isocolumbin, in an attempt to form a compound containing a dichlorocylopropane system (V), gave quantitative recovery of isocolumbin.

Ozonolysis of dihydrocolumbin and dihydroisocolumbin gave the acids (VI; R = OH) and (VI; R = OH, $C_{(8)}$ α H) respectively and reaction of these with rubidium carbonate gave the salts (VI: R = ORb) and (VI; R = ORb, $C_{(8)}$ α H). These salts were isolated as fine white needles and all attempts to grow crystals suitable for X - ray examination failed.

Reaction of the acids (VI; R = OH) and (VI: R = OH, $C_{(8)}$ α H) with p - iodophenacylbromide gave a good recovery of the starting acids.

Attempts/

Attempts were made to form amides of these acids via their acid chlorides. Early attempts were unsuccessful, possibly due to non-formation of the acid chloride, but finally the method described in the experimental section[96] gave the acid chloride. Reaction of the acid chloride with p - bromoaniline gave small amounts of the amide as fine needles and attempts to grow favourable crystals failed.

However reaction of the acid chloride (of the acid derived from dihydrocolumbin) with m - bromoaniline gave the anilide (VI; R = m - bromoaniline) m.p. 228 - 30° as stout prisms and sizeable single crystals were easily obtained. Preliminary measurements indicate that this derivative crystallises in the monoclinic system with four molecules to the unit cell, an arrangement which is not suitable for X - ray study because of symmetry problems.

The reaction scheme however should allow a series of amides to be made with the possibility of obtaining a more suitable derivative. A bulky group attached to the carboxyl in the molecule (VI) should function as the original furan ring in preserving conformations of the ring C lactone.

(b) Experimental.

For general experimental see page 79.

Acetylisocolumbin (I; R = $COCH_3$)

(a) Isocolumbin (200 mg., m.p. 185 - 7° dec.) (prepared as on page) in dimethylaniline (5 ml.) was refluxed with acetyl chloride (3 ml.) overnight. The solution was poured into cold water giving a solid (200 mg.) which on crystallisation from acetone - alcohol gave acetylisocolumbin (I; R = $COCH_3$) (150 mg.) m.p. 226 - 8°, $[\alpha]_D$ + 23°.

(b) Isocolumbin (50 mg.) in chloroform (3 ml.), with a few drops of pyridine, was refluxed with acetyl chloride (0.5 ml.) overnight. Water was added and chloroform extraction followed by crystallisation from acetone - alcohol gave acetylisocolumbin (I; R = $COCH_3$) (30 mg.) m.p. 227 - 9°.

(c) Isocolumbin (50 mg.) in dioxan (2 ml.) was refluxed with acetyl chloride (0.5 ml.) overnight. The mixture was poured into cold water giving a solid which afforded, on crystallisation from acetone - alcohol, acetylisocolumbin (I; R = $COCH_3$) (37 mg.) m.p. 227 - 9°.

Attempted Chloroacetylation of Isocolumbin (I; R = H)

(a) Isocolumbin (50 mg.) as in (a) above but using chloroacetyl chloride gave a black tar. A black tar was formed with chloroacetyl chloride and dimethylaniline alone.

(b) Isocolumbin (50 mg.) as in (b) above using chloroacetyl chloride (0.5 ml.) gave isocolumbin (10 mg.) and oily material which could not be crystallised.

(c) Isocolumbin (50 mg.) as in (c) above using chloroacetyl chloride (0.5 ml) gave isocolumbin (14 mg.) and oily material which could not be crystallised.

(d)/

(d) Isocolumbin (200 mg.) in chloroacetyl chloride (3 ml.) with a few drops of dimethylaniline was refluxed overnight. The mixture was poured into water giving a dark brown gum which could not be crystallised.

(e) Isocolumbin (400 mg.) in dioxan (10 ml.) was refluxed with chloroacetyl chloride (4 ml.) overnight. The mixture was poured into water and chloroform extraction yielded an oil (445 mg.)

The oil was chromatographed on grade V alumina in benzene. Apart from isocolumbin (ca 40 mg.) no crystalline material was isolated.

The infrared spectra of all the products from (b) to (e) still showed hydroxyl (3500 $cm.^{-1}$) absorption and were generally quite different from isocolumbin.

