A T H E S I S

entitled

"STEREOCHEMICAL STUDIES IN THE TERPENOID FIELD"

submitted to the

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Faculty of Science

by

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CONTENTS.

SECTION I

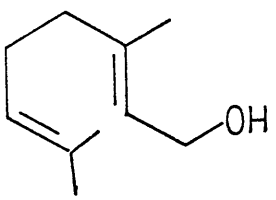
REVIEW of DITERPENOID BIOGENESIS

A review of Diterpenoid Biogenesis.

As this Thesis is principally concerned with diterpenoids of the labdane group, a short review of their biogenesis is in order.

Since the introduction of the term isoprene[1], the structures of a large number of terpenoids have been elucidated, and from consideration of these, it was gradually realised that these compounds were constructed according to a definite architectural plan. This plan is embodied in the Isoprene Rule[2] which states that terpenoid skeletons are obtained by condensation of two or more isoprene or isopentane units. This unity of structure based on a common isopentane building block implies an underlying unity in the biochemical transformations responsible for the synthesis of these compounds.

Ruzicka summarised his views on the interrelations in this group and proposed a generalisation called the "Biogenetic Isoprene Rule", This proposes that terpenoids are formed by preliminary condensations of isopentane (C_5) units to a few compounds; geraniol (1), farnesol (2), geranyl-geraniol (3(a)) and squalene (4), which subsequently cyclise and (where required) rearrange by accepted mechanisms to form the individual members of the mono-, sesqui-, di-, and tri-terpenoids (and sterols) respectively. This statement has formed the basis for the rationalisation of terpenoid biogenesis and has received much support from recent investigations.



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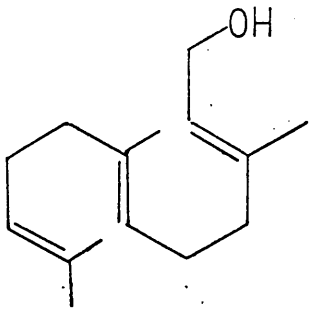

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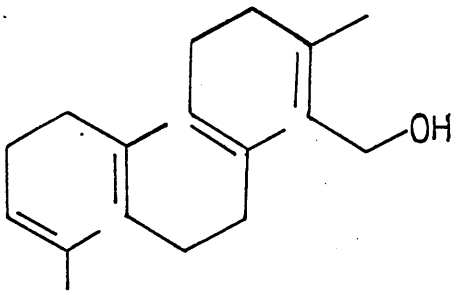

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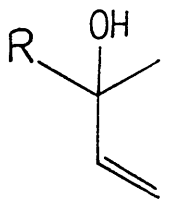

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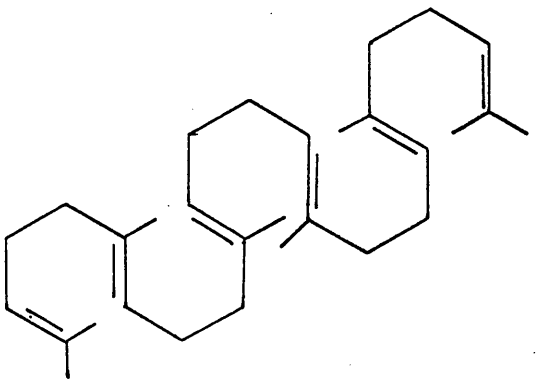

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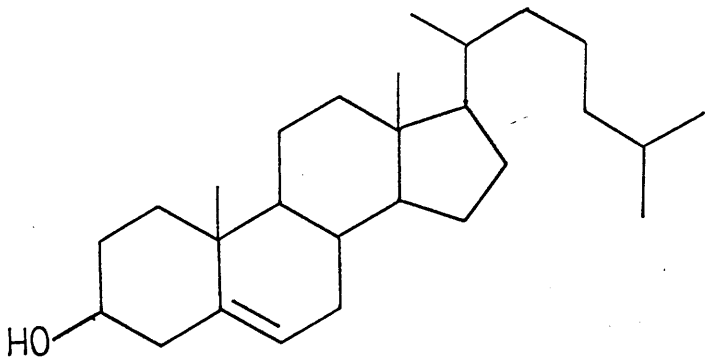
On the basis of the Isoprene Rule, the biogenesis of terpenoids has two problems; the mechanisms involved in the synthesis of the $C_{(5)}$ unit from small molecules such as acetate and the condensations of these C_5 units to the aliphatic terpenoid precursors eg. geraniol (**1**),farnesol (**2**), and the mechanisms of cyclisation and rearrangement of these molecules on their way to the final product.

Early work on sterols[3] indicated that acetate was a precursor of terpenoids and this has been confirmed by subsequent results obtained from the incorporation of C^{14} labelled acetate. Acetic acid has been shown by these means to be a carbon source for the biosynthesis of all the terpenoids investigated eg. geraniol[4], squalene[5], rosenonolactone[6], giberellic acid[7]. This work showed that both carbon atoms of the acetic acid molecule were incorporated and that to a great extent, the two acetic acid carbon atoms preserved their identity in the final structure. This can be seen in cholesterol (5) synthesised from labelled acetate[3], all the carbon atoms in this structure have been shown[3] to be derived from acetate. Study of the labelling pattern in the side chain and ring A showed that the molecular structure is based on a C_5 isopentane unit (6).

The nature of the isopentane unit and its synthesis from acetic acid posed great problems until the isolation[8] of β-hydroxy-β-methyl-δ-valerolactone (MVA) and the demonstration[9] that this substance was converted into cholesterol virtually



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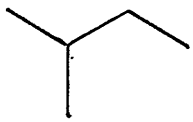

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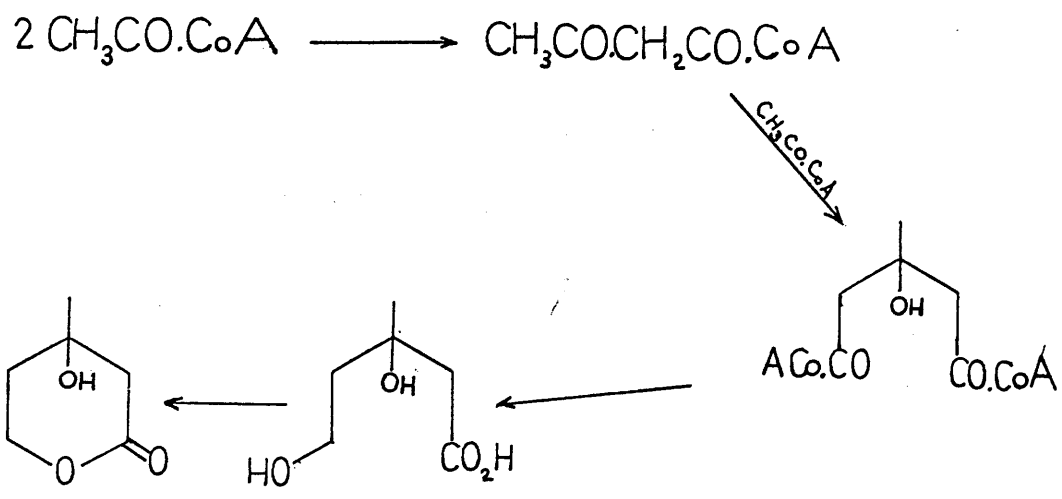
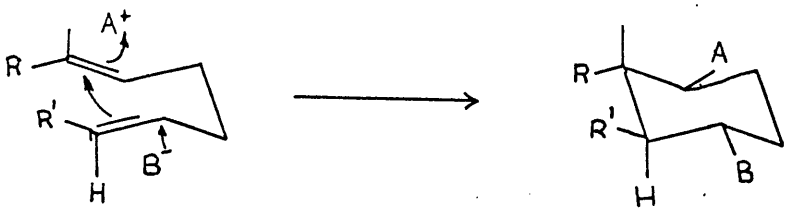
SCHEME I.



M.V.A.

quantitatively, much more efficiently than acetate. MVA which can be derived from acetyl coenzyme A ($CH_3CO.CoA$) as shown in Scheme 1, must be a direct precursor of the active isoprene unit. MVA decarboxylates to a C_5 unit during its conversion into cholesterol, this C_5 unit having the required distribution of atoms. It has been shown[10-12] that the transformation of MVA into terpenoids by cell extracts depends on the presence of ATP, Mn^{2+} or Mg^{2+} and reduced pyridine nucleotides. This led to a search for phosphorylated intermediates and culminated in the determination of the exact nature of the C5 unit. The steps towards formation of the active isoprene unit are shown in Scheme 2. MVA is first phosphorylated to mevalonic acid-5-phosphate(PMVA) then to mevalonic acid-5-pyrophosphate(PPMVA) which decarboxylates to form isopentenyl-pyrophosphate(IsPP)[10,11,13]. IsPP on isomerisation forms dimethyl-allyl pyrophosphate(DmalPP)[14]. IsPP and DmalPP are the active isoprene units of biological systems which can condense to form polyisoprenoid compounds e.g. farnesol(2), squalene(4).

Since both IsPP and DmalPP are required for polyisoprenoid formation, Lynen and Bloch[11,12,14] postulated the mechanism shown in Scheme 3 for the synthesis of farnesyl pyrophosphate (FaPP). The elimination of protons in this mechanism may be concerted and need not proceed via carbonium ion formation. Extending this mechanism, a molecule of FaPP and its isomer nerolidol pyrophosphate(3b) condense to form squalene(4).



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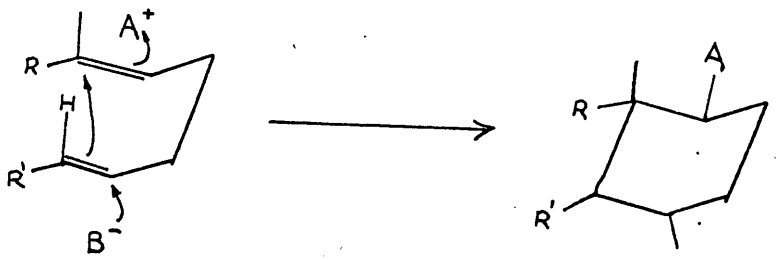

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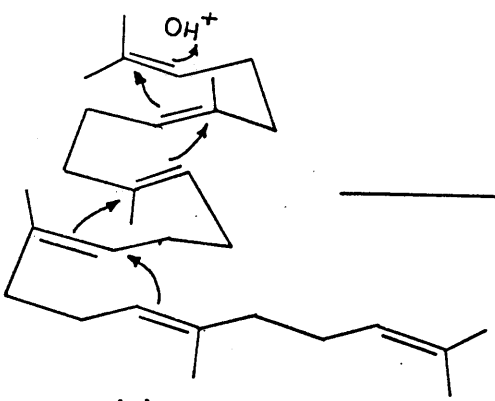

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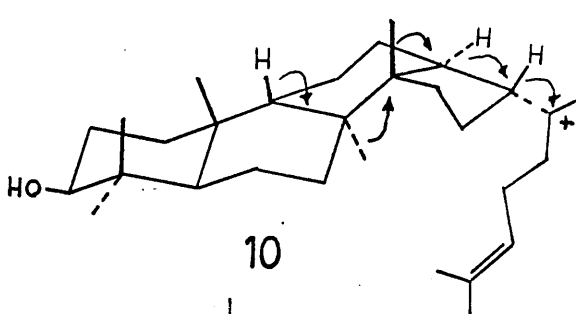

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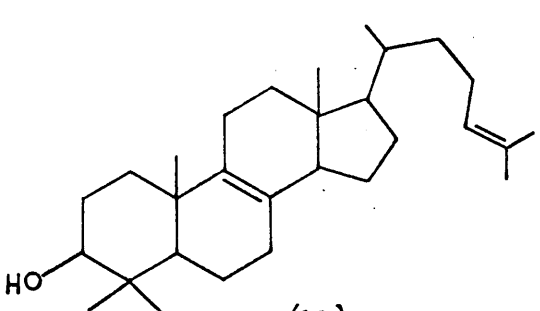
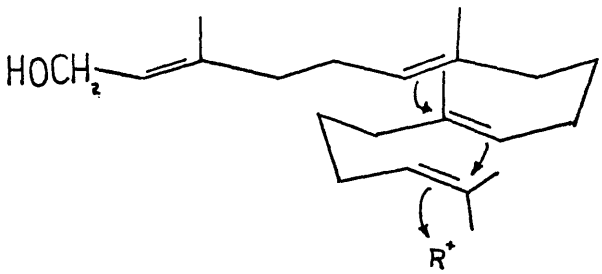


(11)

Squalene (4) cyclises to form eventually cholesterol.[15][16] Cornforth and his associates showed[17,18], by experiments using labelled MVA, that the rearrangements occurring during the formation of cholesterol followed a 1,2 mechanism.

In contrast to the cyclisation of squalene, no detailed study of the condensation of geranyl geraniol (3a) has been performed, but from the experimental evidence existing[19][20], it appears that geranyl geraniol or its isomer geranyl linalool[21] (3b) is the precursor of diterpenoids. The cyclisation of squalene was considered to occur by a trans, anti-parallel mechanism such as is shown in (7) or (8) where the polyisoprenoid is folded (a) in the chair (b) in the boat conformation. When squalene is folded in the chair-boat-chair-boat (9) conformation it cyclises to (10) by a series of trans, anti-parallel condensations which, by trans, anti-parallel concerted 1,2 - shifts of hydride and methyl groups, rearranges to lanosterol (11). By analogy with these cyclisations, geranyl-geraniol adopts the conformation shown (12) which, on cyclisation, yields the bicyclic carbonium ion (13a), all the newly formed bonds being parallel in accordance with the Isoprene Rule. The cyclisation must be initiated by protonation of the 3,4-double bond followed by concerted trans, anti-parallel condensations to form the trans-decalin carbonium ion (13). This carbonium ion can be neutralised by protonation with a solvent molecule, 1,2-hydride shift or proton elimination. These



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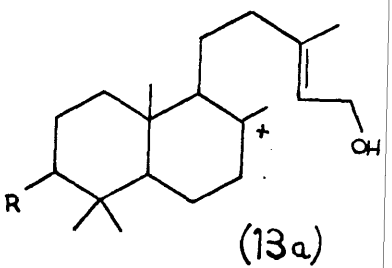
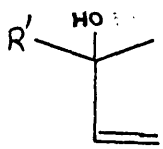


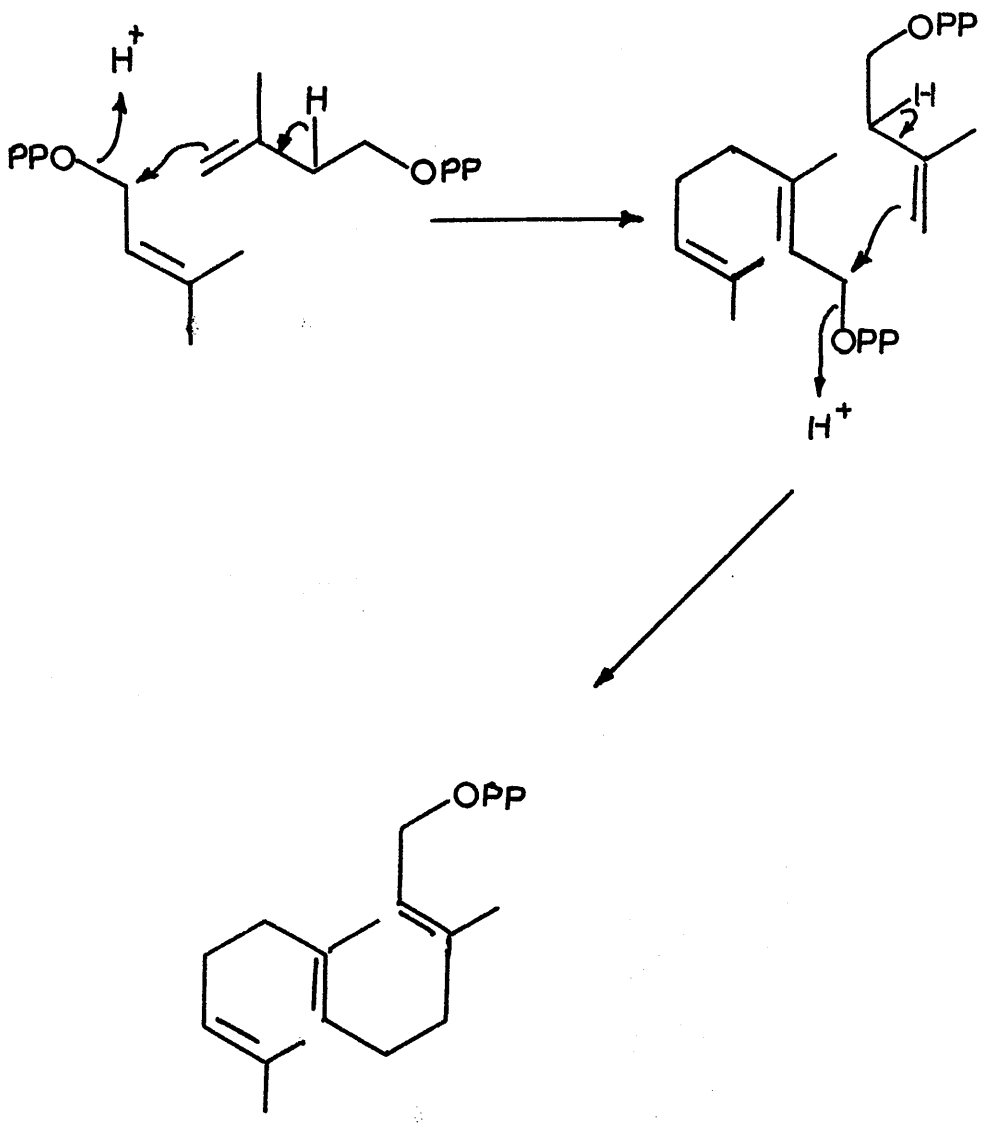
(13a)

OR



(13b)

Scheme 3.

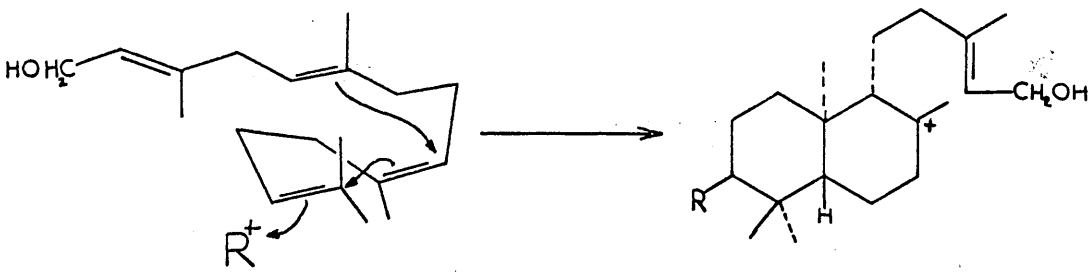


(FaPP)

processes which can be accompanied by allylic shift of the initially formed primary alcohol (13) produce the various types of labdane diterpenoids. From this labdane skeleton, by a further series of condensations and when necessary rearrangements, the various types of diterpenoids can be produced Scheme 4.

Diterpenoids produced by cyclisation of geranyl-geraniol or geranyl-linalool folded in the conformation (12) should possess a trans-anti-trans arrangement predicted by the Isoprene Rule, and in all authenticated cases the stereochemistry at $C_{(5)}$, $C_{(9)}$ and $C_{(10)}$ is in agreement with this prediction. This trans-anti-trans stereochemistry with $C_{(10)}$ β-methyl group is the "normal" stereochemistry. Antipodal compounds are found in the diterpenoid series having the "unnatural" $C_{(10)}$ α-methyl configuration as opposed to the normal $C_{(10)}$ β-methyl configuration. The "unnatural" stereochemistry is formed from cyclisation of geranyl-geraniol folded in the conformation (14). Diterpenoids found in the labdane group with this "unnatural" stereochemistry include: andrographolide[22-24], eperuic acid[25](16), polyalthic acid[26], copalic acid[27] and daniellic acid[28].

A small number of diterpenoids in the labdane group were considered to have a trans-syn arrangement of $C_{(5)}$, $C_{(9)}$ and $C_{(10)}$ (15a) in contradiction with the Isoprene Rule, but all have now been shown to possess the orthodox trans-anti pattern (15b). The compounds in this small group included cafestol[29], kahweol[29], gibberellic acid[30], eperuic acid[25] and rimuene[31].



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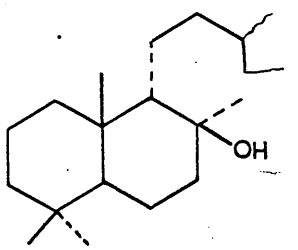
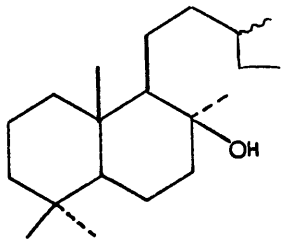
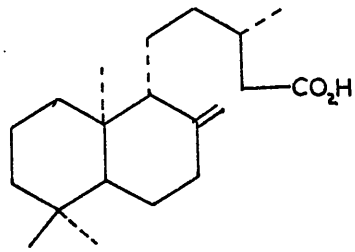


(a)



(b)

(15)



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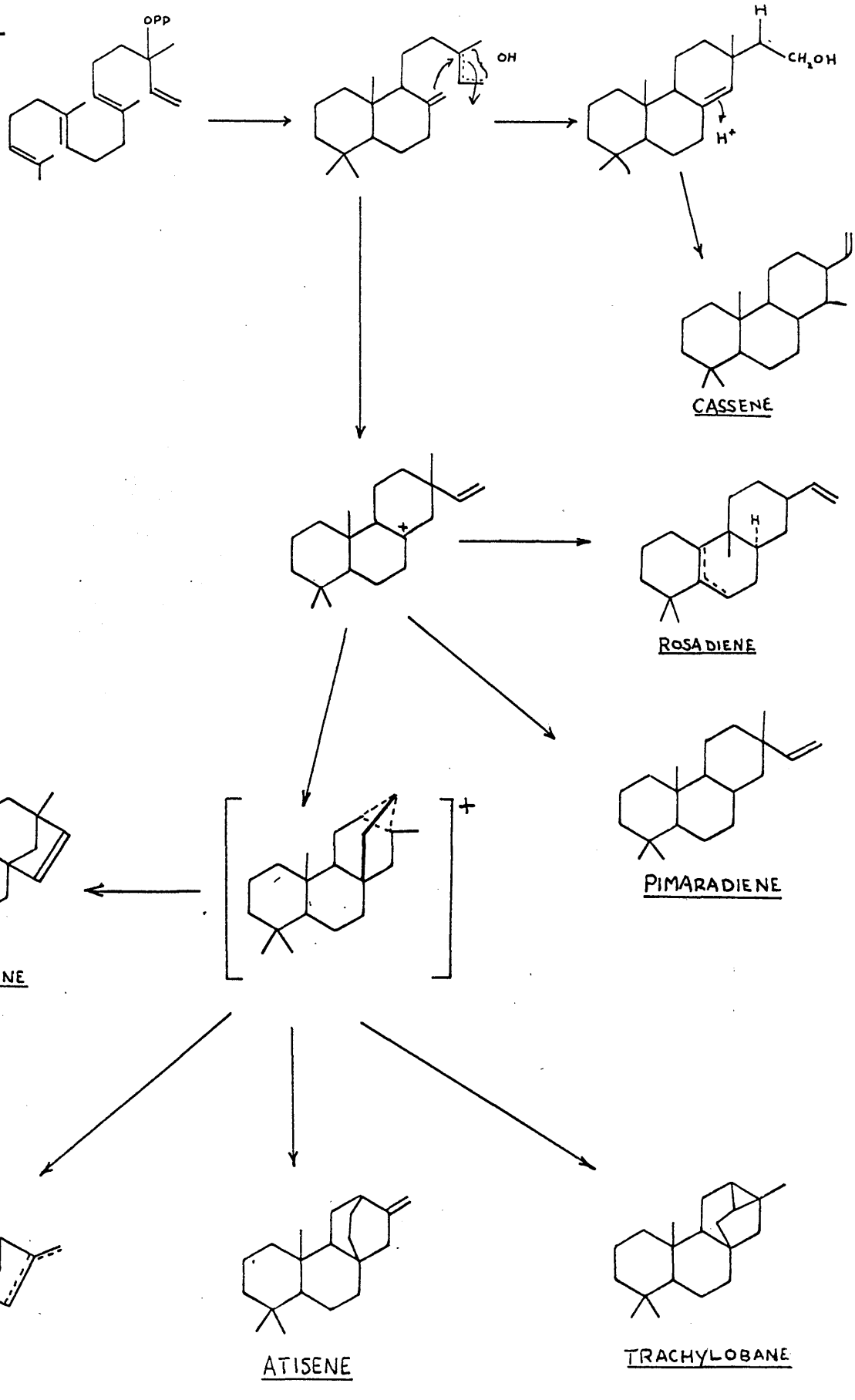
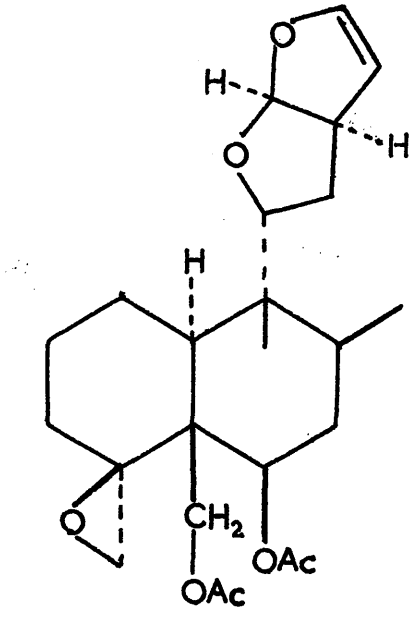
SCHEME 4.