Treatment of Isocolumbin (I; R = H) with β - Bromopropionyl Chloride.

Isocolumbin (135 mg.) in chloroform (5 ml.) with a drop of pyridine was refluxed with β - bromopropionyl chloride (1 ml) overnight. Water was added and chloroform extraction followed by washing with aqueous sodium bicarbonate gave a yellow oil (600 mg.).

This material could not be crystallised and the large amount recovered suggested reaction other than that desired.

Osmate Esters of Isocolumbin (I; R = H)

The general method of making these compounds is exemplified as follows:

(a) Isocolumbin (36 mg.) in dry distilled tetrahydrofuran (2 ml.) with β - picoline (ca 0.2 ml.) was treated at room temperature overnight with osmium tetroxide (25 mg.) in tetrahydrofuran (1 ml.). The solution (if no material had crystallised) was concentrated in vacuo at room temperature. The solvents were decanted and the complex isolated as dark red needles (25 mg.) m.p. ca.230°. Recrystallisation was carried out by dissolving the compound in a large excess of tetrahydrofuran in the cold and concentrating to small volume also in the cold. The compound was again

again isolated as dark red needles m.p. ca 230° which lost solvent of crystallisation at ca 180°, (II; R = β - picoline).

(b) As above, but using α - picoline, gave dark red prisms which lost solvent of crystallisation rapidly at room temperature and decomposed on heating ca 10° above room temperature (II; R = α - picoline).

(c) As above, but using γ - picoline, gave a dark red solution which yielded amorphous material on standing. No crystalline material could be isolated from this reaction. (II; R = γ - picoline).

(d) As above, but using pyridine, again gave an amorphous solid which could not be crystallised (II; R = pyridine).

(e) As above, but using 2 : 4 : 6 - collidine, yielded an amorphous solid which could not be crystallised. (II; R = 2 : 4 : 6 - collidine).

(f) Isocolumbin (36 mg.) in dioxan (2 ml.) with β - picoline (19 mg.) was treated overnight at room temperature with osmium tetroxide (25 mg.) in dioxan (1 ml.). The solution was concentrated at room temperature but no material crystallised from the dioxan solution. The remainder of the dioxan was removed and the residue crystallised from tetrahydrofuran giving the material described in (a) (identical infrared spectrum) (II; R = β - picoline).

Treatment of Isocolumbin with Bromine.

Isocolumbin (36 mg.) in chloroform (10 ml.) was treated at room temperature with bromine (1 mole) in chloroform. When the bromine colour had disappeared (ca 5 mins.) the solvent was removed at room temperature. The semi-crystalline residue could not be crystallised and rapidly decomposed on standing in solution.

Isocolumbindiol (III; R = OH)

Isocolumbin (340 mg) in dry dioxan (10 ml.) was treated with osmium tetroxide (340 mg.) in dry dioxan (10 ml.) at room temperature for 6 hours. The solution was saturated with hydrogen sulphide and filtered through kieselguhr. The solvent was evaporated and/

and the residue on crystallisation from ethanol furnished isocolumbindiol (III; R = OH) (300 mg.) m.p. 260 - 1°, $[\alpha]_D$ + 32° (C, 1.4)

Attempted Condensation of the Diol with Benzaldehyde derivatives.

(a) The diol (50 mg.) in dry chloroform (5 ml) was refluxed with O - bromobenzaldehyde (1.1 moles) and a trace of toluene - p - sulphonic acid for three hours. The solution was cooled and washed with aqueous sodium bicarbonate solution, then aqueous bisulphite solution. Evaporation gave no appreciable recovery of any product. (The diol is quite water soluble and reaction could not have occurred.)

(b) The diol (50 mg.) in dry dioxan (0.5 ml.) was refluxed with a large excess of benzaldehyde in benzene and a trace of toluene - p - sulphonic acid for 1 hour. The benzene was removed by distillation and further amounts of dry benzene (5 ml.) added. The benzene was again removed by distillation and the process repeated for about 1 hour. The solution was cooled and washed with aqueous sodium bicarbonate and aqueous sodium bisulphite. Removal of the benzene gave a small amount of solid material (ca 8 mg.) which on crystallisation from ethyl acetate - methanol gave a compound m.p. 299 - 300° (subl.) as fine needles. This material had no aromatic absorption in the infrared and was not investigated further.