OPP
OH
H
CH₂OH
H⁺
CASSENE
H
ROSADIENE
PIMARADIENE
STACHENE
ATISENE
TRACHYLOBANE
KAURENE

Although the stereochemistry at $C_{(5)}$, $C_{(9)}$ and $C_{(10)}$ in the labdane group is defined by the mode of cyclisation of geranyl-geraniol, the stereochemistry at $C_{(13)}$ is not. Where the double bond at $C_{(13)}$ has been reduced, as in labdanolic acid, or the allylic alcohol has isomerised, as in the case of manool, $C_{(13)}$ can adopt either the R or the S configuration. Examples of both configurations have been found and labdanolic acid and eperuic acid provide a good example of the non-definition of $C_{(13)}$ stereochemistry by cyclisation. Eperuic acid was considered to be antipodal to labdanolic acid at all centres except $C_{(9)}$. Overton and Graham[25] however, showed that these acids were antipodal at all centres except $C_{(13)}$. Although Bigley, Rogers and Barltrop[32] assigned the R-configuration to $C_{(13)}$ in labdanolic acid, their conclusion was based on certain erroneous assumptions and hence the configurations at $C_{(13)}$ in labdanolic and eperuic acids was unresolved. Chemical[33] and X-ray crystallographic[34] work has established the configuration at $C_{(13)}$ in labdanolic acid as R.

Other examples of $C_{(13)}$ epimers are manool (R) and 13-epi-manool (S); eperuic acid (S) and 13-epi-eperuic acid (R).

The regular labdane skeleton can undergo rearrangement to produce structures like clerodin (17), cascarillin (18) and pleuromutilin (19). Cascarillin and clerodin are of interest in connection with the colombo root bitter principles since their structures contain certain stereochemical features which



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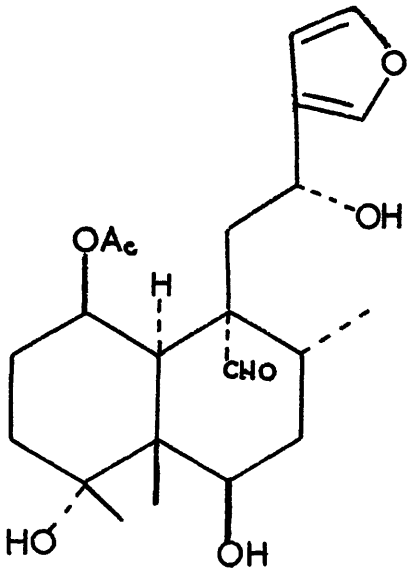
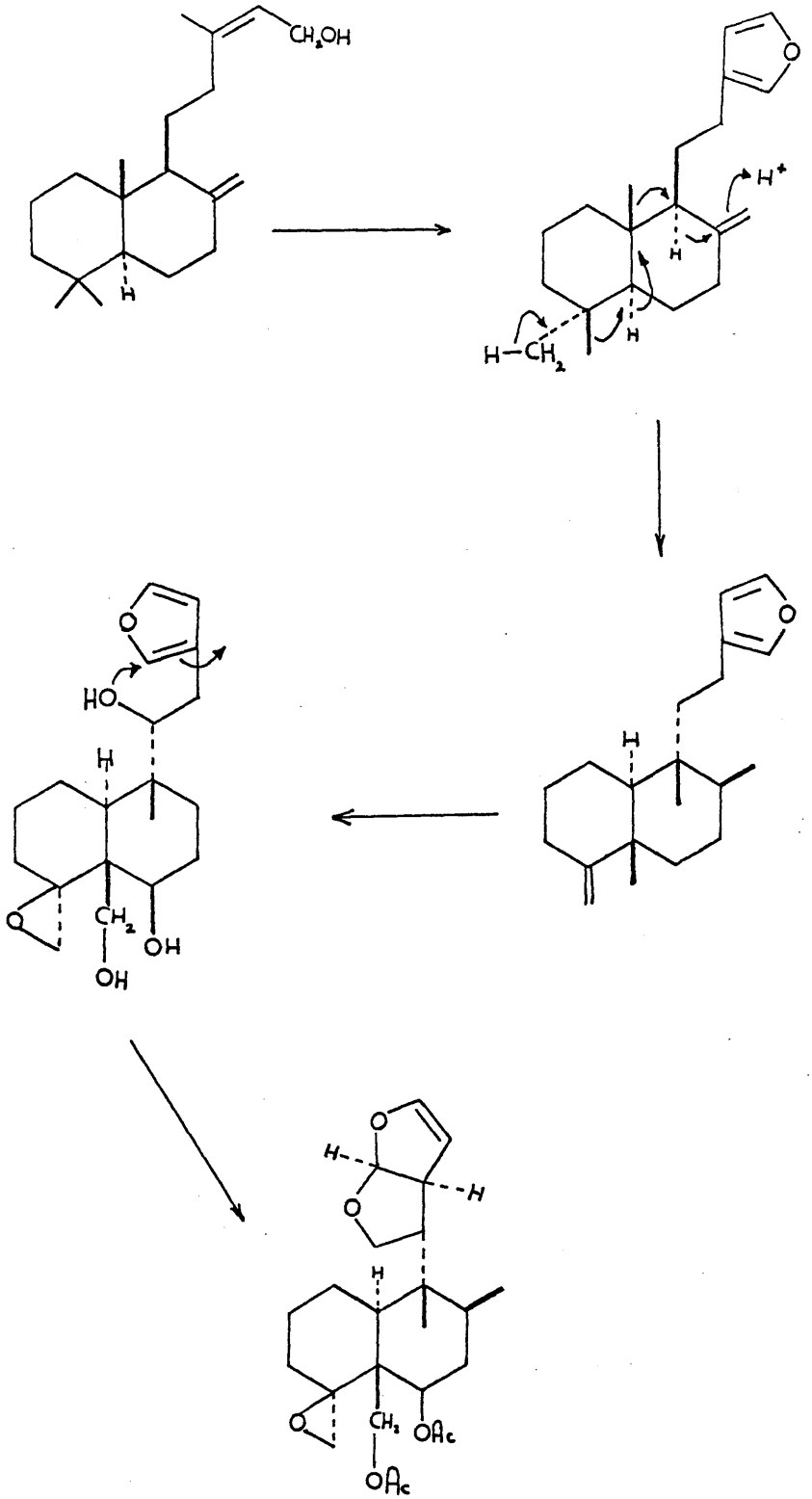


(18)

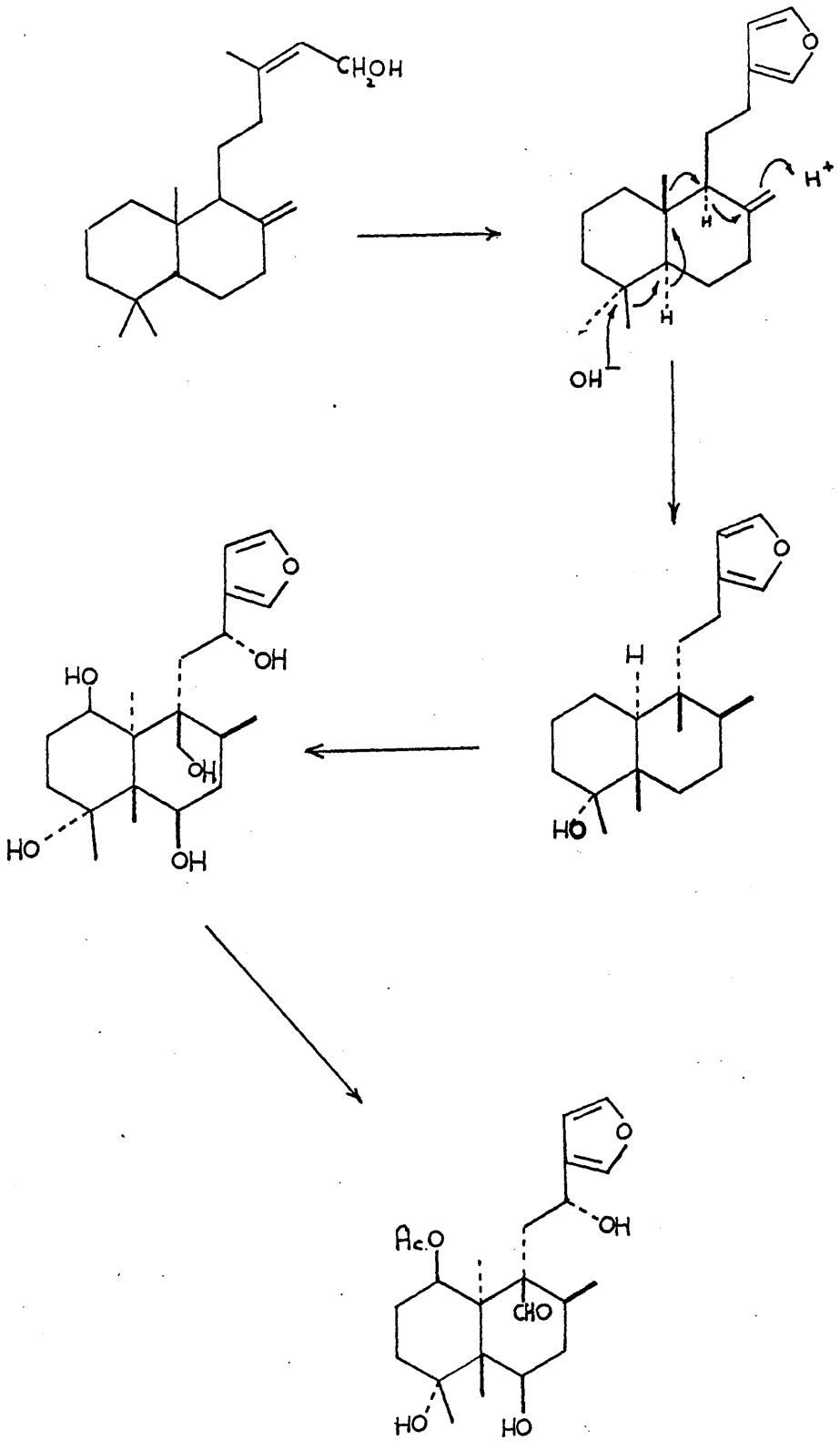
must represent intermediate stages in the rearrangement of the labdane to the columbin skeleton. Protonation of the 8,20 double bond would initiate concerted 1,2-shifts of hydride from $C_{(5)}$ to $C_{(10)}$ and a methyl group from $C_{(4)}$ to $C_{(5)}$. The resulting carbonium ion can be neutralised by a solvent molecule to form an -hydroxy group. Since the 1,2-shifts occur with inversion in each case, the "normal" stereochemistry of trans-anti-trans is maintained. A scheme indicating possible biogenetic routes to cascarillin and clerodin is shown in Route 1 and Route 2 respectively.

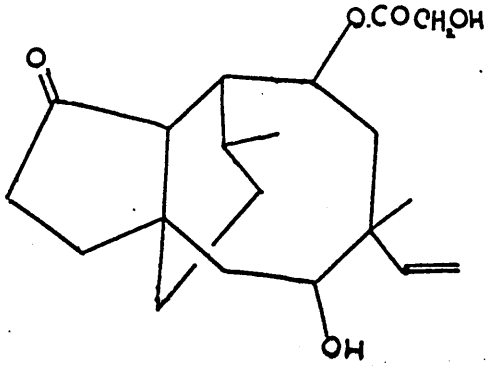
In many cases in the labdane group, the terminal four atoms in the side chain form a β -substituted furan ring, e.g. marrubiin[35], columbin[36], cascarillin[37], sciadin[38], daniellic acid[28] and polyalthic acid[26]. The dihydro furan ring of clerodin (17) and the butenolide system in andrographolide are interesting variants to the furan ring. It has been suggested[39], that rearrangement of the allylic alcohol (20) to (21) with epoxidation and oxidation to (22) with further rearrangement to (23) produces a furan ring as in (24). According to the above mechanism only β -substituted furan rings are expected in terpenoids derived from the labdane skeleton and all known cases comply with this.

Route 1



Route 2





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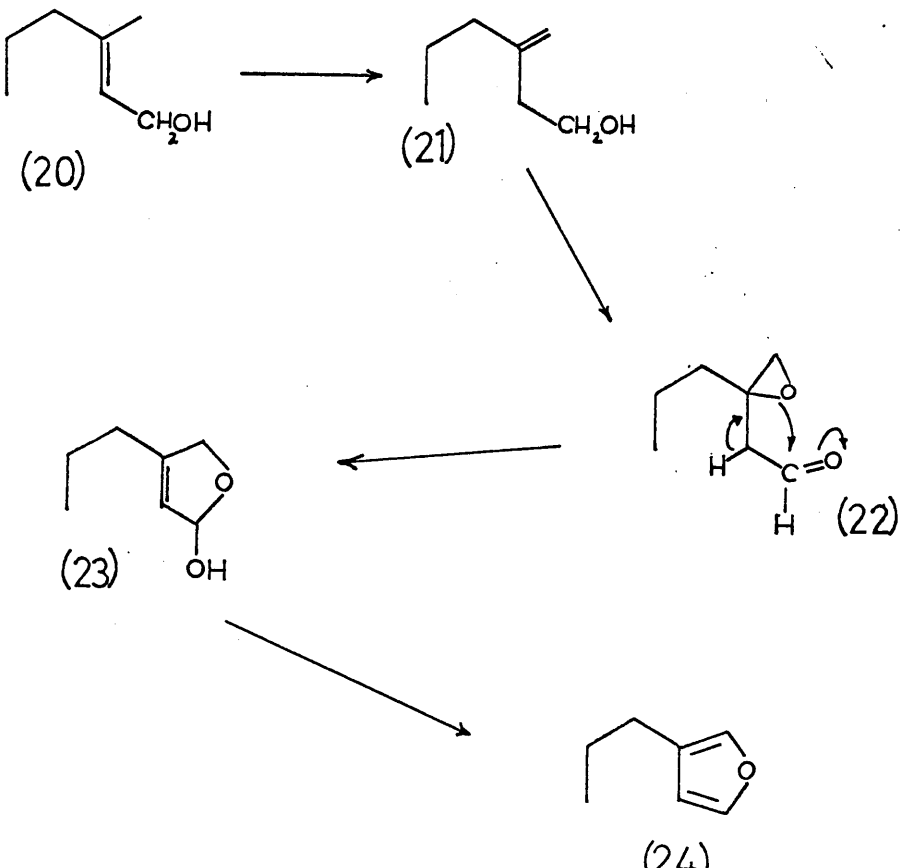


(20) (21) (22) (23) (24)

SECTION II

DETERMINATION

of the

STEREOCHEMISTRY OF COLUMBIN

by

X-RAY METHODS.

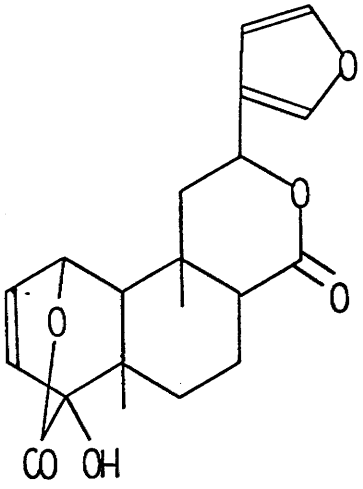
INTRODUCTION:

The root of Jateorrhiza palmata Miers (Colombo Root) yields four neutral bitter principles viz: columbin, chasmanthin, jateorin and palmarin. Of these columbin is the most abundant. Mild alkaline treatment of columbin, chasmanthin and jateorin yields respectively iso-columbin, palmarin and iso-jateorin; thus the palmarin found in the root extract may be an artefact arising during the extraction process.

Prolonged investigations by Wessley[40-45] and Feist[46-51] and their associates did not lead to agreement on the functional groups of these compounds. Since the colombo root bitter principles have such strong structural resemblances, they have the unfortunate ability to form mixed crystals which makes them extremely difficult to obtain in a pure form by crystallization Much of this early work must have been performed on mixtures which would account for the misleading results obtained.

Wessley first obtained a pure sample of columbin (25) and proposed its composition as $C_{20}H_{22}O_6$. The work done by Wessley and Feist showed that columbin has two lactone rings (one of which opens by hydrolysis to a lactone hydroxy acid and by hydrogenolysis to a desoxy-acid), one tertiary, slightly acidic hydroxyl group and three double bonds.

Barton[52] showed that lactone ring A was of the form (26) and that lactone ring C had the part structure (27). Cava [53] also postulated (26) for ring A.



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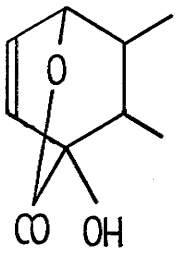

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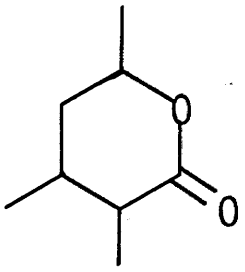

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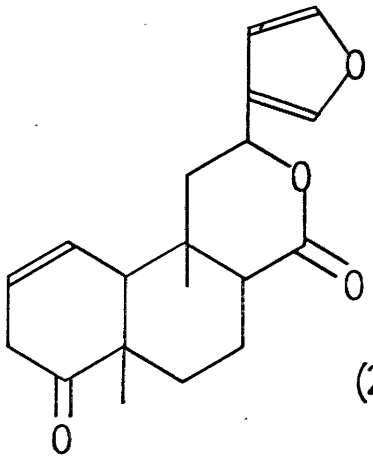


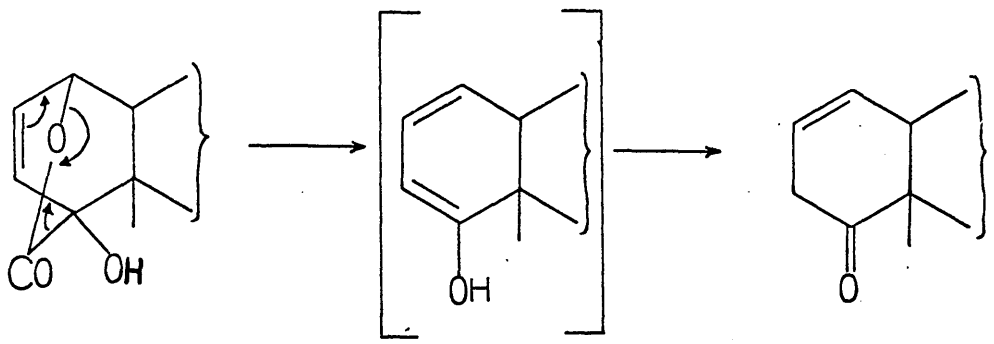
(28)

On melting, columbin decarboxylates to a non-conjugated enone which has lost the tertiary hydroxyl group (28). After hydrogenation, this ability to decarboxylate disappears and the remaining lactone cannot be decarboxylated, hence the lactone system must be altered by hydrogenation. The disappearance of the hydroxyl group and the ease of decarboxylation can be explained by postulating structure (26). A mechanism for decarboxylation was suggested by Barton [54] as (29) which could not function after hydrogenation of the double bond.

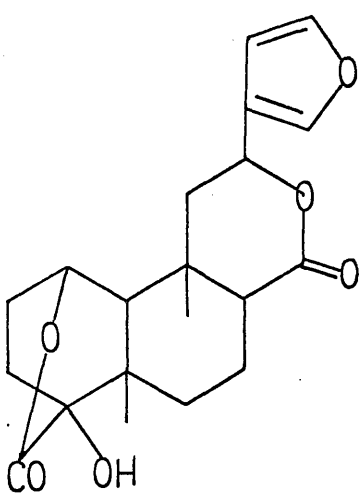
Ozonolysis of dihydro-columbin yields a C_{17} acid (30) and a C_{18} keto-acid (31) which also contains the lactone systems. The formation of these acids can be explained by postulating a β-substituted furan ring and this is supported by spectroscopic data (ν max 1510cm^{-1}, 890cm^{-1}) and (3.52 (1β-furyl proton), 2.60τ (2α-furyl protons)).

Hydrogenation of dihydro-columbin yields an octahydro-acid ($C_{20}H_{30}O_6$) (32) and a hexahydro-lactone ($C_{20}H_{28}O_6$) (33) showing that in the former, hydrogenolysis of one of the lactone rings has occurred. Hydrogenation of decarboxy-columbin (28) also yields an octahydro-mono-acid ($C_{19}H_{30}O_4$) (34) showing that lactone A is not involved in this hydrogenolysis. The alkyl oxygen atom of ring C lactone is therefore probably attached allylically to the β-substituted furan.

The presence of a decalin system in columbin is indicated by the degradation of columbin on zinc dust distillation to 1,2,5- trimethyl naphthalene. Selenium dehydrogenation of



(29)



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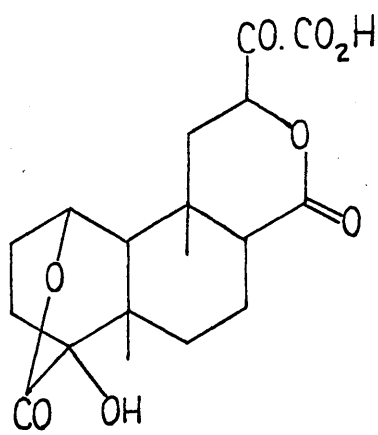

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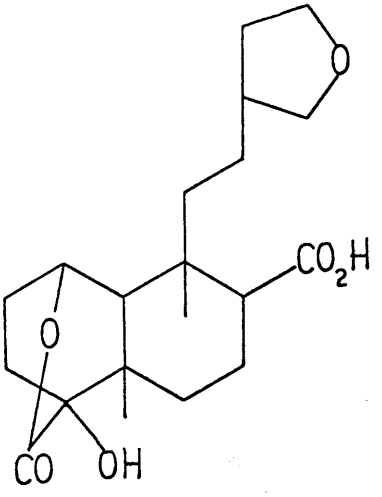

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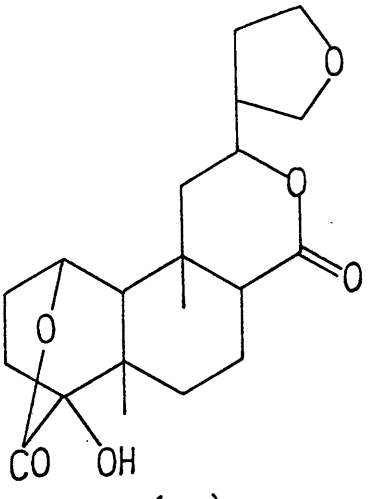

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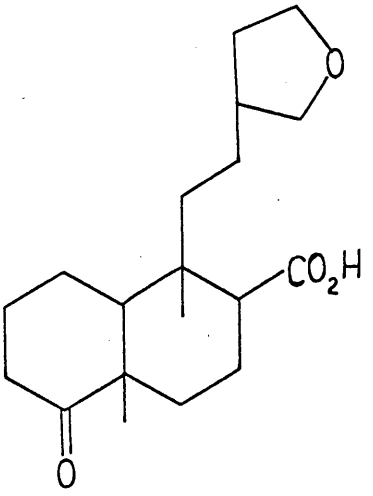
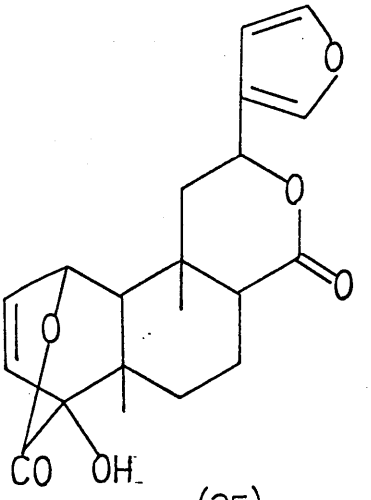


(34)

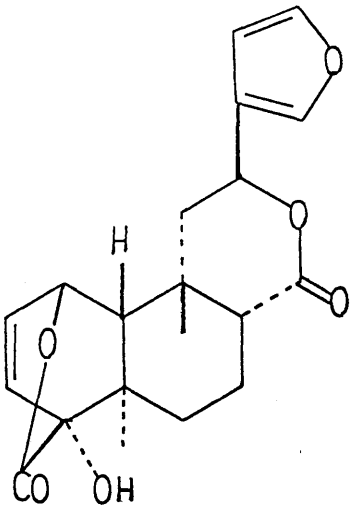


(35)

decarboxy-columbin[52] gives the same degradation product but if the ketone group of decarboxy-columbin is removed by Wolff-Kischner reduction, the product of selenium dehydrogenation is 1-methyl-2-naphthoic acid. The position of the carboxyl group in 1-methyl-2-naphthoic acid fixes the position of the ring C δ-lactone carbonyl group at $C_{(8)}$. When the ketonic group of decarboxy-columbin is reduced to the alcohol and this compound dehydrogenated with selenium, the product is 1,2,5-trimethyl naphthalene. Hence, the methyl group at $C_{(5)}$ of 1,2,5-trimethyl naphthalene, must have arisen from migration of the methyl group, on a neighbouring quaternary centre, to the point of attachment of the hydroxyl group. The tertiary hydroxyl group and hence the ketone group of decarboxy-columbin must be at $C_{(4)}$ with a quaternary methyl group at $C_{(5)}$.

Kuhn-Roth oxidation indicates the presence of two methyl groups in columbin. One methyl group is located at $C_{(5)}$ as shown above, and the other methyl group must be at $C_{(9)}$ to explain satisfactorily the dehydrogenation products obtained. From the above evidence, columbin was formulated as (35).

From speculations on the optical rotatory dispersion curve of octahydro-decarboxy columbinic acid (34), Cava et al[55] postulated the stereochemistry of columbin as (36), with a <u>trans</u> A-B ring junction. Overton et al[36], however, provided chemical, spectroscopic and optical rotatory dispersion evidence that rings A and B of columbin are <u>cis</u> fused and that the absolute stereochemistry is as in (37).



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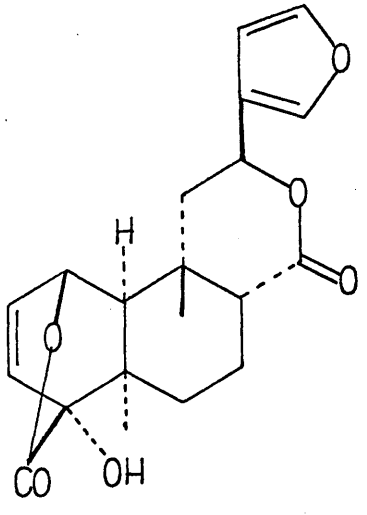
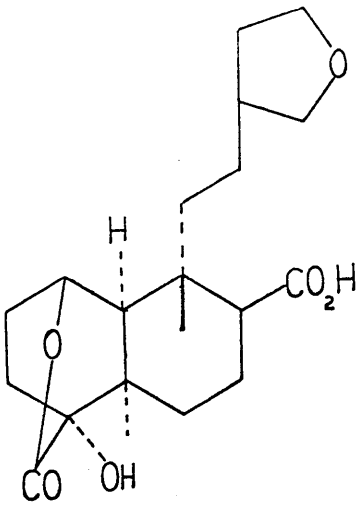


(37)

The work of Overton et al[36] involved hydrogenation of iso-columbin which is obtained by mild alkaline treatment of columbin. The columbin to iso-columbin isomerisation was postulated[54] as being merely epimerisation at $C_{(8)}$. Hydrogenation of iso-columbin to octahydro-iso-columbinic acid (38) and mild alkali isomerisation gave an "iso-lactone" in high yield. This "iso-lactone" was formulated as (39). The corresponding octahydro-columbinic acid did not yield the corresponding iso-lactone, merely opening ring A lactone. The formation of the "iso-lactone" imposes stringent conditions on the stereochemistry of the octahydro acid from which it is formed. Rings A and B must be cis fused, with an α-methyl group at $C_{(5)}$, a β-methyl group at $C_{(9)}$ and an α-carboxyl group at $C_{(8)}$. The stereochemistry at $C_{(12)}$ follows from application of the Hudson-Klyne lactone rule[56] i.e. the furyl side chain is β-oriented.

The fact that rings A and B were cis fused was a startling result, since this was (and apart from the exceptional case of thelepogine, still is) the only known case of a diterpenoid having this unusual configuration.



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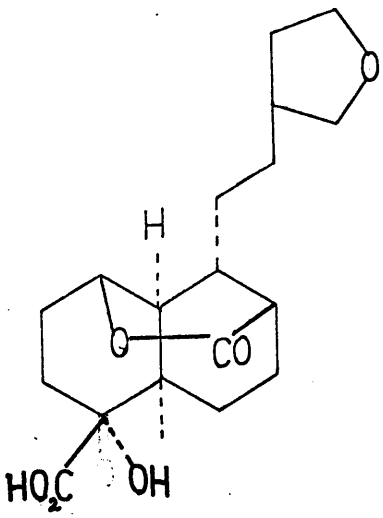


(39)

DISCUSSION.

The stereochemistry of columbin as shown in (37) has been derived[36] using organic chemical techniques, infra-red spectroscopy and the methods of molecular rotation differences and optical rotatory dispersion. The molecule has two interesting features in its cis fused rings A and B, and B and C. Although the arguments for this stereochemistry appeared sound, it was considered necessary to confirm them by an X-ray analysis of a suitable derivative, in view of the fact that a cis fusion of rings A and B had not previously been encountered in the diterpenoid field.

The functional groups in columbin and iso-columbin which are available for derivative formation are the disubstituted double bond in ring A, the tertiary hydroxyl group and the β-substituted furan ring. An X-ray analysis, using the "heavy" atom method, requires that the scattering of X-rays by the crystal be dominated by the "heavy" atom. Since the scattering factor of an atom is proportional to the square of the atomic number, a suitable derivative of columbin must contain a "heavy" atom of atomic number 35 or above. Thus,

$$(\text{A.No.``heavy'' atom})^2 > n_o\,(\text{A.No.}_o)^2 + n_c(\text{A.No.}c)^2 + n_h(\text{A.No.}_H)^2$$

e.g. Bromine $\quad (35)^2 > 6.(8)^2 + 20.(6)^2 + 22.(1)^2.$

Weir[58] made preliminary attempts to procure a suitable X-ray derivative. Since iso-columbin forms an acetate with acetyl chloride with ease, attempts were made to form an

iodo-acetate with the tertiary hydroxyl group. However, no ester formation occurred when chlor-acetyl chloride or β-bromo-propionyl bromide was used.

Derivatives of the double bond were attempted. Attempted bromo-hydrin formation using N-bromo-succinimide, failed; direct bromination also failed to produce a crystalline derivative.

Although osmates had not previously been used for X-ray analysis, osmium promised to be a most suitable "heavy" atom. Accordingly, formation of cyclic osmate esters, incorporating an organic base (40), was attempted, but in only two cases, namely α-and β-picoline were crystalline products obtained. The compound containing α-picoline lost solvent of crystallisation on standing in the atmosphere and the β-picoline compound could not be crystallised in a size suitable for an X-ray analysis. Formation of osmate esters incorporating organic bases other than those used by Weir[58], was attempted. The bases used were γ-collidine, 2-methyl-6-ethyl pyridine, 2,6-lutidene, quinoline and iso-quinoline. Only in the case of 2,6-lutidene was a crystalline product obtained. The material crystallised in the form of dark-red needles which were not sufficiently large for X-ray analysis. The other osmate esters precipitated as black amorphous solids but when crystallisation was attempted from a variety of solvents, no crystals were obtained.

From the osmylation of columbin a cis 2,3-diol is obtained and Weir[58] attempted to prepare cyclic acetals of the type (41),

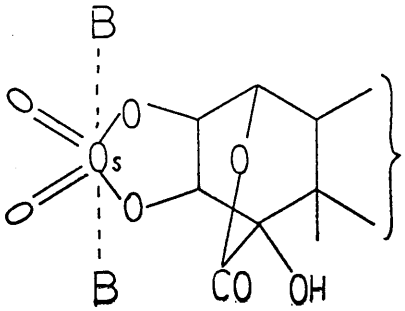
but failed to do so .Attempted esterification of the 2,3-diol with chlor-acetyl chloridealso failed and the action of dichlorocarbene on iso-columbin gave a good recovery of iso-columbin.

It was thought that if the furan ring of dihydro-columbin could be removed by ozonolysis to give a tris-nor acid(42), a bulky ester of this acid could preserve the conformation of ring C-δ-lactone. Consequently, the m-bromo and the p-bromo anilides were prepared. Only the m-bromo anilide crystallised in a suitable form, but owing to the space group (monoclinic, with four molecules per unit cell) this derivative was unsuitable because of symmetry problems.

The "heavy" atom salts of the tris-nor acid (rubidium and cesium) were attempted, but although a nicely crystalline rubidium salt was obtained, it proved to have unfavourable symmetry properties.

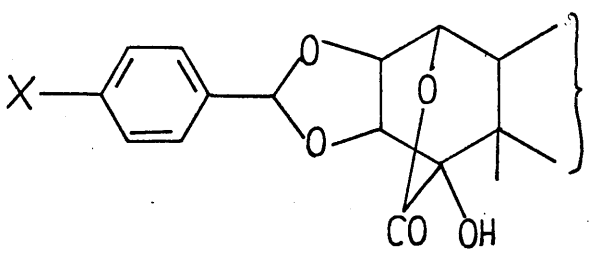
Since these attempts had failed we turned to 1,3-dipolar cyclo addition reactions. A large number of these reactions are known [59], and of this number, three types were selected viz: nitrile oxides [60], azomethine-imines [61] and nitrile imines [62]. Using these 1,3-dipolar cyclo additions derivatives could be prepared with the "heavy" atom distant from the diterpene part of the molecule so that the stereochemistry of the diterpene should not be affected by inclusion of bulky groups. The "heavy" atom used was either bromine or iodine.

Before adduct formation with columbin or iso-columbin was



(40)

B = an organic base



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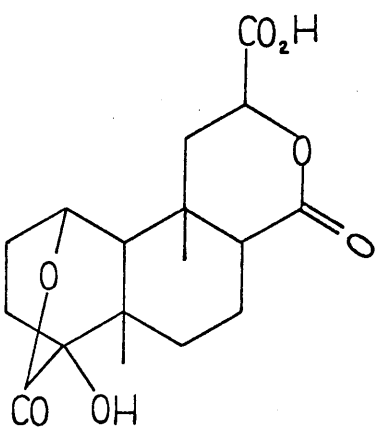
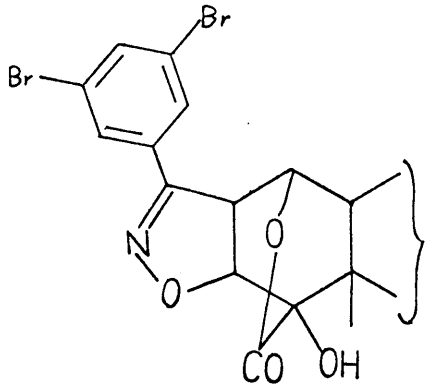


(42)

attempted, derivatives of model compounds norbornene or stilbene were prepared. The 1,3-dipolar reagents were generated from their precursors by tri-ethylamine. The first 1,3-dipolar cyclo-addition reaction attempted, used the nitrile oxide prepared as shown in Scheme 5 , by reduction of p -nitro-toluene (a) to p -amino benzaldehyde (b) which, on bromination and deamination, yielded 3,5-dibromobenzaldehyde (c). The oxime of (c) was chlorinated with chlorine gas to form the chlor-oxime (d) which, on treatment with tri-ethylamine, liberated the corresponding nitrile oxide. The nitrile oxide, in the presence of an olefin, yields an isoxazole derivative.

When a dilute solution of tri-ethylamine in dry tetrahydro-furan (THF) was added slowly to a stirred solution of the chlor-oxime and columbin, an isoxazole derivative was formed. Unfortunately, two such derivatives of columbin were produced, probably isomeric as indicated by Quilico[63], which were of almost identical polarity from thin-layer chromatography. Possibly these derivatives had the structures (43) and (44) or they were α and β isomers. The derivatives could not be separated even on careful thin-layer preparative chromatography. Iso-columbin reacted in a similar fashion, and in both cases the derivative mixtures crystallised in very fine, white needles.

Since the isoxazole derivates of columbin and iso-columbin crystallised as fine needles, it was thought that if the shape of the derivative molecule could be radically altered, the shape of the resultant crystals might be altered to one which would



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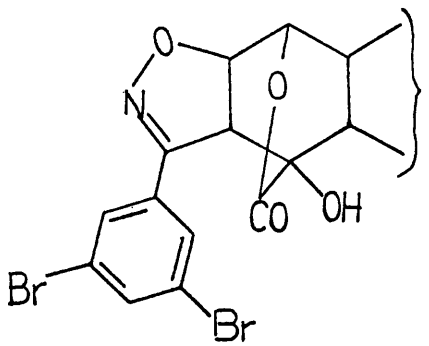

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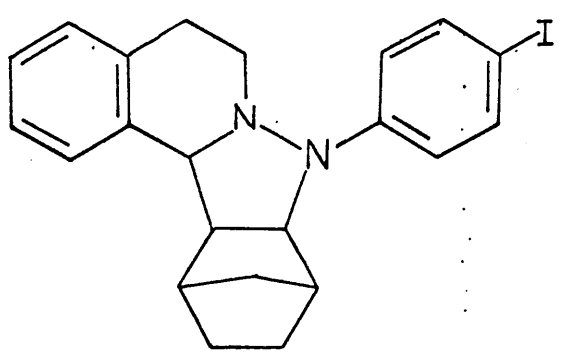


(45)

be more suitable for an X-ray analysis. Consequently, a derivative of the 3,4-dihydro-iso-quinoline azo-methine type[61,64] was attempted.

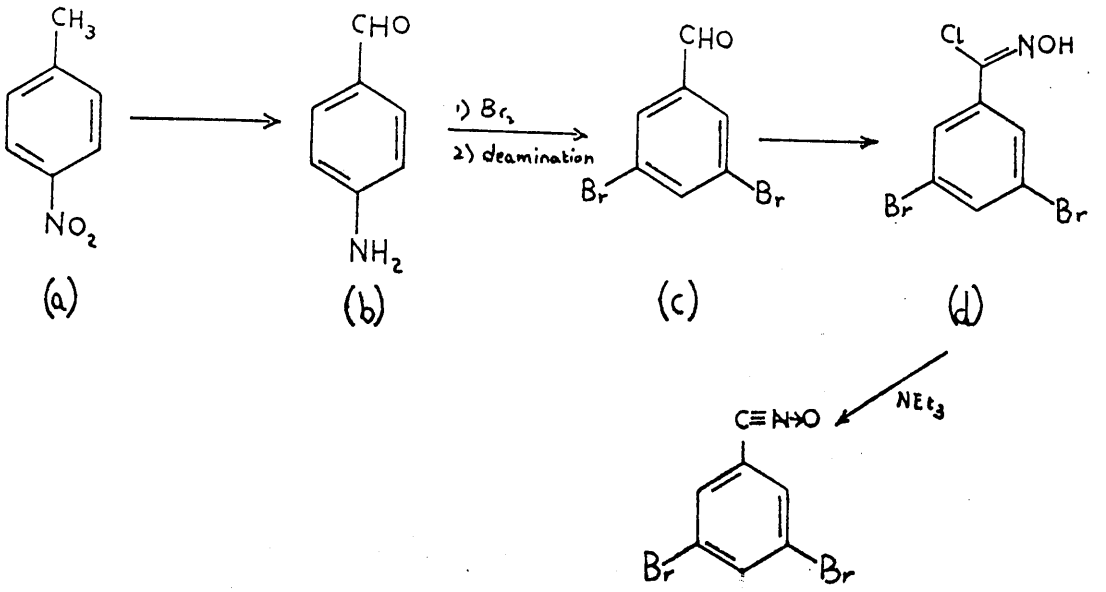
The azomethine imine was prepared as in Scheme 6, by bromination of iso-chroman[65] (b) to β-bromo-2-ethyl benzaldehyde[65] (c) the p-iodo phenyl hydrazone (d) of which, on heating at 180°C for ten minutes, cyclised to the 3,4-dihydro iso-quinoline azomethine imine[64] (e). Treatment of the azomethine dissolved in dimethyl formamide (DMF) with tri-ethyl-amine liberates the azomethine ylide, which forms a pyrazoline with olefins.

Although a pyrazoline derivative of this type was easily formed with nor-bornene (45) no derivative formation occurred with either columbin or iso-columbin. A white crystalline compound was isolated from the reaction mixtures, which appeared to be a condensation of two molecules of the azomethine ylide (only aromatic frequencies showing in the infra-red spectrum, but this was not investigated further.

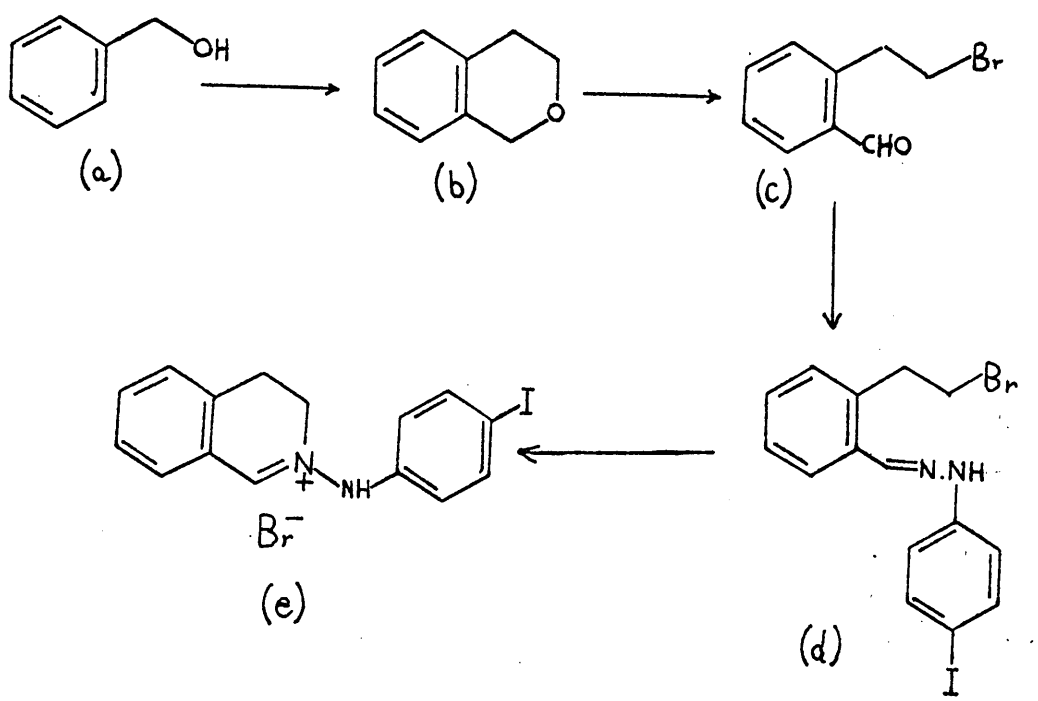
The third 1,3-dipolar cyclo-addition reaction to be tried was of the nitrile-imine type. Several of these reagents were prepared and most of them produced crystalline derivatives. The first type attempted was the diphenyl nitrile-imine (d) prepared as in Scheme 7, by treatment of β-benzoyl phenyl hydrazide (b) with phosphorus pentachloride[62].

On treatment of the α-chloro-benzoyl phenyl hydrazide (c) with tri-ethylamine, the zwitterionic compound (d) is liberated as

SCHEME 5.



SCHEME 6.



shown in Scheme 7, which formed a pyrazoline derivative easily with stilbene, columbin and iso-columbin at room temperature. These adducts, when run on a thin-layer chromatographic plate and sprayed with acidified ceric ammonium nitrate, showed a blue spot turning reddish brown, marking the position of the adducts. These compounds also fluoresced strongly in the ultra-violet and when preparative thin-layer chromatography was used, the positions of the bands were found from this fluorescence. The decomposition products from the reagent (c) Scheme 7 were either non-fluorescent or only weakly fluorescent and could easily be distinguished from the adducts which fluoresced bright blue.

The diphenyl pyrazoline adducts, at this stage, were not suitable for an X-ray analysis as they did not contain a "heavy" atom. Bromination of the diphenyl pyrazoline adduct with one mole of bromine in chloroform gave two products with virtually identical polarities on a thin-layer chromatographic plate. Lengthy attempts were made to separate this mixture using thick-layer and gradient elution chromatography, with little success. However, gradient elution chromatography over grade III neutral deactivated alumina, using benzene:ether = 1:1 added to benzene, as solvent, achieved a partial separation.

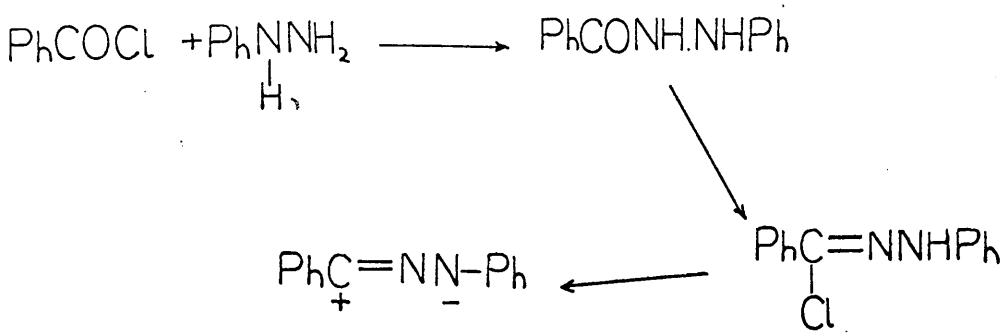
The more polar compound was unchanged adduct, the less polar compound contained bromine (Beilstein test). In an attempt to increase the yield of brominated adduct, the columbin diphenyl pyrazoline adduct was treated with two moles of bromine in chloroform; unfortunately a variety of products was produced.

The brominated adduct separated by gradient elution chromatography was crystallised as stout prisms from a mixture of ethyl-acetate-petrol but, unfortunately, on exposure to the atmosphere, lost solvent of crystallisation with consequent breakdown of the crystal structure. This compound was thus unsuitable for an X-ray analysis. Similar bromination of the iso-columbin diphenyl pyrazoline adduct appeared to give only one product as judged by thin-layer chromatography but, owing to symmetry problems, caused by the monoclinic space group of the crystal, this compound was unsuitable for X-ray analysis.

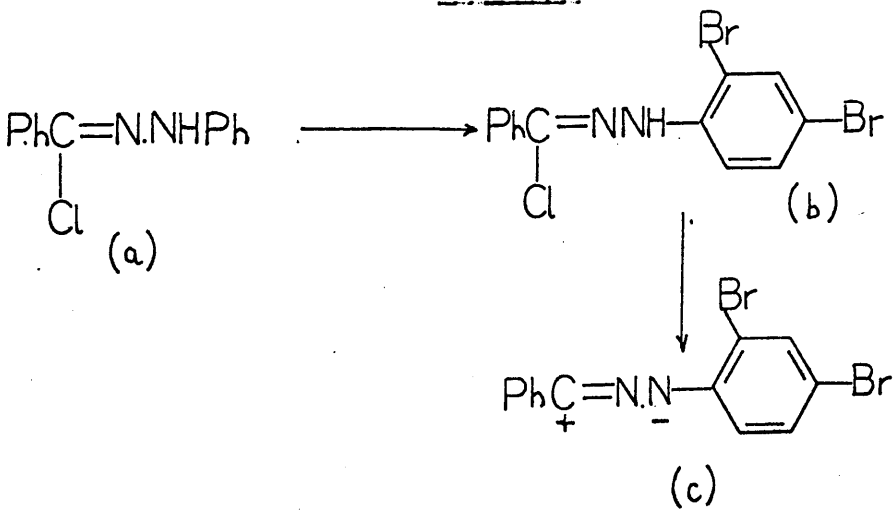
Since this type of 1,3-dipolar cyclo-addition yielded a single crystalline adduct so easily, further attempts were made to incorporate a "heavy" atom. A methiodide of the nitrogen in the pyrazoline ring was attempted[66] but no methiodide formation occurred. Using the benzene rings of the adduct, it was hoped to form a chromium carbonyl complex[67]. However, attempts to form this adduct failed, although some solid material was isolated which showed high carbonyl frequencies in the infra-red spectrum, indicative of a chromium carbonyl complex. On attempted crystallisation, this material decomposed.

As attempts to incorporate a "heavy" atom after formation of the pyrazoline ring had failed, the "heavy" atom was incorporated in the nitrile imine before cyclisation. Direct bromination of ∝-chloro-benz-phenyl hydrazide[68] with bromine in acetic acid yielded the di-brominated compound (b) as shown in Scheme 8. Unfortunately, this compound when treated with

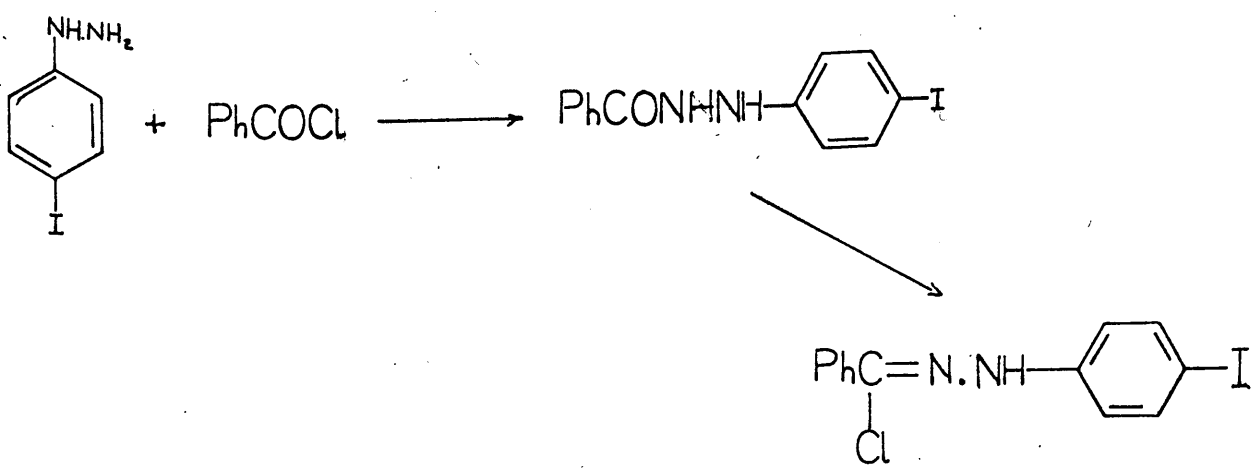
SCHEME 7.