(c) As (b) above but using p - bromoacetophenone gave small amounts of the same non-aromatic material (infrared spectrum).

(d) As (b) above but with no carbonyl compound present gave small amounts of the same non-aromatic material.

Attempted Chloroacetylation of the Diol (III; R = OH)

The diol (90 mg.) in dioxan (1 ml.) was treated overnight with chloroacetyl chloride (2 ml.) and a few drops of pyridine. The mixture was poured into water and chloroform extraction gave a brown gum (70 mg.) which could not be crystallised despite chromatography on grade V alumina.

Reaction/

Reaction of N - Bromosuccinimide with Isocolumbin.

Isocolumbin (100 mg.) in aqueous acetone (2 ml.) with 1 drop 1 N sulphuric acid was treated with N - bromosuccinimide (1 mole). A brown coloration developed immediately and disappeared in about 10 minutes. Aqueous sodium sulphite was added and the acetone removed. A white amorphous solid (ca 90 mg.) was isolated which gave a faint positive Beilstein test. This material m.p. 190 - 200° had strong 3,500 $cm.^{-1}$ (hydroxyl) absorption and no furan absorption in the infrared. The compound decomposed on attempted crystallisation giving a brown oil.

Reaction of N - Bromosuccinimide with Dihydroisocolumbin.

Dihydroisocolumbin (500 mg.) (prepared as on page) in acetone (20 ml.) with water (5 ml.) was treated with N - bromosuccinimide (245 mg.) at 0° for 1 hour. The acetone was removed in vacuo and water (ca 25 ml.) added giving a white amorphous material (400 mg.) m.p. 185 - 200° dec. This material gave a faint positive Beilstein test and had 3,500 $cm.^{-1}$ (hydroxyl) and no furan absorption in the infrared. The material decomposed on warming with solvent giving an oil whose infrared spectrum showed acidic hydroxyl and double bond absorption.

The amorphous material (300 mg.) was treated overnight at room temperature with acetic anhydride (1.5 ml.) in pyridine (6 ml.). The solvents were removed at room temperature and the dark oil taken up in methylene chloride. The extract after washing with dilute hydrochloric acid and aqueous sodium bicarbonate gave an oil (170 mg.). This material showed a decrease in hydroxyl absorption and aromatic absorption in the infrared but could not be crystallised.

Reaction of Dihydroisocolumbin with Mercuric Acetate.

Dihydroisocolumbin (360 mg.) in aqueous ethanol (5 ml.) was treated with mercuric chloride (1 mole) and sodium acetate (4 moles) at room temperature. Dihydroisocolumbin was recovered unchanged after 1.1/2 weeks.

Reaction/

Reaction of Isocolumbin with Mercuric Acetate.[98]

Isocolumbin (140 mg.) in aqueous methanol (2 ml.) was treated with mercuric acetate (100 mg.) at room temperature. Isocolumbin was recovered unchanged after standing at room temperature for 1 day.

Reaction of Dihydroisocolumbin with Dichlorocarbene[94]

Dihydroisocolumbin (100 mg.) in 1 : 2 dimethoxyethane (10 ml.) was treated at reflux with sodium trichloroacetate a) (52 mg.) b) (259 mg.) for 18 hours. The solvent was removed in vacuo at room temperature and the residue taken up in methylene chloride, in both cases dihydroisocolumbin was recovered unchanged. (The generation of dichlorocarbene was detected by the presence of chloride ion in the reaction mixture.)

Reaction of Isocolumbin with Dichlorocarbene[94,95]

a) Isocolumbin (100 mg) in 1 : 2 dimethoxyethane (10 ml.) was treated at reflux with sodium trichloroacetate (104 mg.) overnight. Work up as before gave unchanged isocolumbin (90 mg.).

b) Isocolumbin (100 mg.) in 1 : 2 dimethoxyethane (10 ml.) was stirred with sodium methoxide (2 moles) and methyl trichloroacetate (2 moles) at room temperature overnight. The solution was acidified and methylene chloride extraction gave isocolumbin (70 mg.)