SCHEME 8.



SCHEME 9.



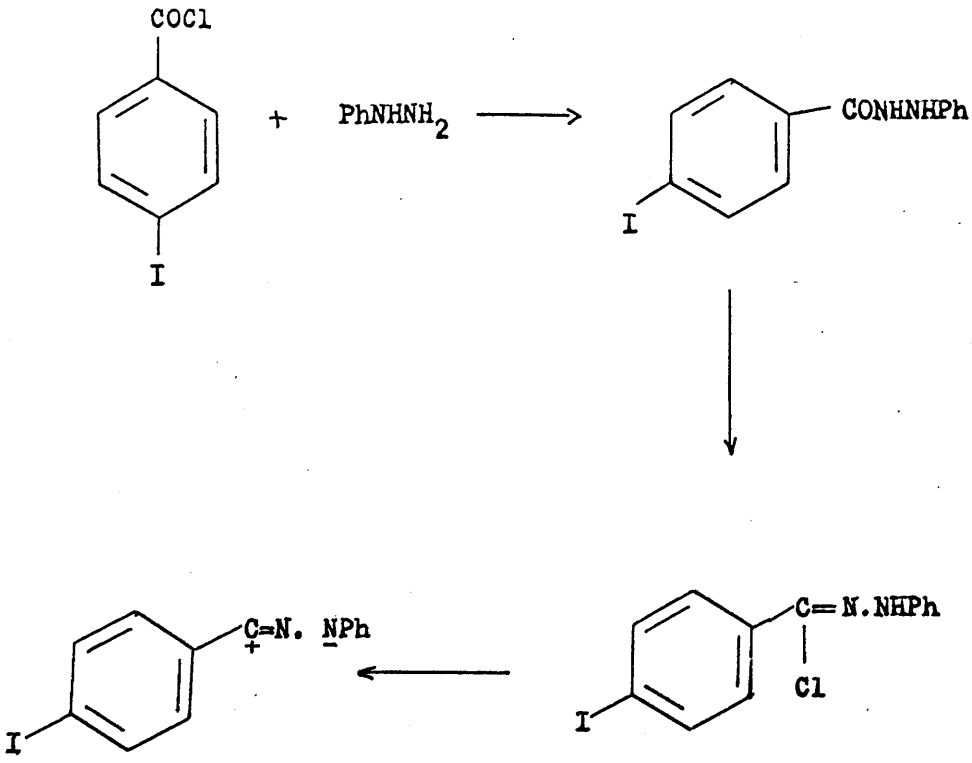
tri-ethylamine failed to form a pyrazoline adduct with stilbene (no blue spot on staining with ceric ammonium nitrate) and attempts to prepare a derivative from this nitrile imine were abandoned.

Two further nitrile imines were prepared as in Scheme 9 and Scheme 10 and the formation of derivatives of columbin and iso-columbin was attempted. At room temperature in acetone, no adduct formation occurred i.e. no blue staining spot on spraying a thin-layer chromatogram with ceric ammonium nitrate. Refluxing the reaction mixtures for 8 hours produced a small amount of adduct and a very complex mixture of decomposition products of the nitrile imine used.

Since the iodo-nitrile imines from Scheme 9 and 10 deteriorated rapidly in the atmosphere, the reactions were repeated under nitrogen to prevent aerial oxidation. This increased the yield of the adduct but the overall yield was extremely small (10%). The solvent was changed from acetone to tetrahydrofuran but no increase in yield was obtained.

The nitrile imine (prepared as in Scheme 9) yielded three derivatives on reaction with columbin. Condensation of this nitrile imine with iso-columbin produced two derivatives, one in much greater yield, both of which were products of the corresponding columbin reaction. Hence, the basic conditions produced by the tri-ethylamine causes some isomerisation of columbin to iso-columbin resulting in the formation of two derivatives which could be exo and endo adducts or they may be

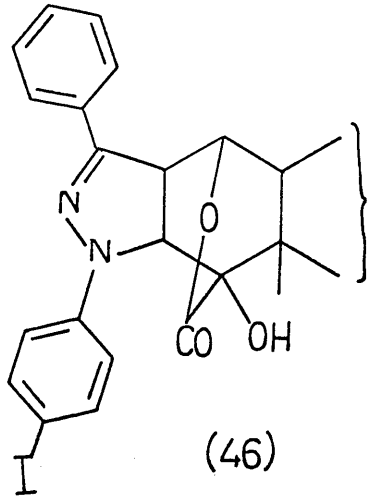
SCHEME 10.



of the form (46) and (47). Of the three adducts of columbin and iso-columbin, only the more abundant of the iso-columbin adducts could be crystallised in a form suitably large for an X-ray analysis.

This adduct crystallised in the tetragonal system as square plates slow evaporation of an acetone solution. m.p.> 340°C with decomposition.

The X-ray analysis of this iso-columbin adduct, which is discussed in detail later in this Thesis, confirmed in every detail, the constitution previously proposed by Barton and Elad, and the relative stereochemistry proposed by Overton, Weir and Wylie. It did not distinguish the absolute configuration deduced by the latter group of workers from its antipode.



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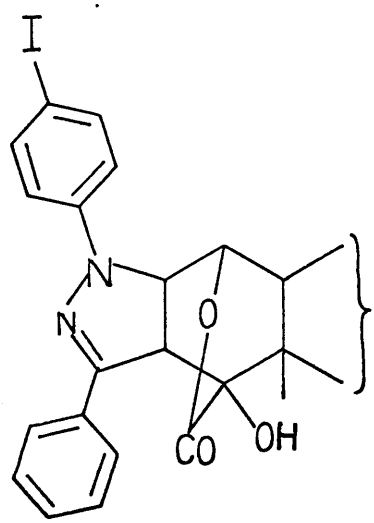


(47)

X-ray THEORY

INTRODUCTION:

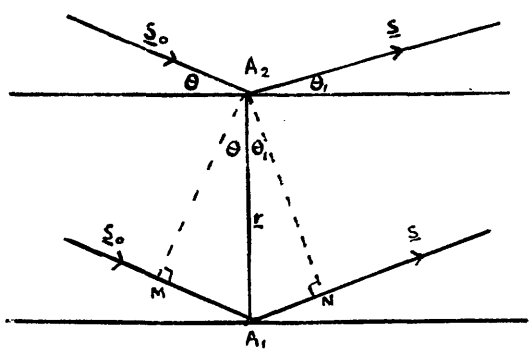
Since the first successful diffraction of X-rays by Von Laue in 1912, the study of crystalline structures, on an atomic basis, has developed rapidly. A vast number of structures have been determined ranging from the very simple in the case of diamond to the highly complex structures of the proteins, hae moglobin and myoglobin.

The procedures of a structure determination employed in this Thesis involved recording the diffraction of the X-rays by the crystal photographically; estimating their intensities visually; determining the structure by the "heavy" atom method and refining the parameters of the atoms in the molecule by Fourier or least squares methods. Brief accounts of the methods involved are given in the following sections.

Diffraction by a lattice.

The simplest three dimensional pattern is an array of points composing a lattice. Consider each point of the lattice to be an electron.

Electrons in the process of accelerating will radiate energy and can disturb the movement of other neighbouring electrons. When X-radiation impinges on an electron, the electron is forced to accelerate and radiate energy, hence it becomes a source of secondary radiation. The wave fronts of the secondary radiation from the electrons give rise to diffraction patterns. Before we can appreciate the total effect of this secondary radiation we must consider the result of diffraction in one direction.



A_1 and A_2 are two lattice points at the end of vector $\underline{r}$

$\underline{r} = u\underline{a} + v\underline{b} + w\underline{c}$

$\underline{a}$, $\underline{b}$, $\underline{c}$ are primitive translations

u v w are integers

A_2M and A_2N are normal to the incident and diffracted wave fronts of the parallel X-ray beam, wavelength λ which falls on the lattice in the direction $\underline{S}_o$ and is diffracted along vector $\underline{S}$,

Path Difference between incident and diffracted waves

$$= A_1M + A_1N$$
$$= (\underline{r}\cdot\underline{S}_o + \underline{r}\cdot\underline{S})$$
$$= \underline{r}\cdot(\underline{S}_o - \underline{S})$$
$$= \underline{r}\cdot\underline{S}' \qquad \text{where } \underline{S}' = \underline{S}_o - \underline{S}.$$

For a maximum the incident and diffracted waves must be in phase i.e. $\underline{r}\cdot\underline{s}$ must be an integer.

thus $(u\underline{a} + v\underline{b} + w\underline{c}) \cdot \underline{S}' = \text{integer}.$

since u,v,w are integers

then $\underline{a} \cdot \underline{S}' = h$

$\underline{b} \cdot \underline{S}' = k$

$\underline{c} \cdot \underline{S}' = l$

These are the Laue equations.

Bragg in 1913 equated these integers (hkl) with the Miller indices, which are used to define crystal planes, and used these equations to interpret X-ray spectra.

$$\underline{a}/h \cdot \underline{S}' = 1 \; ; \; \underline{b}/k \cdot \underline{S}' = 1 \; ; \; \underline{c}/l \cdot \underline{S}' = 1$$

$$\therefore \quad (\underline{a}/h - \underline{b}/k) \cdot \underline{S}' = 0 \; ; \; (\underline{a}/h - \underline{c}/l) \cdot \underline{S}' = 0$$

hence $\underline{S}'$ is perpendicular to the vectors $(\underline{a}/h - \underline{b}/k)$ and $\underline{a}/h \, \underline{c}/l)$ which are in the plane given by the Miller indices hkl, hence $\underline{S}$ is perpendicular to this plane.

$\underline{S}'$ however, is the bisector of the incident $(\underline{S}o)$ and the diffracted beams $(\underline{S})$, thus we can consider diffraction as reflection from the lattice plane.

If the lattice spacing is d, then this is the perpendicular distance of the lattice plane from the origin i.e. the projection of $\underline{a}/h$, $\underline{b}/k$, $\underline{c}/l$ on $\underline{S}'$,

$$\therefore \quad d = \frac{\underline{a}/h \cdot \underline{S}'}{|\underline{S}'|} \qquad \underline{a}/h \cdot \underline{S}' = 1$$

$$|\underline{S}| = \frac{2 \sin \theta}{\lambda}$$

$$\therefore \quad \underline{\lambda = 2d \sin \theta}$$

This is the Bragg equation.

Atomic Scattering Factor.

In a normal lattice, the scattering points are not single electrons as was considered above, but atoms. The scattering power of an atom (f) is expressed in terms of the scattering for a single free electron. The maximum scattering of an atom is equal to its atomic number (z) and this occurs when $\frac{\sin\theta}{\lambda} = 0$. However, owing to the destructive interference between wavelets scattered throughout the volume of the atom, the scattering power (f) will decrease as $\sin\theta/\lambda$ increases. Hence, for a given wavelength (λ) the scattering power of an atom (f), is a function of the angle of diffraction (θ) and the distribution of electrons in the atom. Hartree (1928) showed that these atomic scattering factors could be calculated for different values of θ .

When the wavelength of X-radiation used is just shorter than that of the absorbtion edge of an atom, the amplitude and phase of the scattered radiation is abnormal and Friedal's Law ($f_{hkl} = f_{\bar{h}\,\bar{k}\,\bar{l}}$) no longer holds. This is termed anomalous dispersion. In normal scattering the atomic scattering factors (f) are real and positive; for anomalous scattering, they are complex and may be represented as

$$f_p = f'_p + if''_p .$$

Structure factor and Intensity of X-ray reflections.

The unit cell of a crystal contains different atoms at various positions and the waves scattered by these atoms, in any order of diffraction (hkl) will have phase differences with

respect to each other. The amplitude of the resulting wave from all the atoms in the unit cell, will be determined by the kind of atoms present and their arrangement in the unit cell. The amplitude of this resultant wave is given by

$$F_{hkl} = \sum_{j=1}^{N} f_j \exp 2\pi i (hx_j + ky_j + lz_j)$$

The complex quantity F_{hkl} is termed the structure factor and only its modulus $|F_{hkl}|$ can be determined experimentally. $|F_{hkl}|$ is termed the structure amplitude and is given in terms of the square of its absolute value by

$$|F_{hkl}|^2 = \left[f_r \cos 2\pi (hx_r + ky_r + lz_r)\right]^2 + \left[fr \sin 2\pi (hx_r + ky_r + lz_r)\right]^2$$

The intensity of a reflection depends on a number of physical factors - absorbtion, polarization factor, heat factor, Lorentz factor (L), rotation factor and atomic scattering factor(f) - and on a discontinuous crystallographic factor - structure factor (F). Hence the intensity of a reflection can be used to find the structure factor.

Thermal Vibrations.

The atomic scattering factors have been calculated on the assumption that the atoms are at rest. In practice, atoms have thermal vibrations which smear the electron distribution and so decrease the intensities of the wavelets scattered.

The effect of thermal vibration can be allowed for by modifying the scattering function by including a temperature factor.

$$f = fo \exp - \left(\frac{B \sin^2\theta}{\lambda^2} \right)$$

Where is the Bragg angle, $B = 8\pi^2\bar{u}^2$ and $\bar{u}^2$ is the mean square displacement of atoms from their mean positions.

This expression implies that all identical atoms will have the same thermal vibration and that this vibration will be isotropic. This is not the case since the thermal vibration varies with direction. Cruickshank (1956) describes the anisotropic thermal vibrations in terms of a symmetrical tensor with six independent components U_{ij}. The U_{ij}'s are defined with respect to the reciprocal axes a^*, b^*, c^* and at reciprocal lattice point $S = (ha^*, kb^*, lc^*)$, the temperature factor is expressed as

$$\exp\left[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{23}klb^*c^* + 2U_{31}lhc^*a^* + 2U_{12}hka^*b^*)\right]$$

such that, for instance U_{11} is the mean square amplitude of vibration of the atom parallel to the reciprocal axis a^*.

Phase Problem.

The ultimate goal, in determination of crystal structure, is the determination of the positions of the atoms in the unit cell. Electron density is a measure of the electron population at any point in the unit cell of the crystal and the region of highest electron density is found next to the atomic nucleus. The atomic centres are thus found at maxima of electron density. Since the calculation of atomic scattering factors depends on a knowledge of the electron distribution in the atom we can determine the atomic sites by using electron densities.

Since the electron density (xyz) in a crystal is a periodic function, repeating itself at intervals of one unit cell, it can be represented as a Fourier series.

$$\rho_{(xyz)} = \frac{1}{V\ abc} \sum_{h} \sum_{k}^{\infty} \sum_{l} |Fhkl| \cos\left[2\pi\left(\frac{hx}{a} + \frac{ky}{b} + \frac{lz}{c}\right) - \alpha hkl\right]$$

Where $|Fhkl|$ is the structure amplitude and αhkl is the phase angle. Although $|Fhkl|$ is readily determined, αhkl is not and it is the difficulty of determining α which is termed the "phase problem".

Various methods have been employed to determine these phases, including trial structure method, the "heavy" atom method and the method of isomorphous replacement. Perhaps the most commonly used method is that employing a "heavy" atom.

"Heavy" Atom method.

This technique, first used by Robertson (1937,1940), offers a practical method for determining the structure of a molecule. If a compound contains a few atoms whose atomic numbers are considerably greater than those of the remaining atoms, then their positions can generally be determined from a Patterson map.

The structure factor can be expressed as

$$F = F_H + F_L$$

F_H and F_L are the contributions of the heavy and light atoms respectively.

Frequently the contributions from the heavy atoms approximates to the magnitude of F and hence the phase angle (α_H) of the heavy atom may be a good approximation to the phase angle (α) due to the whole molecule. Consequently, where a Fourier synthesis is computed using the observed structure amplitudes (|Fo|) and the phase angle α_H, the resulting electron density distribution will generally reveal part or whole of the structure.

For this situation to occur, it has been suggested that the square of the atomic number of the heavy atom should be approximately equal to the sum of the squares of the atomic numbers of the remaining lighter atoms.

Patterson Method.

The X-ray method is greatly limited by the fact that the phase of Fhkl cannot be determined experimentally, although |Fhkl| is readily determinable. Patterson (1935) derived a function in the form of a Fourier series which depends only on $|Fhkl|^2$, the phases being zero; the function can be derived directly from the observed intensities. i.e. the Patterson function.

$$A(xyz) = \frac{1}{V^2} \sum_h \sum_k^{\infty} \sum_l (|Fhkl|)^2 \cos 2\pi(hx + ky + lz)$$

Maxima in this distribution represent interatomic vectors in the actual crystal structure.

Fourier Synthesis.

In order to refine a trial structure, it is necessary to alter the positional and thermal parameters to obtain closer agreement between the observed and calculated structure amplitudes and between the calculated and true phases. Positional parameters can be obtained from electron density distribution maxima resulting from Fourier synthesis.

The electron density in a crystal is a periodic function and can be represented by a Fourier series of the form

$$\rho(xyz) = \frac{1}{V} \sum_{h} \sum_{k}^{\infty} \sum_{l} |Fhkl| \cos \left[2\pi (hx + ky + lz) - \alpha hkl \right]$$

αhkl is the phase constant associated with the structure amplitude ($|Fhkl|$) which can be derived from the observed intensities.

On the basis of a trial structure, structure factors and phases are calculated, the phases obtained being attributed to the observed structure amplitude, and a Fourier synthesis is computed. From the electron density distributions, improved coordinates for the atoms are obtained. Successive cycles of phasing calculations followed by Fourier synthesis, are performed, each cycle taking the structure closer to the correct one.

Although the Fourier series given above is infinite, the number of terms employed is limited by experimental conditions and hence the method suffers from the defect that termination of series errors occur. These errors can lead to inaccuracies

in positional parameters, which are most marked in the vicinity of a "heavy" atom but the effect can be minimised by "back-shift" corrections.

When a Fourier synthesis is calculated using observed structure amplitudes $|Fo|$, due to termination of series errors the peaks are displaced from their true positions. A Fourier synthesis is calculated using calculated structure amplitudes $|Fc|$ which should be subject to the same series termination effects as the $|Fo|$ synthesis. Coordinates from the $|Fo|$ synthesis inserted into the $|Fc|$ synthesis should be reproduced but, owing to termination of series errors, the two sets of coordinates will differ by $\Delta x_j, \Delta y_j, \Delta z_j$, for the j^{th} atom. This difference can be subtracted from the $|Fo|$ synthesis coordinates to give their true value. This correction is known as a "back-shift" correction.

The Method of Least Squares.

Hughes (1941) introduced the method of least squares refinement of atomic parameters, as a supplementary method to that of Fourier synthesis, in the final stages of crystal structure analysis.

The theory of errors predicts that, if the errors in the measured structure amplitudes follow the normal Gaussion Law, then the best atomic parameters are those which result from the minimisation of the quantity R'

$$R' = \sum_{hkl} w \left(|Fo| - |Fc| \right)^2 \quad \text{----(1)}$$

|Fo| = observed structure amplitudes.
|Fc| = calculated " "
w = weight of a particular term.

w should be taken as being inversely proportional to the square of the probable error of corresponding observed structure amplitudes.

If p_1, p_2 --- p_n are parameters of Fc to be determined, then for R to be a minimum,

$$\frac{\partial R}{\partial p_j} = 0 \quad (j = 1 \text{ --- } n) \quad \text{----- (2)}$$

i.e. $$\sum_{hkl} w \Delta \frac{\partial Fo}{\partial p_j} = 0 \quad \text{where } \Delta = |Fo| - |Fc| \quad \text{----(3)}$$

If the correct value of p_j is $(p_i + \epsilon_i)$ where ϵ_i is a small correction, then expanding the function of the parameters by Taylor series to first order we have,

$$\Delta(p + \epsilon) = \Delta(p) - \sum_{i=1}^{n} \epsilon_i \frac{\partial Fc}{\partial p_i} \quad \text{----} \quad (4)$$

Substituting (4) into (3) we obtain

$$\sum_{i=1}^{n} \left[\sum_{hkl} w \frac{\partial Fc}{\partial p_i} \cdot \frac{\partial Fc}{\partial p_j} \right] \epsilon_i = \sum_{hkl} w \Delta \frac{\partial Fc}{\partial p_j} \quad \text{----(5)}$$

There are n of these equations for j=1, --- n and they are known as the normal equations. Solving these equations for n unknowns leads to an improved set of coordinates.

For each atom there are either four parameters (3 positional parameters and 1 temperature parameter) or nine parameters (3 positional parameters and 6 anisotropic temperature parameters) and hence the solution of the normal equations for a molecule containing about twenty atoms is a very formidable task even for a large scale digital computor. Approximation to a full matrix solution must therefore be made.

The block diagonal approximation neglects the interactions between atoms and considers only the parts of the equation concerning the one atom. Thus instead of solving a matrix n x n a series 4 x 4 or 9 x 9 matrices for each atom, are solved.

X-RAY ANALYSIS OF 1- p -iodo-phenyl-3-phenyl pyrazoline adduct of iso-columbin.

The X-ray structural analysis of 1- p-iodo-phenyl-3-phenyl pyrazoline adduct of iso-columbin had two objectives; to confirm the biogenetically unprecedented stereochemistry of the A-B ring junction and to study the subtler conformational properties of the carbocyclic and lactone rings. The derivative, white cubic crystals melting above 340°C with decomposition, crystallising in the tetragonal system a = b = 9.68Å, c = 70.8Å was suitable for crystallographic analysis, using the "heavy" atom method. The derivative used was a mono-acetone solvate.

Equi-inclination and zero-layer Weissenberg photographs were taken with CuK radiation and three dimensional intensity data were recorded visually for the layers okl, 1kl ---- 7kl, 1860 of the reflections estimated being used in the structure determination.

The position of the iodine atom was found from a Patterson vector analysis. An electron density distribution with phases calculated for the iodine atom and three subsequent electron density distribution calculations established the positions of all atoms in the molecule, including the atoms of the acetone solvate molecule. The discrepancy factor (R) was 23.3% at this stage.

$$R = \frac{\sum(|Fo| - |Fc|)}{\sum|Fo|}$$

Six cycles of isotropic least squares calculations with all

planes being given unit weight and two final cycles of anisotropic least squares calculations using the weighting scheme

$$w = \frac{1}{(a + |Fo| + c\,|Fo|^2)^{\frac{1}{2}}} \qquad \begin{matrix} a = 14.4 \\ c = 0.006 \end{matrix}$$

reduced the value of R to 9.5%. This concluded the refinement.

DISCUSSION OF STEREOCHEMISTRY.

The stereochemistry of 1- p -iodo phenyl-3-phenyl pyrazoline adduct of iso-columbin is as shown in (48) with rings A, B and C adopting the boat conformation to minimise non-bonded interactions.

Ring A has a β -oriented 1,4- δ -lactone bridge system, the five atoms of the lactone system $C_{(1)}$ $O_{(25)}$ $C_{(17)}$ $O_{(24)}$ $C_{(4)}$ being planar. The equation of the plane is given by

$$-0.9378\,X - 0.2626\,Y - 0.2272\,Z + 1.6344 = 0$$

The atoms in this plane deviate from strict planarity as follows:

$C_{(1)}$ is + 0.064Å, O_{25} - 0.075Å, $C_{(17)}$ - 0.028Å, $O_{(24)}$ + 0.055Å and $C_{(4)}$ - 0.017Å from the δ-lactone plane. Since the estimated standard deviations are 0.02-0.03Å the deviations of these atoms from the δ -lactone plane may be significant. When a Dreiding model of this compound is examined, it appears that the $C_{(9)}$ β -methyl group can have some slight interaction with $O_{(25)}$ which could disturb the planarity of the δ-lactone system. The distances of $C_{(2)}$ and $C_{(3)}$ from the δ -lactone plane are -1.18Å and -1.22Å while the distances of $C_{(5)}$ and $C_{(10)}$ are + 1.34Å and +1.36Å, respectively, showing that ring A is in the boat conformation; Ring A is cis fused to rings B and E.

The 1,4 δ -lactone bridge causes only slight distortion in ring A; angle $C_{(3)}C_{(4)}C_{(5)}$ being 114° instead of the expected tetrahedral angle of 109.5°, the other angles in ring A at C_1, C_2, C_3, C_5 and C_{10} are close to the expected value.

The methyl group at $C_{(5)}$ is axial and has the α-configuration and the methyl group at $C_{(9)}$ is also axial but has the β-configuration. The hydroxyl group at $C_{(4)}$ is quasi-equatorial.

The conformation of the cage structure of ring A is indicated in (49). The intramolecular distance between $O_{(23)}$ and $N_{(28)}$ is 2.97Å and between $O_{(23)}$ and $O_{(24)}$ is 2.70Å, both distances are within the range for hydrogen bonding and there are no intermolecular distances between $O_{(23)}$ and other atoms that suggest hydrogen bonding. However, the angles $O_{(24)}$ $O_{(23)}$ $C_{(4)}$ and $N_{(28)}$ $O_{(23)}$ $C_{(4)}$ are only 65.9 and 55.1° respectively, indicating that the hydrogen atom would lie well off the line joining either $O_{(23)}$ and $O_{(24)}$ or $O_{(23)}$ and $N_{(28)}$.

Ring B also has the boat conformation cis fused to ring A at $C_{(5)}$ and $C_{(10)}$ and trans fused to ring C at $C_{(8)}$ and $C_{(9)}$. This boat conformation is imposed to alleviate interaction between the β-methyl group at $C_{(9)}$ and the ring A 1,4-δ-lactone. However, adoption of the boat conformation results in some non-bonded interaction between the $C_{(9)}$ methyl group and the $C_{(6)}$ axial hydrogen atom. This interaction causes a flattening of ring B by increasing the bond angles from the tetrahedral value to the value shown below:

Angle	$C_{(10)}$ $C_{(9)}$ $C_{(8)}$ = 113°	Angle	$C_{(7)}$ $C_{(6)}$ $C_{(5)}$ = 113°
"	$C_{(9)}$ $C_{(8)}$ $C_{(7)}$ = 113°	"	$C_{(6)}$ $C_{(5)}$ $C_{(10)}$ = 112°
"	$C_{(8)}$ $C_{(7)}$ $C_{(6)}$ = 114°	"	$C_{(5)}$ $C_{(10)}$ $C_{(9)}$ = 115°

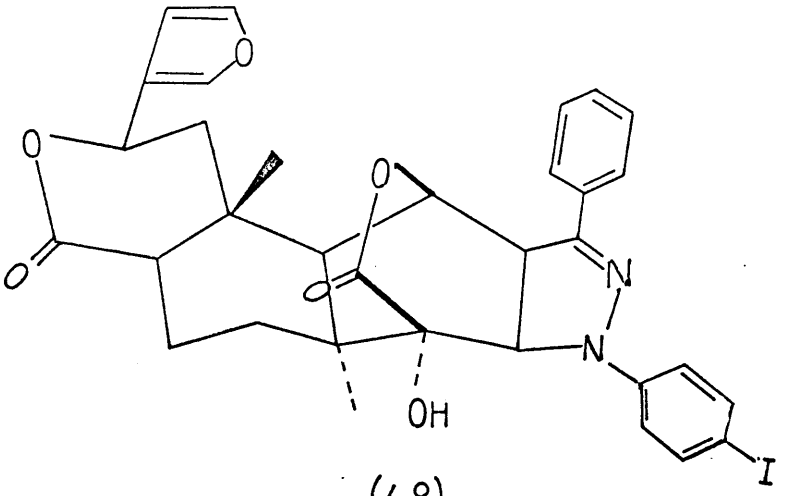
The $C_{(9)}$ β -methyl group ($C_{(19)}$) is repelled towards the angle $C_{(10)}C_{(9)}$ $C_{(11)}$ since the angles $C_{(19)}$ $C_{(9)}$ $C_{(10)}$ and and $C_{(19)}$ $C_{(9)}$ $C_{(8)}$ have been increased to 113° while angle $C_{(19)}$ $C_{(9)}$ $C_{(11)}$ is only increased to 111° from the tetrahedral value of 109.5°. The methyl group on $C_{(9)}$ is thus repelled towards the 1,4- δ -lactone bridge on ring A which helps to account for the observed slight twisting of 1,4- δ -lactone.

Ring C, trans fused to ring B at $C_{(8)}$, $C_{(9)}$, has the boat conformation, and the δ -lactone system $C_{(8)}$ $C_{(20)}$ $O_{(21)}$ $O_{(22)}C_{(12)}$ is strictly planar. The equation of the mean plane through the δ -lactone system is given by,

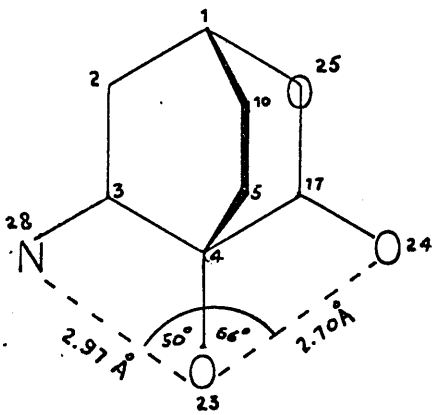
$$0.0197\,X - 0.6950\,Y - 0.7188\,Z + 1.4215 = 0$$

and the deviations of the atoms $C_{(8)}$, $C_{(20)}$, $O_{(21)}$, $O_{(22)}$ and $C_{(12)}$ from the plane are all within standard deviations. $C_{(9)}$ and $C_{(11)}$ are - 1.22 and - 1.25Å from the δ -lactone plane showing that ring C has the boat conformation.

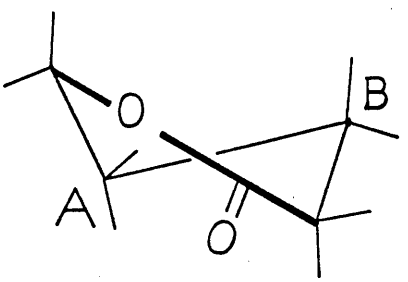
It has been shown[75] that δ -lactone systems can exist in two conformations; a half-boat (50) and a half-chair (51), and that the carbonyl frequencies of δ -lactones in the infra-red correlates with the conformation adopted. The higher carbonyl frequencies, in the range 1758-1765 cm^{-1} appear to correspond to the half-boat conformation and the lower carbonyl frequencies, in the range 1730-1750cm^{-1} correspond to the half-chair conformation. Confirmation for these assignments has been found from X-ray structural analyses of compounds containing such -lactone groupings : iridomyrmecin[76], iso-iridomyrmecin[77];



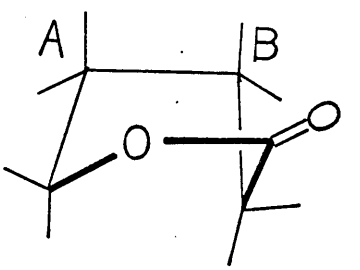
(48)



(49)



(51)



(50)

iso-columbin[78], limonin (A lactone)[79] all with the half-boat conformation, and swietenine[80], glaucarubin[81] and simaroilide[82] all with the half-chair conformation. The normal conformation adopted is the half-chair, the half-boat is only adopted in response to special conditions.

In iso-columbin, both δ-lactone systems adopt the half-boat conformations : ring A and C $\nu_{max}^{CHCl_3}$ 1755cm^{-1}. Ring A 1,4- δ -lactone necessarily exists in the half-boat conformation. Ring C δ-lactone adopts the half-boat conformation in response to non-bonded interactions. In the chair conformation, the furan ring will seriously interact with the β- $C_{(9)}$ methyl group, but this interaction can be removed when ring C adopts the boat conformation. However, a 'bowsprit' interaction between $H_{(12)}$ and $H_{(8)}$ is introduced when ring C is in this conformation. As a result of this interaction ring C is slightly flattened, the flattening being shown in an increase in size of the bond angles in ring C viz: $C_{(8)}C_{(9)}C_{(11)} = 111^{o}$, $C_{(9)}C_{(11)}C_{(12)} = 111^{o}$, $C_{(11)}C_{(12)}O_{(21)} = 115.5^{o}$, H_8 --- H_{12} repulsion increases angle $C_{(12)}O_{(21)}C_{(20)}$ to 115^{o} from the normal value of 103^{o}.

There appears to be some slight rotation about $C_{(12)}$ to reduce the $H_{(8)}$ --- $H_{(12)}$ 'bowsprit' interaction since the furan ring is tilted upwards from the δ-lactone system in ring C; angle $C_{(13)}C_{(12)}C_{(8)}$ is 164^{o} instead of 180^{o} which might be expected from an underformed structure. Tilting the furan ring upwards also tilts $H_{(12)}$ away from $H_{(8)}$ reducing

the 'bowsprit' interaction. The dihedral angle between the furan ring and the plane of ring C δ-lactone is 60.5° to reduce the interactions between the protons of the furan ring and the $C_{(11)}$ protons.

The stereochemistry of iso-columbin has now been completely resolved confirming the conclusions of Overton et al[36]. The trans B-C ring junction in iso-columbin implies that rings B and C in columbin must be cis fused since the columbin-iso-columbin isomerisation has been attributed to epimerisation at $C_{(8)}$.

EXPERIMENTAL.

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The following instruments were used for spectroscopic determinations. I.r.: Unicam S P 100 double beam and P.E. 237 spectrophotometers; U.V.: Unicam SP 500 recording spectrophotometer ; N.m.r.: Varian HA 100 and P.E. R 10 spectrometers; Mass spectrum: A.E.I. MS 9 spectrometer.

Micro-analyses were by J.M.L.Cameron and his staff. Woelm alumina, deactivated to the appropriate Brockmann grade[104] was used for column chromatography. Chromatoplates for thin layer chromatography(t.l.c.) were prepared according to Stahl[105] using Kieselgel G (Merck) and Kieselgel H (Merck).

Unless otherwise stated the petrol referred to in the text had a boiling range between 60- and 80 °C.

Isolation of Columbin:

100g. of a brown ethereal extract of Colombo Root was heated with 1 ℓ. of acetone on a steam bath for 5 min. After cooling, insoluble material (11g.) was filtered off.

The filtrate was poured into a flask which had been marked 200 ml. 600 ml. --- 1.7 ℓ. Ethanol (700 ml.) was added and the volume was reduced to 1.5 ℓ. and allowed to cool. No precipitation occurred and 600 ml. ethanol was added and the volume reduced to 1 ℓ. On cooling, amorphous material (0.7g.) was filtered off and the volume reduced to 500 ml.

On cooling, white needles (14g.) crystallised (I) and were filtered and washed with ethanol (2 x 25 ml.), the washings being added to the filtrate. The volume was further reduced to 300 ml. and mixed needles and prisms (2.4g.) (II) were obtained which were filtered and washed with ethanol (2 x 10 ml.), the washings being added to the filtrate. Further concentration to 200 ml. precipitated mixed needles and prisms (8.1g.) (III) which were filtered and washed with ethanol (2 x 10 ml.) and the combined washings and filtrate were concentrated to 150 ml. After 7 days this solution had deposited gummy crystals (15g.) (IV). The filtrate was reduced to 100 ml. and after a further 7 days, a brown solid (6g.) (V) was filtered. No further crystalline material was isolated from the remaining gum.

Crops I and II contained columbin as the major product, but chasmanthin, palmarin, iso-jateorin and iso-columbin were also present.

Crop III contained the same materials as crops I and II but the concentration of columbin relative to the other constituents had greatly diminished.

Crops IV and V contained chasmanthin as their major product with small quantities of iso-columbin, palmarin and iso-jateorin being present.

Crystallisation of crop I from ethanol-acetone (2:1) yielded material (6.8g.) which contained columbin with a little palmarin and chasmanthin. Chromatography of this material over grade IV acid alumina eluted pure columbin with chloroform-benzene (2:5) (3.1g.). $[\alpha]_D = +55^\circ$.

Isomerisation of Columbin.

Columbin (10g.) $[\alpha]_D = +43.5^\circ$, was mixed with ethanol (140 ml.) and N sodium hydroxide (70 ml.) and heated on the steam bath until solution was complete (ca. 5 min.) then for a further 2 min. After cooling, the solution was acidified with 2N hydrochloric acid to pH 3. The solid material obtained in the first crop (7g.) was crystallised from aqueous ethanol yielding iso-columbin (4.35g.) $[\alpha]_D = +77^\circ$.

Osmate ester complexes of 2,3-dihydroxy columbin (40).

Columbin (50 mg.) was dissolved in dry tetrahydrofuran (THF) (3 ml.) and osmium tetroxide (35.5 mg. : 1 molar equivalent)

in dry THF (2 ml.) was added with 2 molar equivalents of organic base. The mixture was left at room temperature overnight, then the solvent was allowed to evaporate slowly in the fume cupboard.

Several different bases were used, viz: γ-collidine, 2-methyl-6-ethyl pyridine, 2,6-lutidene, quinoline and iso-quinoline. Only when 2,6-lutidene was used, was crystalline material obtained (small dark-red needles), black amorphous material was deposited from the other reactions. Slow crystallisation from THF led to no increase in the size of the dark-red needles obtained from the 2,6-lutidene reaction.

3,5-dibromo-α-chloro benzaldoxime (Scheme 5.)[69] [70]

3,5-dibromo-benzaldoxime[71] (1g.) in AnalaR chloroform (50 ml.) was cooled in ice and a dry, hydrogen chloride free stream of chlorine was bubbled through. The solution turned emerald green in colour then changed to a golden yellow. When this occurred the chlorine stream was shut off and the solvent removed in vacuo. The remaining yellow oil was taken up in carbon tetrachloride and fine white needles were deposited. Recrystallisation from petrol yielded the desired α-chloro oxime (780 mg.) mp. 107-109°. (Found: C,26.6%; H,1.44%; N,4.50%; for $C_7H_4NO\ Br_2Cl$ requires C,26.68%; H,1.28%; N,4.49%.)

Isoxazole adduct of norbornene (72)

Redistilled triethylamine (50 mg.) in dry ether (5 ml.) was added dropwise over 1 hour to a stirred solution of norbornene (47 mg.) and 3,5-dibromo-α-chloro-benzaldoxime (244 mg. : 1.5 molar equivalents) in dry ether (8 ml.). The mixture was stirred for a further 1 hour. The triethylamine hydrochloride was filtered and the solvents removed in vacuo. The residue was taken up in benzene-petrol (1:4), filtered through a short column of neutral alumina (grade IV, neutral) and crystallised from benzene-petrol as white rods (172 mg.) mp. 153-156°C. (Found: C,45.20%; H,3.28%; N,3.73%; for $C_{14}H_{11}NO\,Br_2$ required; C,45.15%; H,3.48%; N,3.76%.).

Isoxazole derivative of columbin (43,44).

Redistilled triethylamine (25 mg. : 1 molar equivalent) in 10 ml. dry THF was added dropwise over 1 hour to a stirred solution of columbin (89 mg.) $[\alpha]_D = +45^\circ$, and 3,5-dibromo-benzaldoxime (122 mg. : 1.5 molar equivalents) in dry THF (10 ml.). The mixture was left at room temperature 16 hours. After filtration the reaction mixture was crystallised from ethyl acetate-petrol yielding white plates (65 mg.) mp. 233-236°C.

(Found: C, 51.23 %; H, 4.06 %; N, 2.36 %.

$C_{27}H_{25}NO_7Br_2$ requires: C, 51.02%; H, 3.94%; N, 2.2%.)

This material was a mixture of two components not separated by chromatography on a thick layer plate.

Isoxazole derivative of iso-columbin.

Iso-columbin (89 mg.) $[\alpha]_D$ = + 70° and 3,5-dibromo-α-chloro-benzaldoxime (122 mg.) were dissolved in dry THF (10 ml.). To this stirred solution redistilled triethylamine (25 mg.) in dry THF (10 ml.) was added dropwise over 1 hour. The mixture was allowed to sit at room temperature for a further hour. The residue after filtration and removal of solvent crystallised from ethyl acetate-petrol as white plates mp. 278-282°C.

A thin layer chromatoplate showed this material to be a mixture of two compounds with almost identical polarities, possibly isomeric[63]. Chromatography on an 0.8 mm, 20 x 20 cm silica chromatoplate with ethyl acetate-chloroform (1:4) as eluant failed to separate the compounds.

2-(β-bromo ethyl)-4'-(p-iodo phenyl)-benzhydrazone (Scheme 6. b).

a)[64] 2-(β-bromo ethyl) benzaldehyde (10.2 g.) was added dropwise over 30 min. to a stirred solution of p-iodo phenyl hydrazine[73] (11.3g.) in 20% hydrochloric acid (5 l.). Formation of a red oil occurred at the bottom of the flask during the addition of the hydrazine, which contained a number of compounds, as judged by TLC.

An ether extract (3 x 100 ml.) of the acidic solution was washed with water (3 x 50 ml.) and, on evaporation of solvent, yielded a little starting aldehyde. The acidic

solution was basified with 2N sodium hydroxide solution and extracted with ether (4 x 100 ml.) washed with water (3 x 100 ml.) and dried over anhydrous sodium sulphate. This solution yielded a red oil with the same characteristics as the red oil obtained previously.

Chromatography over silica gel BDH eluted a material (20 mg.) which crystallised as yellow plates from ethanol mp. 200-202°C. This material was eluted with chloroform-benzene (1:4).

b)[74] p-iodo phenyl hydrazine (375 mg.) and 2-(β-bromo ethyl)-benzaldehyde (305 mg.) were refluxed in anhydrous pyridine (10 ml.) for 3 min. The pyridine was removed in vacuo at room temperature leaving a yellow solid. Attempted crystallisation from ethanol deposited yellow material mp. 199-202°C. (510 mg.).

c) 2-(β-bromo ethyl) benzaldehyde (1.94g.) was added to a solution of p-iodo phenyl hydrazine (1.96g.) in benzene (10 ml.). The solution was left at room temperature 2 hours and the benzene removed in vacuo. The resultant red oil was triturated with THF and the yellow solid obtained was filtered and crystallised from ethanol. Yellow plates (3.9g.) mp. 200.5 - 201.5°

(Found: C, 41.87%; H, 3.01%; N, 6.51%: $C_{15}H_{14}N_2BrI$ requires: C, 42.1%; H, 3.26%; N, 6.53%.)

N-(p-iodo phenyl amino)-3,4 dihydro isoquinolinium hydrobromide (Scheme 6.c).

Finely ground β-bromo 2-ethyl (p-iodo phenyl) benzhydrazone (Scheme 6.d.) was heated in a test tube in an oil bath at 180° for 10 min. During this time the material changed colour from yellow to red-brown. mp. 188-189°C (Found: C, 42.39 %; H, 3.31 %; N, 6.63 %: $C_{15}H_{14}N_2BrI$ requires: C, 42.1%; H, 3.26%; N, 6.53%.).

Azomethine Imine (Scheme 6.d.) adduct to Norbornene.

Norbornene (25mg.) and (Scheme 6.b.) (228 mg.: 2molar equivalents) were dissolved in dimethyl formamide (DMF)(3 ml.). To this stirred solution, triethylamine (0.4 ml.) in DMF(5ml.) were added slowly dropwise. The mixture was left at room temperature 10 hours, filtered and the solvents removed in vacuo. A chromatoplate of the reaction mixture showed a brightly U.V. fluorescent spot which stained blue on spraying with ceric ammonium nitrate. The fluorescent material was separated from the reaction mixture by preparative TLC, eluant benzene. Crystallisation from ethanol afforded white rods mp. 134-136°C. (Found: C, 59.86%; H, 5.21%. $C_{22}H_{23}IN_2$ requires: C, 59.60%; H, 5.42%.).

Attempted formation of Azomethine Imine (Scheme 6.d.) adduct to Columbin.

a) Columbin $[\alpha]_D$ +45° (50 mg.) was dissolved in DMF (5ml.) along with (Scheme 6.c.) (240mg. : 4 molar equivalents). To this stirred solution, triethylamine (0.5 ml.) in dry DMF(5ml.) was added dropwise over 2 hours, then left at room temperature

a further 10 hours. Water was added and the reaction mixture extracted with chloroform (3 x 10 ml.), washed with water (3 x 5 ml.) and dried over anhydrous sodium sulphate. A chromatoplate of the reaction mixture showed a small blue spot on staining with ceric ammonium nitrate.

Columbin (45 mg.) was recovered, using preparative TLC.

b) Triethylamine (0.2 ml.) in DMF (15 ml.) was added dropwise over 2 hours to a stirred solution of columbin (200mg.: 4 molar equivalents) $[\alpha]_D$ $+45^o$, and (Scheme 6.c.) (60mg. : 1 molar equivalent) in DMF (15 ml.). The reaction mixture was left at room temperature 10 hours, diluted with water and extracted with chloroform (3 x 10 ml.). The chloroform extract was washed with water (3 x 5 ml.) and dried over anhydrous sodium sulphate. A chromatoplate showed only a small amount of a blue staining compound.

Columbin (192mg.) was recovered by preparative TLC.

β-benzoyl hydrazide formation (Scheme 7.b.).

Redistilled benzoyl chloride (16.6g.) was slowly added to an ice cooled solution of phenyl hydrazine (25.5g : 2 molar equivalents) in dry ether (130 ml.). The white precipitate obtained was collected after 2 hours and boiled in water to remove phenyl hydrazine hydrochloride. The insoluble residue crystallised from ethanol to yield β-benzoyl hydrazide as white plates (23.5g.) mp. 168^oC.

Preparation of α-chloro benzylidene phenyl hydrazide (Scheme 7.c.).

β-benzoyl hydrazide (20g.) was refluxed with phosphorus pentachloride (24g.) in dry ether (25 ml.) for 10 hours. Phenol (40g.) in dry ether (30 ml.) was run into the almost clear solution and, after cooling, methanol (40 ml.) was added. The combined solvents were removed until the residue boiled at 60-70°C at atmospheric pressure. After refrigeration for 16 hours, white crystals were deposited. Recrystallisation from aqueous acetone yielded white plates (12.2g.) mp. 129-130°C.

Preparation of N,N'-diphenyl pyrazoline adduct of columbin.

Columbin $[\alpha]_D$ +45° (50 mg.) was dissolved in dry acetone (5 ml.) with α-chloro benzylidene phenyl hydrazide (67 mg.) and the mixture treated with triethylamine (0.15 ml.) at room temperature for 16 hours. The resulting red solution was filtered to remove triethylamine hydrochloride and the solvent removed in vacuo. The residue was taken up in benzene and chromatographed over silica gel BDH (8g.). The adduct, which was U,V. fluorescent and stained blue on spraying a chromatoplate with ceric ammonium nitrate, was eluted with benzene-chloroform (9:1). Crystallisation from ethanol afforded white needles (65 mg.) mp. 183-186°C. $[\alpha]_D$ -410°. (Found: C, 72.01%; H, 6.02%; N, 5.19%, for $C_{32}H_{32}O_6N_2$: requires: C, 71.72%; H, 5.80%; N, 5.07%.).

Bromination of N,N'-diphenyl pyrazoline adduct to columbin.

The adduct (500 mg.) in AnalaR chloroform (5 ml.) was cooled in ice and treated with 1 molar equivalent of bromine in chloroform. The mixture was left to warm to room temperature over 3 hours, then washed with 2% potassium hydroxide solution (2 x 2 ml.) and water (3 x 2 ml.) and dried over anhydrous sodium sulphate. A chromatoplate of the reaction mixture showed two products with almost identical polarities. Using an 0.8 mm . 20 x 100cm silica plate with chloroform as eluant partially separated the mixture. The brominated adduct was recrystallised from ethanol mp. 248-249°C, as white needles. $[\alpha]_D$ -423°.

In another experiment, the two products were separated using gradient elution chromatography using an automatic fraction collector, eluting with benzene, ether-benzene (1:49) ---- ether-benzene (1:1). The most polar product was found to be the unbrominated adduct (mp., infra-red spectra and R_f values identical) and the least polar adduct was the brominated adduct (Beilstein test positive) mp. 248-249°C.

The crystalline bromo-adduct lost solvent of crystallisation quickly and was thus unsuitable as an X-ray derivative.

Preparation of the N,N'-diphenyl pyrazoline adduct of iso-columbin.

Iso-columbin $[\alpha]_D$ + 74°(343 mg.) and α -chloro benzylidene phenyl hydrazide (37- mg.) and triethylamine (1 ml.) were dissolved in AnalaR acetone and left at room temperature 16 hours.

The solution was filtered, the solvents removed and the residue crystallised from acetone-ethanol. (321 mg.) mp. 311-314° $[\alpha]_D$ - 409°. (Found: C, 71.64%; H, 6.16%; N, 5.19%: for $C_{33}H_{32}N_2O_6$ requires: C, 71.72%; H, 5.80%; N, 5.19%.)

Bromination of the diphenyl pyrazoline adduct of iso-columbin.

The diphenyl pyrazoline adduct of isocolumbin (115 mg.) in chloroform (5 ml.) was treated with 1 molar equivalent of bromine in chloroform for 4 hours. The solution was washed with 2% potassium hydroxide (3 x 2 ml.) and water (3 x 2 ml.) and dried over anhydrous soldium sulphate. After removal of the solvent the product was crystallised from ethanol-benzene to furnish large yellow plates mp. 323°C with decomposition. $[\alpha]_D$ -32.55°. (Found: C, 61.98 %; H, 5.29 %; N, 4.59 %; for $C_{33}H_{31}N_2O_6Br$ requires: C, 61.54%; H, 5.07%; N, 4.58%).

Attempted formation of a chromium carbonyl complex of diphenyl pyrazoline adduct of columbin [67].