Ozonolysis of Dihydrocolumbin.

Dihydrocolumbin (2.5 gms) in chloroform (75 ml.) was treated with ozone at 0° for 8 hours. Water (12 cc) was added and the mixture stood at room temperature overnight. The solid material (1.05 gms.) was filtered and crystallisation from ethyl acetate - petrol gave the acid (VI; R = OH) m.p. 230 - 3°.

Ozonolysis of Dihydroisocolumbin.

Dihydroisocolumbin (1.3 gms) in ethyl acetate (500 ml.) was treated with ozone for 9 hours at - 70°. The solution was evaporated to ca 200 ml. and water (10 ml.) added./

added. The remaining ethyl-acetate was removed and the oily material extracted with sodium bicarbonate giving (on acidification) the acid (VI; R = OH, $C_{(8)}$ α H) (800 mg.). On crystallisation from ethyl acetate - petrol this gave material m.p. 251 - 3°, $[\alpha]_D$ - 12° (C, 1.2 $CHCl_3$) (Found: C, 60 : 4; H, 6.8. $C_{17}H_{22}O_7$ requires C, 60.3; H, 6.55%).

Attempts at Forming Derivatives of the Trisnor Acids from the Ozonolysis of Dihydro - and Dihydroisocolumbin.

1. p - iodophenacyl esters[97]

(a) The acid (VI: R = OH, $C_{(8)}$ α H) derived from dihydroisocolumbin, (100 mg., m.p. 251 - 3°) in acetone (10 ml.) was shaken overnight with p - iodophenacyl-bromide (100 mg.) and an excess of potassium carbonate. The solution was filtered and starting acid recovered in high yield together with the alcohol derived from the phenacylbromide.

(b) The acid (VI; R = OH, $C_{(8)}$ α H) in acetone - methanol (6 ml., V : V; 1 : 1) was treated with 1 equivalent of sodium hydroxide. The salt was heated on the steam bath for 2 hours with p - iodophenacyl bromide (100 mg.) in acetone (4 ml.). The solvents were removed giving the starting acid in high yield.

(c) The acid (VI; R = OH, $C_{(8)}$ α H) in methanol (4 ml.) with 1 equivalent of aqueous sodium carbonate was refluxed overnight with 1 mole of p - iodophenacyl-bromide. The acid was recovered unchanged.

2. The acid (VI; R = OH), derived from dihydrocolumbin, on treatment as in 1(a), (b) and (c) was also recovered unchanged.

3. Rubidium Salts.

(a) The acid (VI; R = OH), derived from dihydrocolumbin, (20 mg.) in aqueous methanol (5 ml.) was treated with 1 mole of rubidium carbonate. The solvents were removed and the residue gave the rubidium salt as very fine needles m.p. 180 - 5°. This material could not be obtained in a different crystalline form from any solvent or solvent mixture tried.

(b) /

(b) The acid (VI; R = OH, $C_{(8)}$ α H), derived from dihydroisocolumbin, on treatment as above gave the rubidium salt m.p. 190 - 3° as very fine needles.

4. Bromoanilides.

(a) The acid (VI; R = OH) (100 mg.) in aqueous ethanol was treated with 1 mole of sodium bicarbonate. The solvents were removed and the residue dried by azeotroping with dry benzene. The sodium salt was suspended in benzene and a trace of pyridine added. The mixture was treated overnight at room temperature with an excess of oxalyl chloride. The solvents were removed and the residue, in benzene, was treated with an excess of m - bromoaniline overnight. Water was added and ethyl acetate extraction, followed by washing with hydrochloric acid and aqueous sodium bicarbonate gave the m - bromoanilide (85 mg.). Crystallisation from ethyl acetate - petrol gave material m.p. 228 - 30° as stout prisms, $[\alpha]_D$ + 21° (Found: C,56.35; H, 5.95; N, 2.9. $C_{23}H_{26}O_6N$ Br requires C, 56.1; H, 5.35; N, 2.85%)

(b) Reaction as in (a) above but using p - bromoaniline gave small amounts of the anilide as fine needles m.p.220 - 5°.

V. REFERENCES.

IV A HEAVY ATOM DERIVATIVE OF COLUMBIN.

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