Diphenyl pyrazoline adduct of columbin (276 mg.) and chromium hexacarbonyl (240 mg. : 2 molar equivalents) were dissolved in diethylene glycol dimethyl ether (10 ml.) and refluxed in an atmosphere of nitrogen for 3 hours. During the reflux the solution became turbid and turned greenish-yellow. The solvent was removed in vacuo, and the residue taken up in methylene chloride and filtered through grade III neutral alumina (0.5g.). Crystallisation of this material from methylene chloride-di-iso propyl ether produced a yellow amorphous solid

ν_{Max}^{nujol} 1880, 1960cm^{-1}.

This material decomposed on attempted slow crystallisation from methylene chloride - di-isopropyl ether to N,N' - diphenyl pyrazoline adduct of columbin.

Attempted formation of the methiodide of diphenyl pyrazoline adduct of columbin [66].

a) Diphenyl pyrazoline adduct of columbin (110 mg.) was dissolved in methyl iodide (2.5 ml.) and sealed inside a Carius tube. This was heated at 60°C for 4 hours. The tube was cooled and opened and the excess methyl iodide removed in vacuo. No methiodide formation occurred and the starting material recovered unchanged.

b) Diphenyl pyrazoline adduct of columbin(75mg.) was dissolved in methyl iodide(0.3ml.) and AnalaR acetone (0.5ml) and refluxed for 48hrs. On dilution with ether a green amorphous precipitate was produced which could not be obtained in a crystalline form.

Preparation of α-chloro benzylidene-2,4-dibromo-phenyl hydrazide(Scheme 8.c) (68).

α-chloro benzylidene phenyl hydrazide (100mg) was suspended in AnalaR acetic acid (10ml). To the stirred suspension, bromine (137mg) in AnalaR acetic acid (5ml) was added slowly over 15min. The mixture was stirred at room temperature until the suspended material had dissolved. The acetic acid was removed in vacuo, and the residue was crystallised from aqueous acetone as white plaates (150mg.)

m.p. 106-108°C (lit. 109°C)

Attempted formation of the adduct of (Scheme 8.c) with stilbene.

Stilbene (20mg.) and α-chloro benzylidene-2,4-dibromo-phenyl hydrazide (85mg.: 2 molar quantities) were dissolved in AnalaR acetone (2ml.). Triethylamine(0.2ml.) was added and the mixture was allowed to stand at room temperature for 16hours. The solvents were removed in vacuo and the residue was chromatographed on a 0.5mm chromatoplate eluant, benzene-petrol(1:6). Stilbene (18mg.) was recovered.

Preparation of N'-(p-iodo phenyl) benzhydrazide (Scheme 9.b.).

p-iodo phenyl hydrazine (mp. 101-103°C) was prepared in 65% yield by reduction of p-iodo benzene diazonium chloride with stannous chloride[73].

To an ice-cooled solution of p-iodo phenyl hydrazine (1.5 g.) in dry ether (20 ml.) was added, dropwise with stirring, redistilled benzoyl chloride (0.35 ml.) in dry ether (5 ml.). The precipitated solid was collected after 2 hours and boiled with water to removed p-iodo phenyl hydrazine hydrochloride. The insoluble residue crystallised from ethanol to afford N'-(p-iodo phenyl) benzhydrazide (803 mg.) mp. 169-170°C. (Found: C, 46.35%; H, 3.55%; N, 8.41%. $C_{13}H_{11}IN_2O$ requires: C, 46.15%; H, 3.25%; N, 8.45%.).

Preparation of N-α-chlorobenzylidene-N'-p-iodo phenylhydrazide (Scheme 9.c.).

N'-(p-iodo phenyl) benzhydrazide (3.62g.) and phosphorus pentachloride (5.07g.) were refluxed in dry ether (10 ml.) for 16 hours. Phenol (7.9g.) in dry ether (10 ml.) was then run into this solution and, after cooling, methanol (72 ml.) was added. The combined solvents were removed until the residue boiled at 60-70°C at atmospheric pressure. The solid which separated on refrigeration was crystallised from ethanol, affording the desired enol chloride (1.87g.) mp. 112-113°. (Found: C,43.7%; H, 3.25%; N, 8.15%. $C_{13}H_{10}Cl\ IN_2$ requires: C, 43.8%; H, 2.8%; N, 7.9%.).

Adduct of columbin and iodo diphenyl nitrilimine[62].

Columbin (200 mg.) $[\alpha]_D$ = + 55°, the above enol chloride (800 mg.) and redistilled triethylamine (3 ml.) were refluxed in AnalaR acetone under nitrogen for 8 hours. The solvents were removed in vacuo and the product was chromatographed over silica gel (B.D.H, 10g.) benzene-chloroform (3:1) eluted two adducts, which were separated by thin layer chromatography, eluant chloroform-benzene (1:4). The less polar compound (12 mg.) crystallised from acetone as fine white needles, mp. 243-5°C. (Found: C, 58.45%; H, 4.62%; N, 3.87%. $C_{33}H_{31}IN_2O_6$ requires C, 58.49%; H, 4.59%; N, 4.13%.).

The more polar compound (75 mg.) crystallised from acetone as diamond shaped plates mp. softens with decomposition at 168° but does not melt below 340°C. (Found: C, 58.05%: H, 4.84%; N, 4.06%. $C_{33}H_{31}IN_2O_6$ requires C, 58.49%; H, 4.59%; N, 4.13%.).

Preparation of adduct of iso-columbin and iodo diphenyl-nitrilimine[62].

The reagent was prepared in situ in presence of iso-columbin as follows: Isocolumbin (100 mg.) $[\alpha]_D$ = + 75°, the above enol chloride (400 mg.) and redistilled triethylamine (1.5 ml.) were refluxed in dry AnalaR acetone (5 ml.) under nitrogen for 5 hours. The product, obtained in the usual way, was chromatrographed over silica gel (B.D.H., 5g.). Benzene-chloroform (7:3) eluted the adduct [46,47] (160 mg.) which slowly crystallised from acetone in flat prisms, mp. softens

with decomposition near 165°C but does not completely melt below 340°C. (Found: C, 58.79%; H, 4.75%; N, 3.85%. $C_{33}H_{31}O_6N_2I$ requires: C, 58.5%; H, 4.59%; N, 4.13%.).

SECTION III

ISOMERISATION

of the

COLOMBO ROOT BITTER PRINCIPLES.

ISOMERISATION OF THE COLOMBO ROOT BITTER PRINCIPLES.

The isomerisation of columbin under mild alkaline treatment has been attributed to epimerisation at $C_{(8)}$ by Barton and his associates[54] but the extent of isomerisation has not been investigated. Iso-columbin has been confirmed as the $C_{(8)}$ epimer of columbim from the X-ray analysis of 1-p-iodo phenyl-3-phenyl pyrazoline adduct of iso-columbin.[78]

When the two main constituents of the colombo root bitter principles, columbin and chasmanthin, are isomerised by warming in 50% ethanol/M sodium hydroxide for four minutes, columbin is transformed, in part, into iso-columbin but chasmanthin is wholly transformed into palmarin. From a thin-layer chromatogram of the columbin isomerisaation product, it appeared that the concentrations of columbin and iso-columbin were approximately equal. It was thought, at first, that in view of the complete transformation of chasmanthin into palmarin under the same conditions, the reaction was only 50% complete. However , increase in the reaction time did not increase the yield of iso-columbin, merely resulting in some decomposition of the product. The amount of decomposition increased as the reaction time increased but the relative concentrations (as judged by thin-layer chromatography) of columbin and iso-columbin remained unaltered. In order to determine the actual extent of isomerisation, a quantitative study of the isomerisation was carried out.

Gas-liquid chromatography could not be used in the quantitative study of the isomerisation product since, aside from their thermal decomposition, the melting points of the compounds were too high. Consequently it was decided to examine the isomerisation by polarimetry.

Pure columbin $[\alpha]_D = +55^\circ$ was isomerised under mild alkaline conditions for four minutes and eight minutes. On extraction with chloroform, the total product was washed with sodium bicarbonate solution and the resulting neutral material was filtered through acid alumina grade IV and checked by thin-layer chromatography to ensure that it contained only columbin and iso-columbin. The rotation was measured and found to be $[\alpha]_D = +64.5^\circ$. This isomerisation procedure was applied to pure iso-columbin $[\alpha]_D = +74^\circ$ and the rotation of the equilibrium mixture was found to be $[\alpha]_D = +65^\circ$. This rotation of $[\alpha]_D = +65^\circ$ corresponds to a 50% mixture of columbin and iso-columbin.

Cheung, Overton and Sim have shown[75] that δ-lactones can exist in one of two conformations; a half-chair (51) and a half-boat (50). The half-boat conformation results in higher, lactone carbonyl, infra-red frequencies (1758-1765cm^{-1}) than the half-chair (1730-1750cm^{-1}). Ring C of iso-columbin has been shown by X-ray analysis[78] to exist in the half-boat form $\nu_{max}^{CCl_4}$ 1761cm^{-1}. Columbin with a cis fused ring B-C junction has an infra-red frequency of 1750cm^{-1} for ring C δ-lactone carbonyl function, indicating that this δ-lactone exists in the half-chair conformation.

Dreyer[83] has postulated that ring C in columbin is in the boat conformation. In the n.m.r. spectrum of columbin, the proton to the furan ring on $C_{(12)}$ at 4.60τ is a quartet J = 11 cps and 5 cps. The splitting of the $H_{(12)}$ signal to a quartet is caused by axial-axial and axial-equatorial couplings with the protons on $C_{(11)}$. Study of a Dreiding model of columbin indicates that, in order to have such couplings, then ring C must be in the quasi-chair conformation. When a Dreiding model of dihydro-iso-columbin is studied, with ring C in the quasi-boat conformation, then, from consideration of the dihedral angles involved, the coupling of $H_{(12)}$ with the protons on $C_{(11)}$ is expected to be $J_{a,a}$ = 10 cps, $J_{a,e}$ = 5 cps. In the n.m.r. spectrum of dihydro-iso-columbin, such a quartet appears at 4.61τ J = 11 cps and 4 cps, which must arise from the $H_{(12)}$ signal. Thus the lactone ring C of iso-columbin must exist in the quasi-boat conformation. This has been confirmed by X-ray analysis[78].

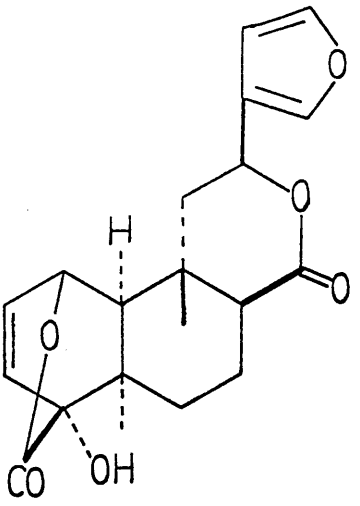
There is gratifying correspondence between the conformation of the iso-columbin derivative (48) revealed by X-ray examination and the probable conformation predicted from a Dreiding model. Models were therefore used freely in deciding conformations arising from n.m.r. spectra and in assessing intramolecular interactions.

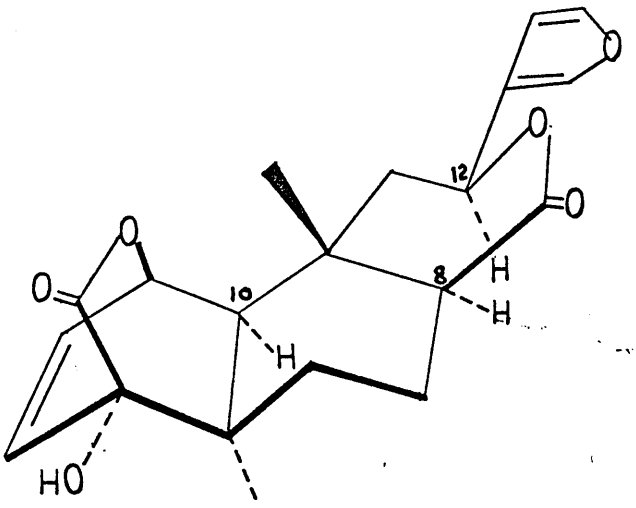
Comparison of Dreiding models of columbin and iso-columbin shows that the intramolecular strain in the molecules, caused by non-bonded interactions, is approximately the same. Rings A

and B in the two molecules are identical. In ring C of iso-columbin, the only serious non-bonded interaction is between $H_{(12)}$ and $H_{(8)}$ (52) and in columbin $H_{(12)}$ interacts with $H_{(10)}$ (53). Since a cis or trans B-C ring junction gives rise to almost identical intramolecular strain, arising from non-bonded interactions, then an equilibrium mixture of columbin and iso-columbin should consist of approximately equal amounts of the two isomers.

Chasmanthin on isomerisation, under the same conditions as for the isomerisation of columbin, is quantitatively converted to palmarin, indicating that the intramolecular strain in palmarin is much less than in chasmanthin. Ring C-δ-lactone in chasmanthin has a high carbonyl infra-red frequency ($1756cm^{-1}$) which indicates that the δ-lactone exists in the quasi-boat conformation. Conversely, ring C δ-lactone of palmarin has an infra-red carbonyl frequency of $1745cm^{-1}$ indicating that the δ-lactone adopts the quasi-chair conformation. When Dreiding models of these two compounds, chasmanthin (54) and palmarin (55) are compared, it can be seen that the intramolecular strain in palmarin is considerably less than in chasmanthin. In both compounds, the furan ring at $C_{(12)}$ has the α-configuration. To maintain the planarity of the lactone system, including $C_{(8)}$, the furan ring in chasmanthin must be involved in serious non-bonded interactions with the hydrogen atoms on $C_{(1)}$ and $C_{(10)}$. These serious interactions disappear in palmarin however, when rings B and C are trans fused and ring C δ-lactone is in the

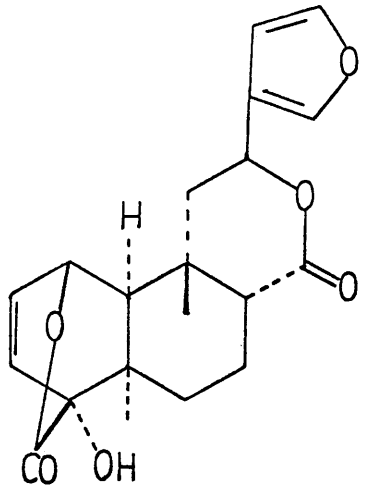
ISO-COLUMBIN.

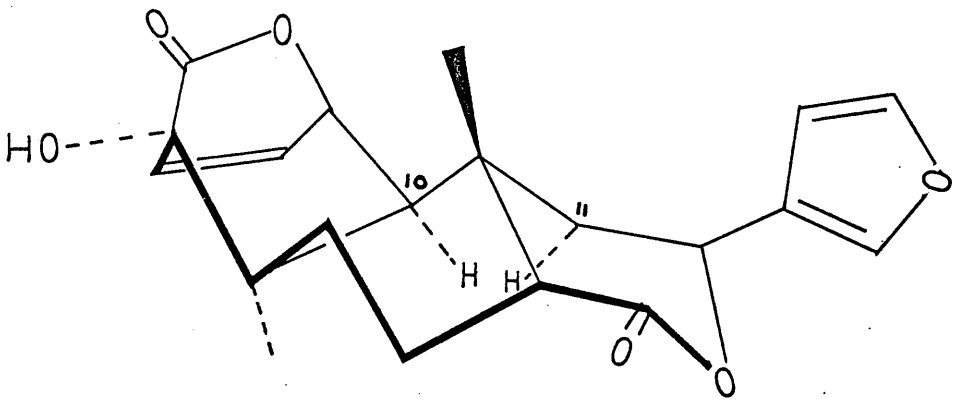




(52)

COLUMBIN.





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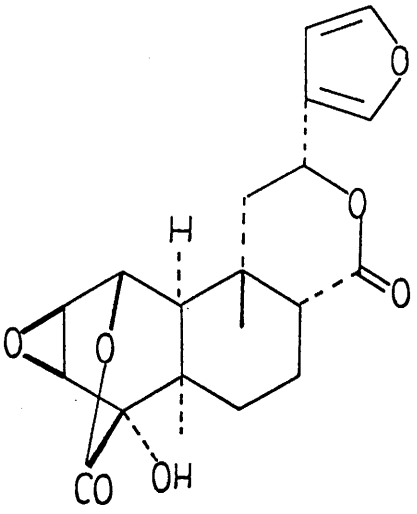
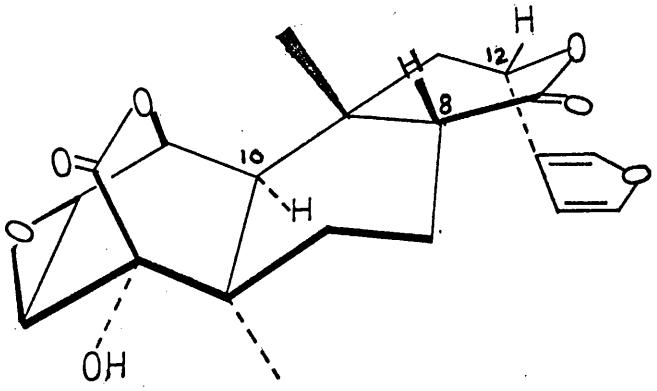
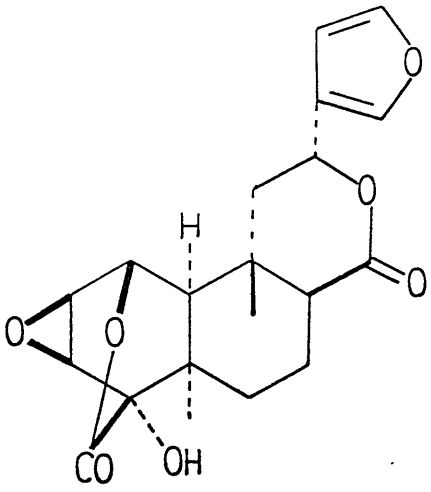
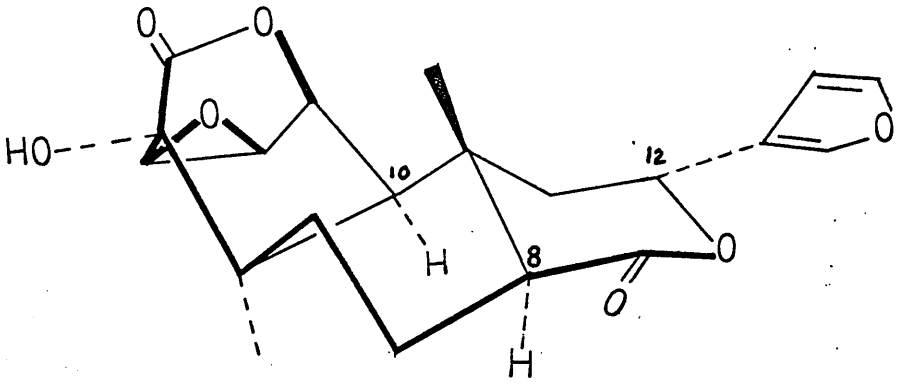


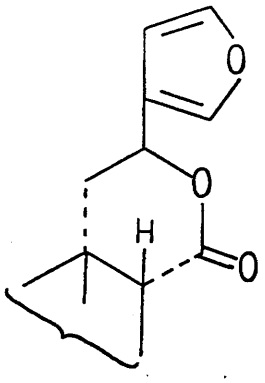


(54)

PALMARIN.







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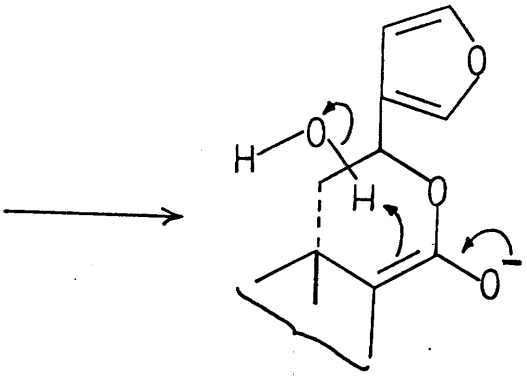

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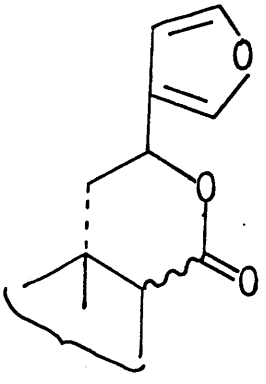


(58)

SECTION IV

DETERMINATION

of the

EPOXIDE CONFIGURATION IN PALMARIN.

INTRODUCTION:-

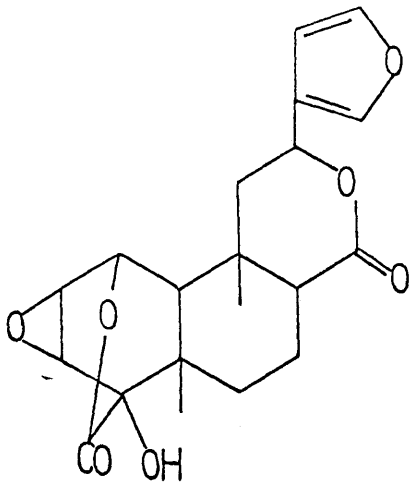
Although columbin is the main constituent of the colombo root bitter principles, two other bitter principles also occur, chasmanthin and jateorin. Owing to their chemical similarity these compounds form mixed crystals in a most tenacious fashion which makes it impossible to obtain pure samples of each using crystallisation techniques. However, if a mixture of colombo root bitter principles rich in chasmanthin is isomerised by mild alkali, palmarin (iso-chasmanthin) and iso-jateorin can be isolated in a pure state by fractional crystallisation from ethyl acetate. A very small amount of palmarin occurs in the root extract but this may well be an artefact arising during extraction.

Wessley[41,45] and Feist[46-51] and their co-workers reported the existence of chasmanthin but much of their work was performed on mixtures of chasmanthin and jateorin. As a result of this, their results on the constitution of chasmanthin, were confusing. Since Barton et al[84] considered that the relationship between chasmanthin and palmarin and between jateorin and iso-jateorin was a simple one and, like that between columbin and iso-columbin, they carried out their degradative studies on palmarin and iso-jateorin. Unlike chasmanthin and jateorin, palmarin and iso-jateorin can be obtained in a pure state fairly readily, by crystallisation.

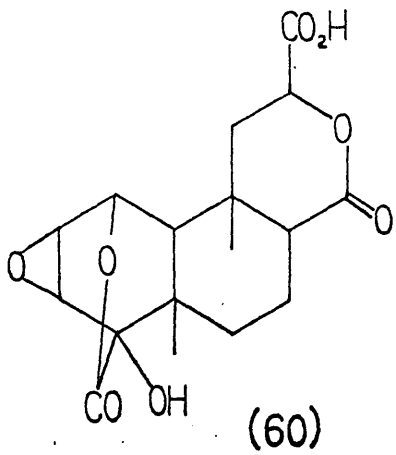
Palmarin ($C_{20}H_{22}O_7$) (59) contains a furan ring, max 1510, 860 cm^{-1}, and two δ-lactone systems, one of which has an infra-red carbonyl frequency more indicative of a γ-lactone, ν_{max} 1775 cm^{-1}. Like columbin, a tertiary hydroxyl group is present which can be methylated with dimethyl sulphate to form a mono-methyl ether. On hydrogenation over palladised charcoal, two products are obtained, a hexa-hydro acid and a neutral tetra-hydro compound. Thus as in columbin hydrogenolysis occurs.

Dehydrogenation of hexa-hydro palmarin with selenium, produced 1,2,5-tri-methyl naphthalene and 1,5 dimethyl-2-naphthoic acid and from this evidence, Feist and his co-workers(deduced that palmarin contained a decalin system. The hexa-hydro acid from palmarin and the octahydro acid from columbin yield the same products on dehydrogenation with selenium, hence columbin and palmarin must have a close structural relationship.

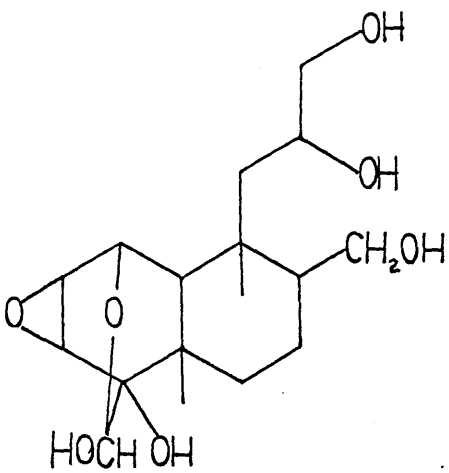
Ozonolysis of palmarin yields a tris-nor acid (60) $C_{17}H_{20}O_8$, which no longer contains the furan ring. An acid, $C_{18}H_{20}O_9$, containing the grouping $-CO.CO_2H$ is also produced showing that the furan ring is mono-substituted and n.m.r. evidence shows that the furan ring is β-substituted. Reduction of tris-nor palmarinic acid with lithium aluminium hydride yields a hemiacetal (61) which on reaction with two moles of sodium periodate oxidises to a keto-formate (62). Oxidation of (62) with chromic acid transforms the hemiacetal group to a δ-lactone (63). From the above evidence, palmarin and columbin probably have the same ring A, hydroxy lactone system and also the same side chain on C(9).


O
O
O
O
CO
OH

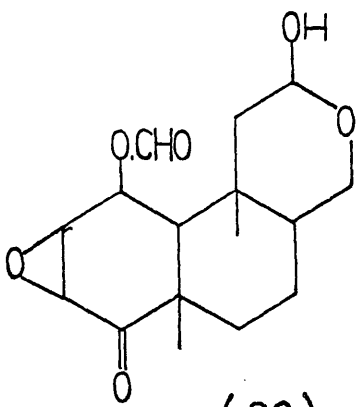
(59)


CO2H
O
O
O
CO
OH

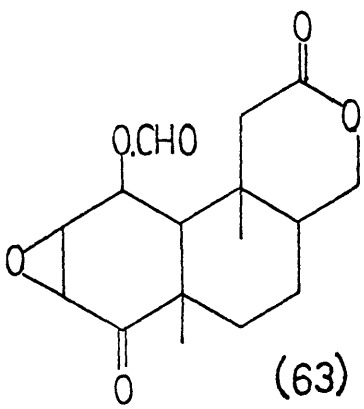
(60)


OH
OH
CH2OH
O
O
HOCH
OH

(61)


OH
O.CHO
O
O
O

(62)

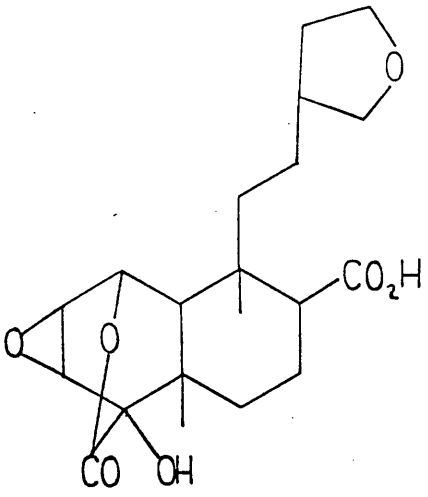

O
O.CHO
O
O
O

(63)

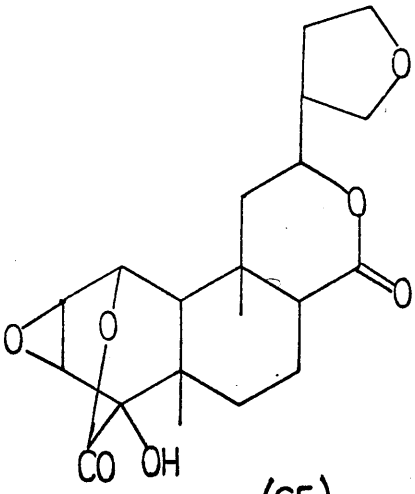
Iso-jateorin, when subjected to the same series of reactions as (59) - (63), also yielded the keto formate, δ-lactone (63) which indicates that palmarin and iso-jateorin differ only in the configuration at C(12). This result was confirmed by hydrogenation of palmarin and iso-jateorin over palladised charcoal, where, in both cases, the same hexa-hydro acid (64) was produced but the neutral tetrahydro-compounds produced (65) were not identical. The hexa-hydro acids must arise from hydrogenolysis of the ring C, δ-lactone system which, by removing $C_{(12)}$ as an epimeric centre, ensures that the hexa-hydro acids derived from palmarin and iso-jateorin are identical. No hydrogenolysis, however, occurred during the formation of the tetrahydro-derivatives and hence these two compounds must be $C_{(12)}$ epimers.

Palmarin differs by one oxygen atom from columbin, this oxygen function being inert. At first, it was considered[85] that this inert oxygen function was in a five or a six membered ether ring but Barton et al[86] showed that this oxygen atom was part of a 2,3 epoxide system. When palmarin is converted to the keto, formate lactone (63) and treated with sodium carbonate, (63) rearranges to a lactone alcohol (66). Reduction of (66) with chromous chloride forms the α,β-unsaturated ketone (67) demonstrating the presence of an 2,3-epoxy ketone group in (63) and hence in palmarin.

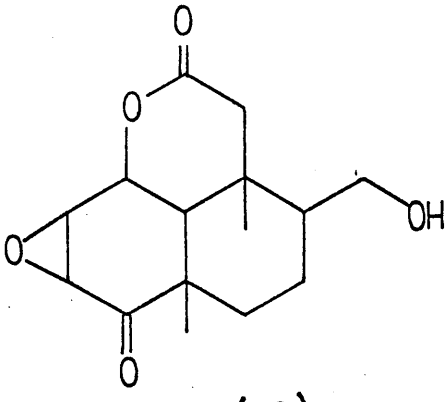
When palmarin is treated with lithium aluminium hydride, the ring A δ-lactone system is reduced only as far as the hemiacetal stage, compound (68.) being formed. Cleavage of (68)



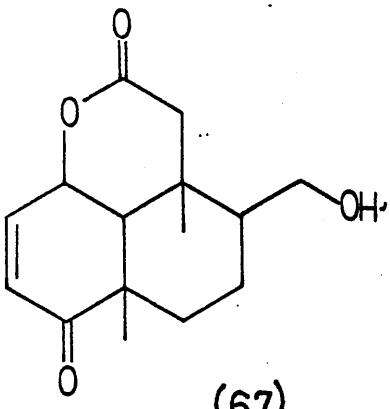
(64)



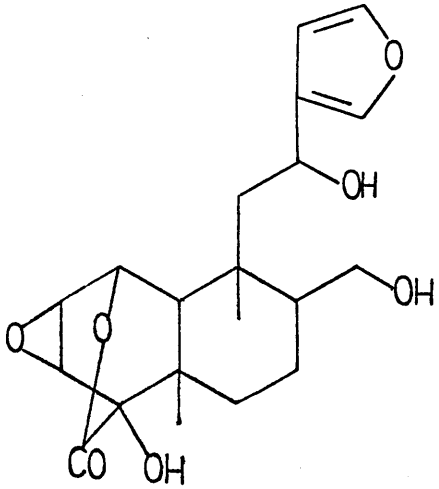
(65)



(66)



(67)

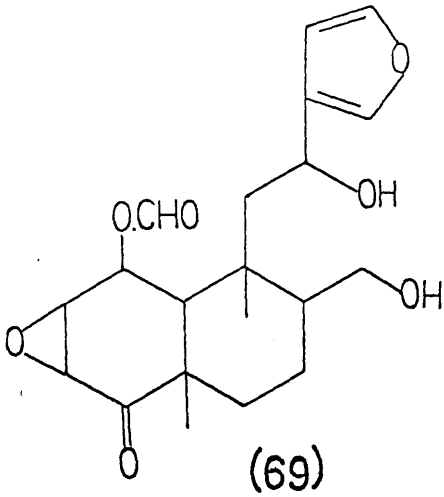


(68)

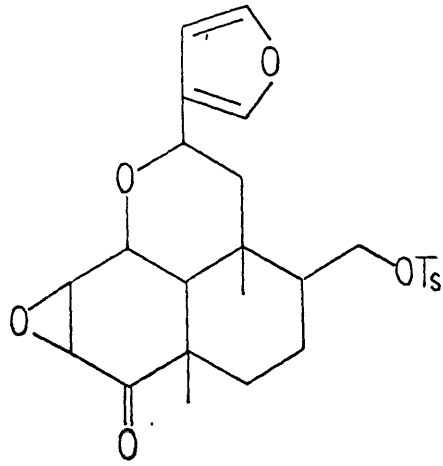
to the keto formate (69) and treatment of (69) with p-toluene sulphonyl chloride in pyridine forms an anhydromono-tosylate (70). This must arise from solvolysis of an initially formed di-tosylate which produces an incipient carbonium ion α to the furan ring, which then cyclises intra-molecularly with the suitably situated hydroxyl group on C(1). The mono-tosylate (70) on treatment with chromous chloride was reduced to an α,β unsaturated ketone (71) showing, as before, that the 'inert' seventh oxygen atom of palmarin was contained in a 2,3-epoxide system.

When iso-columbin was subjected to the same series of reactions, without chromous chloride reduction, a product was obtained which, although not pure, seemed to correspond to the compound analogous to (71) derived from iso-jateorin. This was confirmed by using dihydro iso-columbin and subjecting it to the same series of reactions as iso-columbin where the end product is identical with the reduced α,β-enone (72) derived from iso-jateorin. Thus, although palmarin and iso-columbin have the same hydroxy lactone system in ring A, the configuration at C(12) is different.

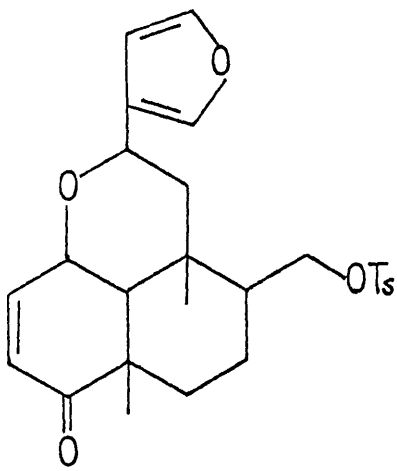
The stereochemistry of palmarin can be represented as (75), the only underfined portion being the configuration of the 2,3-epoxide ring. An interesting feature of palmarin is its extremely high δ-lactone frequency in ring A νmax 1775 cm^{-1} which is more indicative of a γ-lactone. This high frequency must result from the strain involved in the 2,3-epoxy 1,4-δ-lactone (boat) ring A system. The epoxide ring is insensitive



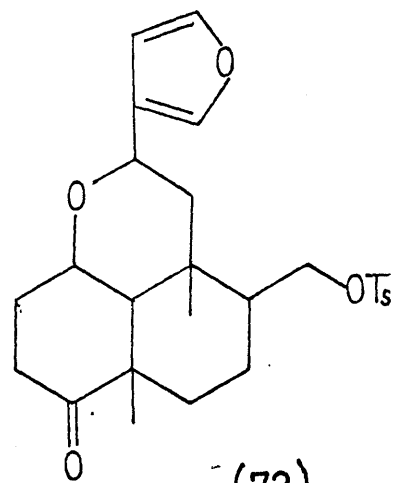
(69)



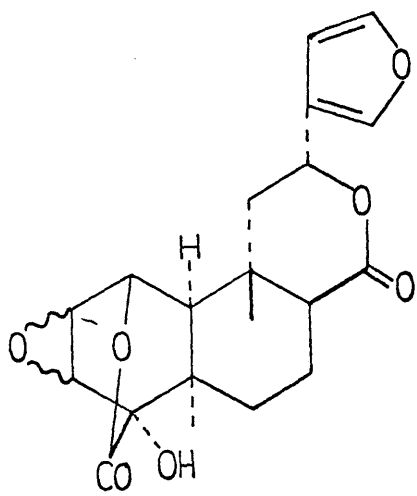
(70) $Ts = CH_3C_6H_4.SO_2-$



(71)



(72)



(73)

to both lithium aluminium hydride and mineral acid which suggests that the close proximity of the lactone bridge protects it from attacking groups. This close proximity of lactone bridge and epoxide must contribute to the resulting strain to cause the high lactone carbonyl infra-red frequency.

DISCUSSION.

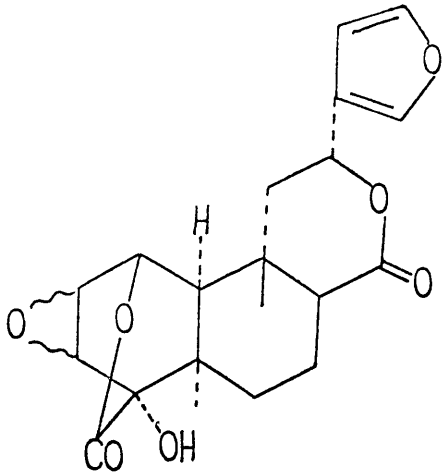
The structure of palmarin has been shown to be as in [73][84][86], the only unknown feature of stereochemistry being the configuration of the epoxide ring.

The palmarin epoxide ring is inert to attack by lithium aluminium hydride and mineral acid, and must therefore be heavily shielded by neighboring groups in the molecule. The δ-lactone bridge in ring A is also resistant to reduction by lithium aluminium hydride and hydrolysis by mineral acid which may indicate that the lactone and the expoxide mutually shield each other from attack. If the epoxide were β-oriented, then the ring A δ-lactone bridge would effectively block any approach of an attacking group from the β-face of the molecule while the $C_{(5)}$ α-methyl group would, to some extent, prevent attack from the α-face. If, however, the epoxide had the α-configuration a similar situation would exist; the $C_{(5)}$ α-methyl group would prevent attack at the α-face and the lactone bridge would shield the epoxide from attack at the β-face. An α-epoxide would not, however, shield the δ-lactone bridge from attack and hence it was suspected that the epoxide was β-oriented. It was hoped that, by using nuclear magnetic resonance (n.m.r.) spectroscopy, the configuration of the epoxide could be determined.

When palmarin or a derivative still containing the ring A δ-lactone bridge intact is reduced with lithium aluminium hydride, the lactone in ring A is reduced only to the hemiacetal stage to furnish (74). It was thought that the $C_{(9)}$ β-methyl group would shield the lactone group from attack by hydride ion, forcing attack to take place on the opposite side of ring A.

If the epoxide ring were β-oriented then the n.m.r. signal of the hemiacetal proton should be moved downfield by the ring current of the epoxide since this proton would be forced into close proximity to the epoxide ring (75)[87]. However, if the hydride ion attack did not occur as predicted and the hydroxyl group of the hemiacetal was oriented in the direction of the epoxide ring, then there would be evidence of hydrogen bonding in the infra-red spectrum. From examination of Dreiding models, the epoxide oxygen atom and the oxygen atom of the hemiacetal hydroxyl group are ca.2.6Å apart which is close enough for hydrogen bonding to occur. If the epoxide had the α-configuration then the $C_{(5)}$ α-methyl group would be deshielded by ca.0.07 [89], and the hemiacetal proton would be unaffected. In order to show that deshielding by the epoxide group had occurred, the epoxy hemaicetal was to be compared with the corresponding hemiacetal from dihydro-iso-columbin (76).

Palmarin (73) on ozonolysis in ethyl acetate at $-10^{\circ}C$ yields a tris-nor acid (77) which, on reduction by lithium aluminium hydride, furnishes a crystalline tetrol hemiacetal (78).



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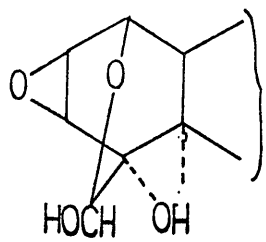

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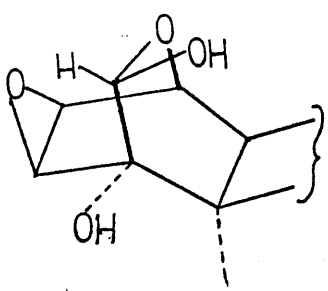

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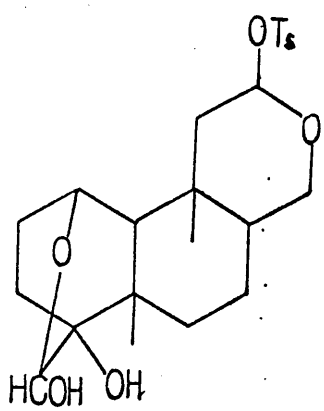
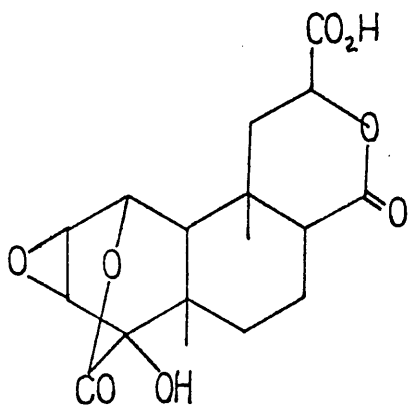


$Ts = -O_2S-C_6H_4.CH_3$

(76)



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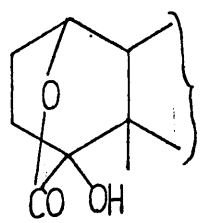
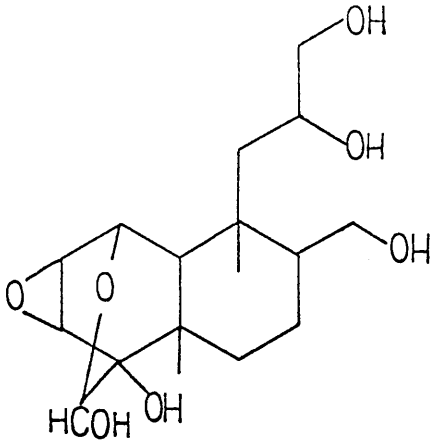


(77a)

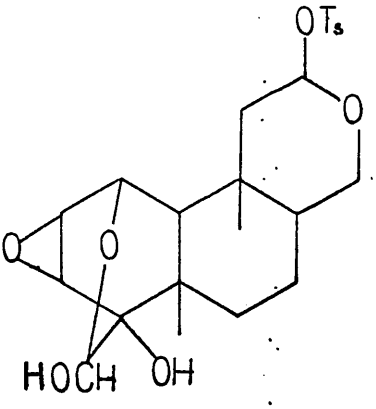
Owing to the low solubility of this compound in organic solvents it was converted to the p-toluene sulphonate by treatment with p-toluene sulphonyl chloride in pyridine at room temperature for two hours. Chromatography of the total reaction product over grade IV neutral alumina eluted a mono-tosylate, formulated as (79), the eluant being benzene:ether (1:1.) This mono-tosylate was a clear gum which decomposed when attempts were made to purify it for analysis. The n.m.r. spectrum of this compound in deutero-chloroform showed the presence of four aromatic protons(2.1-2.7τ), and a methyl group at 7.52 τ which must be the methyl group of the p-toluene sulphonate. The two other methyl groups in the molecule at $C_{(5)}$ and $C_{(9)}$ have signals at 8.74 and 9.03 τ respectively and the spectrum integrated for the desired number of thirty protons.

The hemiacetal proton signal should appear as a singlet and such a signal occurs at 5.06 τ which does not disappear on equilibration with D_2O and hence this is not a hydroxyl proton occurring at low field. As a suitable analysis could not be obtained for this compound, this approach to the problem was abandoned. However, the appearance of a singlet signal at 5.06 τ lower than the value expected for such a proton (ca.5.3τ)[88] seems to indicate that the epoxide ring is β-oriented.

Further attempts were made to obtain a suitable compound from the tetrol hemiacetal for which a suitable analysis could be obtained. Ortho-esterification of the triol system,



(78)



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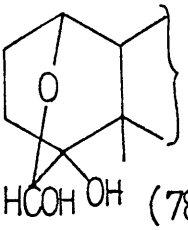
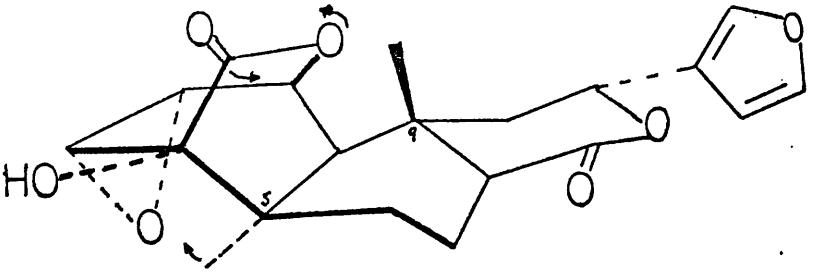


(78a)



(80)

obtained by ring C reductive cleavage was attempted but met with no success. The acetonide of the $C_{(12)}$,$C_{(13)}$ diol system was prepared, but this compound was also a gum which decomposed on attempted purification.

Palmarin and iso-jateorin differ only in their stereochemistry at $C_{(12)}$ but iso-jateorin is much more soluble in chloroform and also in deuterochloroform, consequently the n.m.r. spectrum of iso-jateorin and dihydro-iso-columbin were compared. It was thought that if the epoxide ring were α, then the α-methyl group at $C_{(5)}$ would be close enough, ca. 2Å, to the epoxide ring to be deshielded[89]. By analogy with iso-columbin[78], ring B in the palmarin molecule and also iso-jateorin would be in the boat conformation and hence there would be a bowsprit interaction between the $C_{(6)}$ axial hydrogen atom and the $C_{(9)}$ methyl group. This interaction could be relieved, to some extent, by upward rotation of $C_{(5)}$ and slight twisting of the ring A δ-lactone bridge which would move the $C_{(5)}$ α-methyl group towards the α-epoxide system (80). The amount of deshielding expected was ca. 0.1 [89], however, no such downfield shift of the $C_{(5)}$ methyl group in iso-jateorin was observed; see Table 1. This negative evidence could indicate that the epoxide ring had the β-configuration.

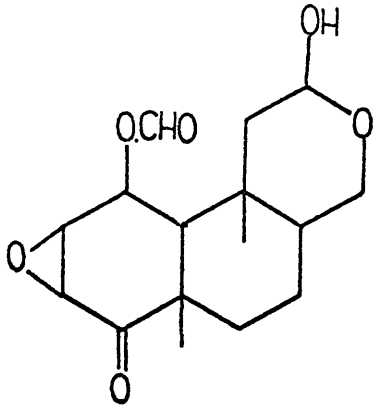
Since it is known[90] that epoxide rings will shield protons suitably situated in close proximity above them, the line of approach was altered to bring the $C_{(9)}$ methyl group nearer to the epoxide ring. If the ring A lactone bridge is

opened to a keto-formate[84], the formate ester will become equatorial to avoid interaction with the $C_{(9)}$ methyl group. This will force ring A into the boat conformation and will bring the $C_{(9)}$ methyl group close to the epoxide ring (80).

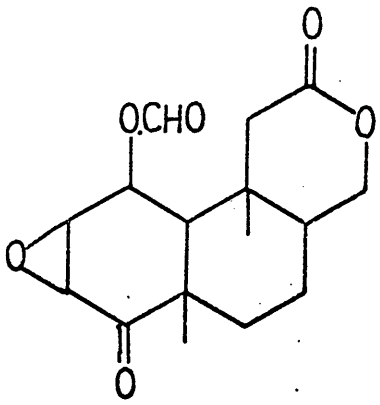
When the tetrol-hemiacetal (78) is cleaved with sodium periodate, the ring A hemiacetal bridge is opened to form the hemiacetal-keto-formate (81), which, on oxidation with chromium trioxide in pyridine, produces the formate lactone (82). Examination of Dreiding models of this keto-formate lactone indicated that the $C_{(9)}$ methyl group is sufficiently close to the β -epoxide ring to be shielded by it[90], and hence by comparing the n.m.r. spectra of (82) and the corresponding compound derived from dihydro-iso-columbin (83) it was hoped to observe an upfield shift of the $C_{(9)}$ methyl group signal in (82).

The expected upfield shift was 0.6τ[90] but a shift of only ca. + 0.1τ was observed (Table 1.) Further consideration of Dreiding models showed that ring A, which is fairly flexible, can be deformed so that the interaction between the β -epoxide ring and the $C_{(9)}$ methyl group can be reduced by bending the interacting groups apart. This deformation of ring A would account for the observed small shielding of the methyl group.

When the epoxy-formate lactone (82) is hydrolysed with sodium carbonate, there results the lactone alcohol (84b) which was converted into the acetate (84a). Formation of this new lactone ring imposes greater rigidity on ring A and, from



(81)



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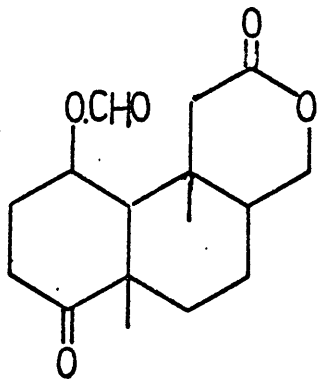

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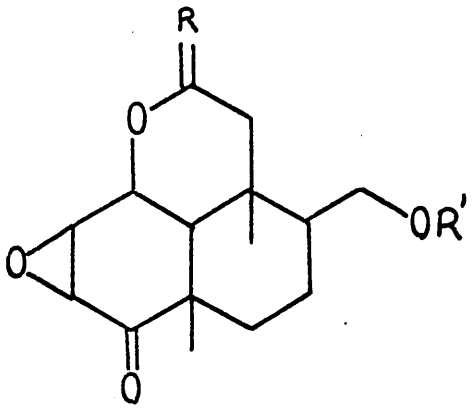
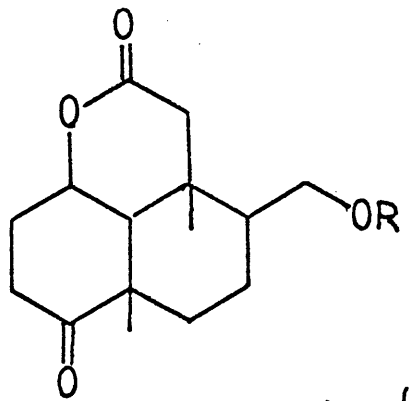


(84) (a) R = O ; R' = $O.COCH_3$

(b) R = O; R' = H

(c) R = H, OH; R' = H

(d) R = O; R' = $O.COCH_2Cl$



(85) (a) R = H

(b) R = $COCH_3$

Dreiding models, the $C_{(9)}$ methyl group is forced almost on top of the β-epoxide. It was considered that, if the epoxide was β-oriented, then this compound (84a) would surely show shielding of the $C_{(9)}$ methyl group, as compared with the corresponding compound derived from dihydro-iso-columbin (85). However, as in the formate lactones (82) and (83), the shielding effect was only ca. + 0.1 (Table 1.). From this result, the epoxide could be in either the α- or the β-orientation, but if it has the β-configuration, then ring A must be considerably deformed in order to separate the $C_{(9)}$ methyl group and epoxide sufficiently, to reduce the shielding of the methyl group to such a low level. However, consideration of the proton signals of $H_{(1)}$, $H_{(2)}$, $H_{(3)}$ and $H_{(10)}$ and their coupling constants, indicated that the epoxide ring was β-oriented.

The protons ($H_{(1)}$, $H_{(2)}$, $H_{(3)}$ and $H_{(10)}$) form an AMNX system in the lactone acetate (84a) which has the following characteristics:-

$H_{(1)}$ a double doublet at 4.66 τ ($J_{1,2}$ = 3.4 cps. $J_{1,10}$ = 9 cps.)

$H_{(2)}$ a triplet at 6.21τ ($J_{1,2}$ = 3.4 cps. $J_{2,3}$ = 3.6 cps.)

$H_{(3)}$ a doublet at 6.62 τ ($J_{2,3}$ = 3.6 cps.)

$H_{(10)}$ a doublet at 7.81 τ ($J_{1,10}$ = 9 cps.)

The coupling of the epoxide protons $J_{2,3}$ = 3.6 cps. is of the order of magnitude expected for such protons. The methylene group of the primary acetate appears as the A B part of an

TABLE 1.

Compound	$C_{(9)}$ methyl n.m.r. signal τ	$C_{(5)}$ methyl n.m.r. signal τ
dihydroformate (83)	8.61	8.92
epoxy-formate (82)	8.72	8.98
dihydro lactone acetate (85b)	8.69	9.04
epoxy lactone acetate (84a)	8.78	9.10
epoxy lactone ester (86a)	8.78	9.05
iso-jateorin (91)	8.61	8.94
dihydro-iso-columbin (30)	8.60	8.91

ABX system at 6.1 τ which tended to interfere with the $H_{(1)}$ triplet at 6.21 τ so the corresponding methyl ester (86 a) was prepared.

The methyl ester (86 a) contains the same AMNX system as the lactone acetate (84 a):-

$H_{(1)}$ double doublet at 4.67 τ ($J_{1,2}$ = 3.6 cps. $J_{1,10}$ = 9 cps.)

$H_{(2)}$ triplet at 6.26 τ ($J_{1,2}$ = 3.6 cps. $J_{2,3}$ = 3.8 cps.)

$H_{(3)}$ doublet at 6.63 τ ($J_{2,3}$ = 3.8 cps.)

$H_{(10)}$ doublet at 7.73 τ ($J_{1,10}$ = 9 cps.)

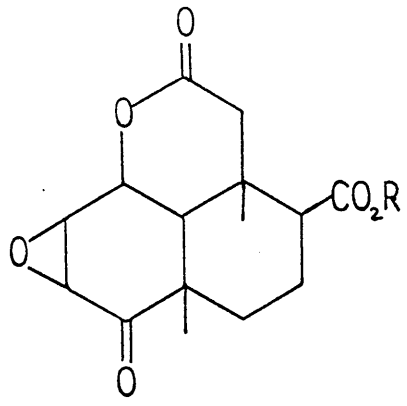
These assignments were confirmed by double irradiation experiments, irradiating at $H_{(1)}$ and $H_{(2)}$. Irradiating at $H_{(1)}$ causes $H_{(10)}$ to collapse to a singlet and $H_{(2)}$ to a doublet (residual coupling with $H_{(3)}$ J = 3.6 cps.). Irradiation at $H_{(2)}$ causes $H_{(1)}$ to collapse to a doublet (residual coupling $J_{1,10}$ = 9 cps.). (8 7).

When Dreiding models of the α-and β-epoxides are studied several interesting differences between these two compounds are revealed. With ring A in the half-chair conformation and the molecule undistorted, the dihedral angles between $H_{(1)}$, $H_{(2)}$ and $H_{(10)}$ are:-

α-epoxide = $\theta_{H_1H_{10}} = 55^\circ$ $\theta_{H_1H_2} = 105^\circ$

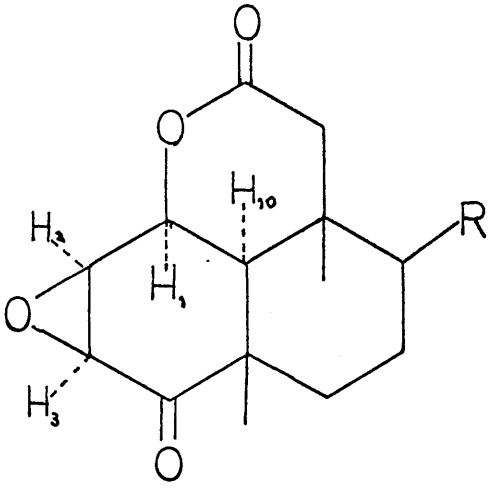
β-epoxide = $\theta_{H_1H_{10}} = 65^\circ$ $\theta_{H_1H_2} = 65^\circ$

The calculated[89,91] coupling constants for these angles are:- $J_{H_1H_{10}}$ = 4 cps. [89,91] $J_{H_1H_2}$ = 0.5 cps. for the α-epoxide, and, $J_{H_1H_{10}}$ = 2 cps. $J_{H_1H_2}$ = 1 cps. for the β-epoxide.

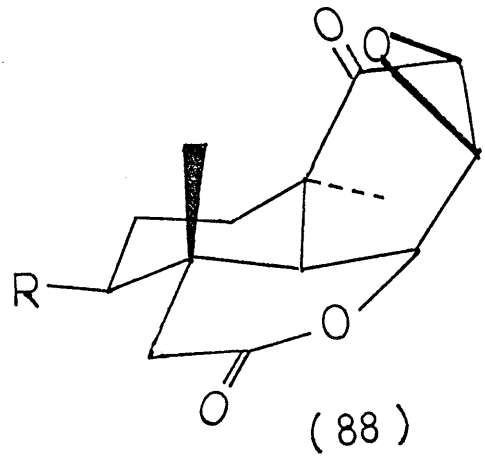


(a) R = CH_3

(86) (b) R = H



(87)



(88)

The observed coupling constant of J = 9 cps. for the coupling between $H_{(1)}$ and $H_{(10)}$ can only be accounted for if there is severe distortion of ring A. If the epoxide were β-oriented, then such distortion could occur to relieve the severe non-bonded interaction between the methyl group and the β-epoxide. This interaction can be relieved by an upward rotation of $C_{(1)}$ until $H_{(1)}$ $H_{(10)}$ is approximately 25°, which would force the epoxide well away from the $C_{(9)}$ methyl group (88) but would markedly distort the bond angles in ring A. This molecular distortion must flatten ring A so that the bond angles $C_{(1)}$ $C_{(2)}$ $C_{(3)}$, $C_{(10)}$ $C_{(1)}$ $C_{(2)}$, $C_{(2)}$ $C_{(3)}$ $C_{(4)}$ and $C_{(3)}$ $C_{(4)}$ $C_{(5)}$ will approach 120° rather than the tetrahedral value of 109.5°; in this situation H_1H_2 is approximately 35°. The calculated coupling constants for such dihedral angles are $J_{1,10}$ = 8 cps., $J_{1,2}$ = 3.5 cps. which are in reasonable agreement with values actually observed.

The α-epoxide does not have the same driving force towards distortion, for although there would be some interaction between the $C_{(9)}$ methyl group and the β-hydrogen atoms on $C_{(2)}$ and $C_{(3)}$(mainly with the $C_{(3)}$ hydrogen atom) this interaction would not be sufficient to cause the severe distortion observed. The calculated values of the coupling constants for the undistorted conformation ($J_{1,10}$ = 2 cps., $J_{1,2}$ = 1 cps.) would thus be expected to occur, since the distortion of ring A would be slight.

The same AMNX system, $H_{(1)}$, $H_{(2)}$, $H_{(3)}$ and $H_{(10)}$, appears in the n.m.r. spectrum of the epoxy-formate-lactone (86) but this spin-system is further complicated by the presence of the aldehydic proton of the formate group. This spin-system has the following characteristics.

$H_{(1)}$ octet 3.87 τ ($J_{1,2}$ = 3.4 cps. $J_{1,10}$ = 9 cps. $J_{1,17}$ ca. 1cps.)

$H_{(2)}$ triplet 6.40 τ ($J_{1,2}$ = 3.4 cps. $J_{2,3}$ = 3.6 cps.)

$H_{(3)}$ doublet 6.65 τ ($J_{2,3}$ = 3.6 cps.)

$H_{(10)}$ doublet 7.87 τ ($J_{1,10}$ = 9 cps.)

Double irradiation experiments to confirm these assignments were also performed on this compound.(75b) Irradiation, at $H_{(10)}$ causes the $H_{(1)}$ signal to collapse to a doublet coupling $J_{1,2}$ = 3.4 cps., and on irradiation at $H_{(2)}$ the $H_{(1)}$ signal collapses to a doublet, $J_{1,10}$ = 9 cps. The $H_{(10)}$ and $H_{(2)}$ signals collapse to a singlet and a doublet ($J_{2,3}$ = 3.6 cps.) respectively on irradation at $H_{(1)}$. These supplementary results also indicate that the epoxide has the β-configuration.

The n.m.r. spectrum of iso-jateorin on re-examination also contained information showing that the iso-jateorin and hence palmarin had a β-epoxide:

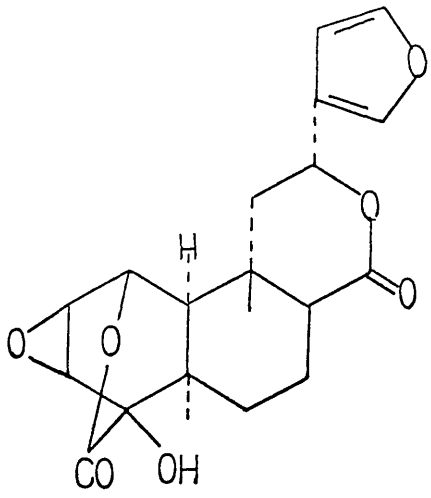
$H_{(1)}$ double doublet at 5.15 τ ($J_{1,10}$ = 4 cps., $J_{1,2}$=2 cps.). This implies that the dihedral angles $\theta_{H_1, H_{10}}$ is ca.50°, and $\theta_{H_1H_2}$ is ca. 60°. A Dreiding model with the epoxide in the α-configuration indicates that the dihedral angle θ_{H_1, H_2} is ca. 20° which would result in a coupling constant $J_{1,2}$ ca. 7 cps.

From the evidence overleaf it is concluded that the epoxide in palmarin is β-oriented and hence the complete stereochemistry of palmarin can be represented as in (89).

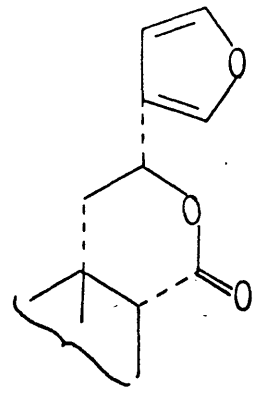
Palmarin (89), chasmanthin (90) and iso-jateorin (91) have high δ-lactone ring A carbonyl frequencies in the infra-red: $\nu_{max}^{CCl_4}$ 1778, 1776, 1778 cm^{-1} respectively, compared with the corresponding lactone carbonyl frequency in columbin of 1762 cm^{-1}. This difference in infra-red frequencies must be a result of the strain introduced into ring A by the interaction between the β-2,3-epoxide and the lactone bridge.

Although the n.m.r. evidence from the formate lactone (82), the lactone acetate (84), the methyl ester (86) and iso-jateorin (91) indicated that the epoxide group was β-oriented, it was decided to confirm these conclusions and also study the distortion in ring A by conducting an X-ray analysis of a suitable derivative of palmarin.

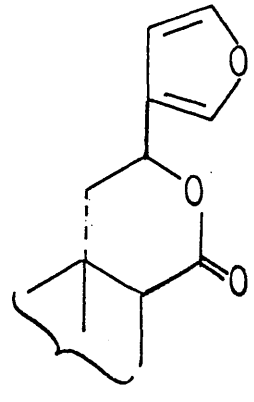
The preparation of the cesium salt of tris-nor palmarinic acid (77) was first attempted. Unfortunately, crystallisation from a variety of solvents yielded only very fine needles, too small to be used in an X-ray analysis. The acid-chloride of tris-nor palmarinic acid was prepared from the sodium salt and the p-iodo anilide was formed. No crystalline amide was isolated, however. Treatment of the sodium salt of the tris-nor acid with p-bromo-S-benzyl thiuronium bromide yielded the corresponding salt of the acid. On slow evaporation from aqueous ethanol, small white



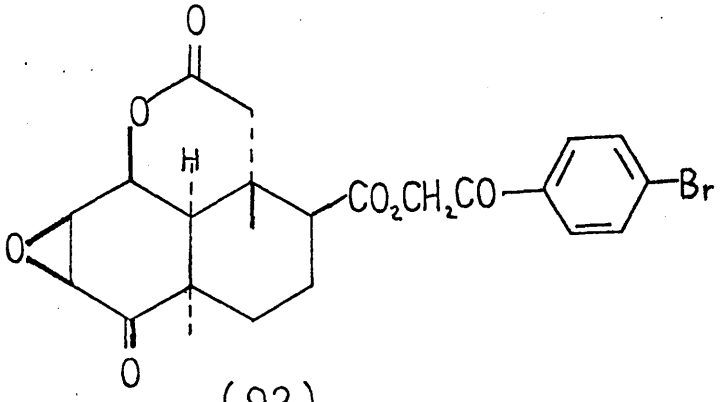
(89)



(90)



(91)



(92)

needles were grown m.p. 279-281°C but these were too small for an X-ray analysis.

Derivatives of the lactone alcohol (84b) were next attempted. Treatment of this alcohol with chlor-acetyl chloride overnight at room temperature produced a chloracetate which, on refluxing with sodium iodide in acetone, was transformed into an iodo-acetate. Slow crystallisation from a variety of solvents produced only very fine needles, too small to be suitable for an X-ray analysis. A needle, obtained by crystallisation from acetone/petrol was mounted and an X-ray oscillation photograph was taken, but the spots on the film were split showing that the crystal was not single. All the crystals of this derivative were split in this fashion.

The lactone acid (86a) was converted to the acid chloride and the p-iodo anilide prepared. However, only fine plates could be obtained on crystallisation which, unfortunately, had an irregular stacking pattern. This derivative was abandoned.

Refluxing the sodium salt of the lactone acid (86 a) with p-bromo phenacyl bromide yielded the corresponding ester. Slow crystallisation over seven days from a chloroform-toluene mixture produced stout rods m.p. 252-3°C which were suitable for an X-ray structural analysis. This analysis confirmed the stereochemistry as (92) and hence the stereochemistry of palmarin as (89).

X-ray Analysis of the p-bromo phenacyl derivative of (86a)

From the n.m.r. spectrum of (86b) it was postulated that there must be considerable distortion of ring A. A convincing cause for this distortion exists if the 2,3-epoxide is β-oriented but not if the epoxide is α-oriented. In order to confirm the stereochemistry and to determine the degree of molecular distortion, an X-ray analysis was conducted on the p-bromo phenacyl derivative of (86a).

Crystals of this derivative are orthorhombic with four molecules($C_{23}H_{23}O_7Br$) to the unit cell of dimensions:

$a=10.76$, $b=8.95$, $c=21.79\text{Å}$.

From systemic absences and Harker sections the space group was determined as $P_{2_1}2_12_12_1$.

Equi-inclination and zero layer Weissenberg photographs were taken with CuKα radiation, and three dimensional intensity data were measured visually for the layers $0k\ell$, $1k\ell$--- $9k\ell$. 1900 intensity data were measured.

The position of the bromine atom was found by the Patterson vector method but since the bromine atom lies close to $y=1/4$ the first electron density distribution, derived from the observed data and the "heavy" atom phases, was complicated by the presence of pseudo mirror planes. However, by careful selection of the atomic sites from this map and consideration of intermolecular contacts, the complete structure was revealed

Four further electron density distribution calculations located all atomic sites and the dis crepancy factor (R) was 0.21 at this stage.

After four cycles of isotropic least squares calculations with all planes being given equal weight a difference fourier synthesis was computed. This, revealed the positions of seventeen hydrogen atoms which were included in the anisotropic least squares calculations. Eight cycles of least squares calculations reduced the valuee of R to 0.078, and R' to 0.011. The refinement was now concluded.

The weighting scheme used during anisotropic least squares calculations was;

$$w = 1/\left(p_1 + |F_o| + p_2 |F_o|^2 + p_3 |F_o|^3\right)^{\frac{1}{2}}$$

where p_1, p_2 and p_3 are parameters which were altered during the process of refinement.

Stereochemistry of the p-bromo phenacyl ester of (86 a):

The stereochemistry of this derivative was predicted from nuclear magnetic resonance spectroscopic evidence, obtained from the methyl ester of(86b).

From this n.m.r. evidence, it was expected that ring A of the compound must be severly distorted, and to explain the distortion involved, an upward rotation of $C_{(1)}$ was postulated. This would have the effect of flattening ring A and increasing the distance between the $C_{(14)}$ β -methyl group and the epoxide ring. These predictions have now been confirmed by an X-ray analysis of the p-bromo phenacyl ester of(86) and the configuration of the epoxide ring in palmarin is now shown to be β.

The bond lengths and angles of the derivative are shown in (93) and (94) respectively.

The upward rotation of $C_{(1)}$ has caused a flattening of ring A to such an extent that the bond angles $C_{(10)}$ $C_{(1)}$ $C_{(2)}$= 120.1°, $C_{(1)}$ $C_{(2)}$ $C_{(3)}$ = 120.6°, $C_{(2)}$ $C_{(3)}$ $C_{(4)}$ = 119.4°. As $C_{(4)}$ is trigonally hybridised, having $C_{(4)}$ carbonyl group, the value of 117.5° is slightly reduced from the expected value of 120°. In ring A $C_{(1)}$ $C_{(2)}$ $C_{(3)}$ $C_{(4)}$ are thus strictly planar, the equation of the plane being given by:
+ 0.1402 X - 0.7302 Y - 0.6687 Z + 4.6435 = 0. The extent of ring A distortion is shown by $C_{(14)}$··$C_{(2)}$ = 3.57Å, $C_{(9)}$··$C_{(1)}$ = 2.61Å, $C_{(1)}$··$C_{(3)}$ in a normal cyclohexane chair is 2.53Å. The same upward rotation of $C_{(1)}$ also flattens the lactone ring slightly

making $C_{(1)}$ $C_{(10)}$ $C_{(11)}$ $C_{(12)}$ $O_{(4)}$ planar, $C_{(9)}$ is the only atom in the lactone ring out of plane. The equation of the plane is given by:

$$+ 0.8379\ X - 0.4188\ Y - 0.3500\ Z - 3.7289 = 0$$

The distortion of the bond angles also has an effect on the bond lengths in this region of the molecule. C_1-C_2, C_2- C_3, C_3- C_4, C_4- C_5 are 1.444, 1.449, 1.496, 1.499Å respectively, all shorter than the theoretical distance of 1.54Å. The shortening of these bonds helps to increase the distance between the $C_{(9)}$ β-methyl group and the epoxide ring.

The C – O bonds in the epoxide ring are not of identical length, $C_{(2)}$ - O = 1.451Å, $C_{(3)}$ - O = 1.475Å and hence the epoxide ring must have a slight twist to it which makes the dihedral angle between $H_{(2)}$ and $H_{(3)}$ approximately 35° which was expected from consideration of the n.m.r. coupling constant $J_{2,3}$ = 3.8 cps.

The lactone ring is in the quasi-chair conformation, with the system $C_{(1)}$ $C_{(10)}$ $C_{(11)}$ $C_{(12)}$ $O_{(4)}$ planar.

In order to reduce the severe non-bonded interaction between the $C_{(9)}$ methyl group and the epoxide ring, the $C_{(14)}$ methyl group is repelled towards C_{11} of the lactone ring as can be seen from consideration of the bond angles around $C_{(9)}$: $C_{10}C_9C_{14}$ = 118.6°, $C_{14}C_9C_8$ = 113°.

The severe non-bonded interaction between $C_{(14)}$ and the epoxide oxygen is reflected in their separation, 2.92Å.

The $C_{(5)}$ methyl group is not subject to any such non-bonded interactions since the bond angles around $C_{(5)}$ involving the methyl group are normal.

Ring B is in the chair conformation the best plane through the ring being given by:

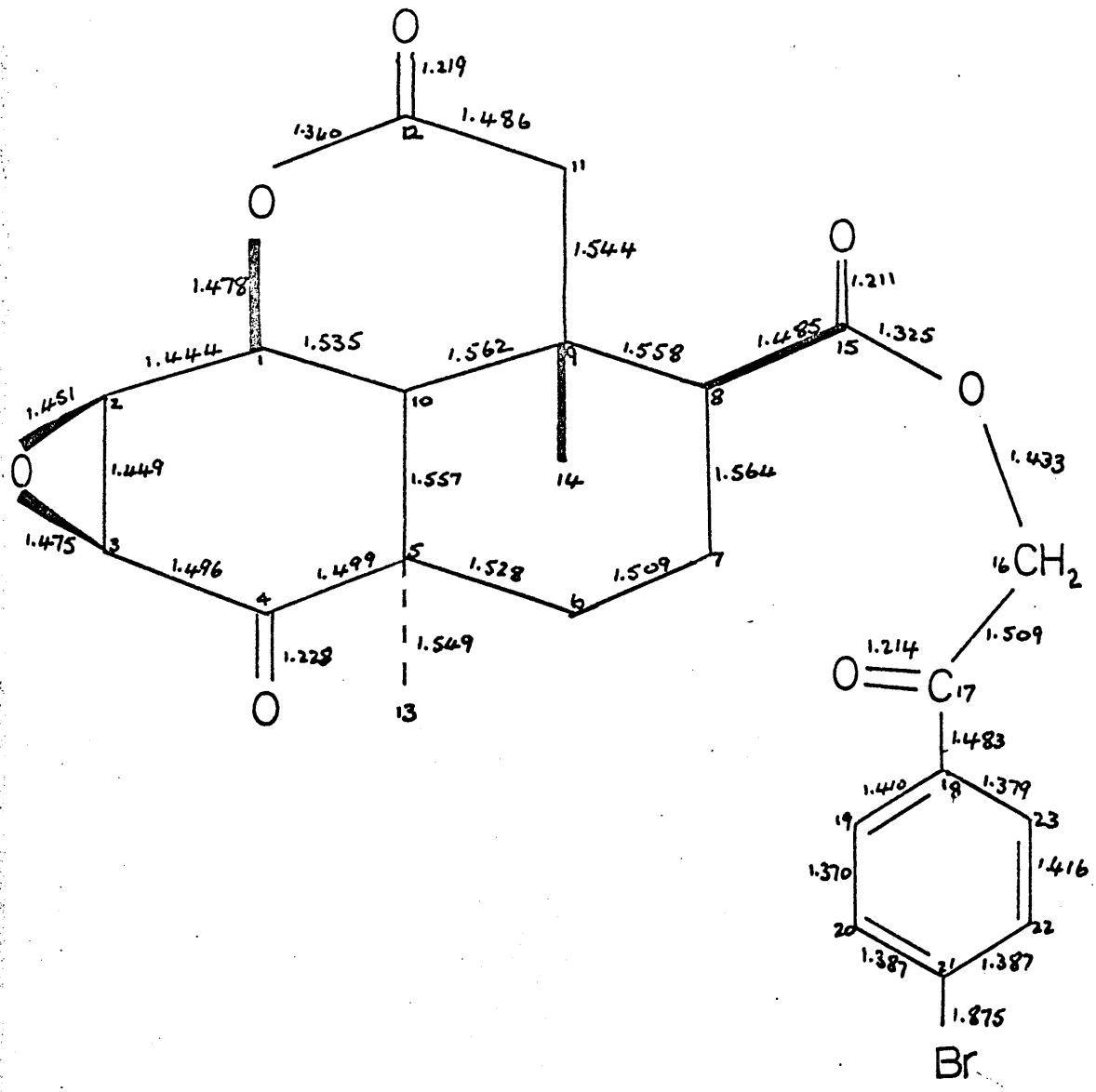
$$0.9541\ X + 0.0633\ Y - 0.2928\ Z - 7.1476 = 0$$

$C_{(6)}$	+ 0.0152	
$C_{(7)}$	- 0.0152	Since there is an alternation, above
$C_{(9)}$	+ 0.0147	and below the plane, through ring B,
$C_{(10)}$	- 0.0147	of the atoms $C_{(6)}$ $C_{(7)}$ $C_{(9)}$ $C_{(10)}$, then ring B must be in the chair conformation.

The benzene ring is planar with the carbonyl $C_{(17)}$ function lying 4.6^{o} off this plane.

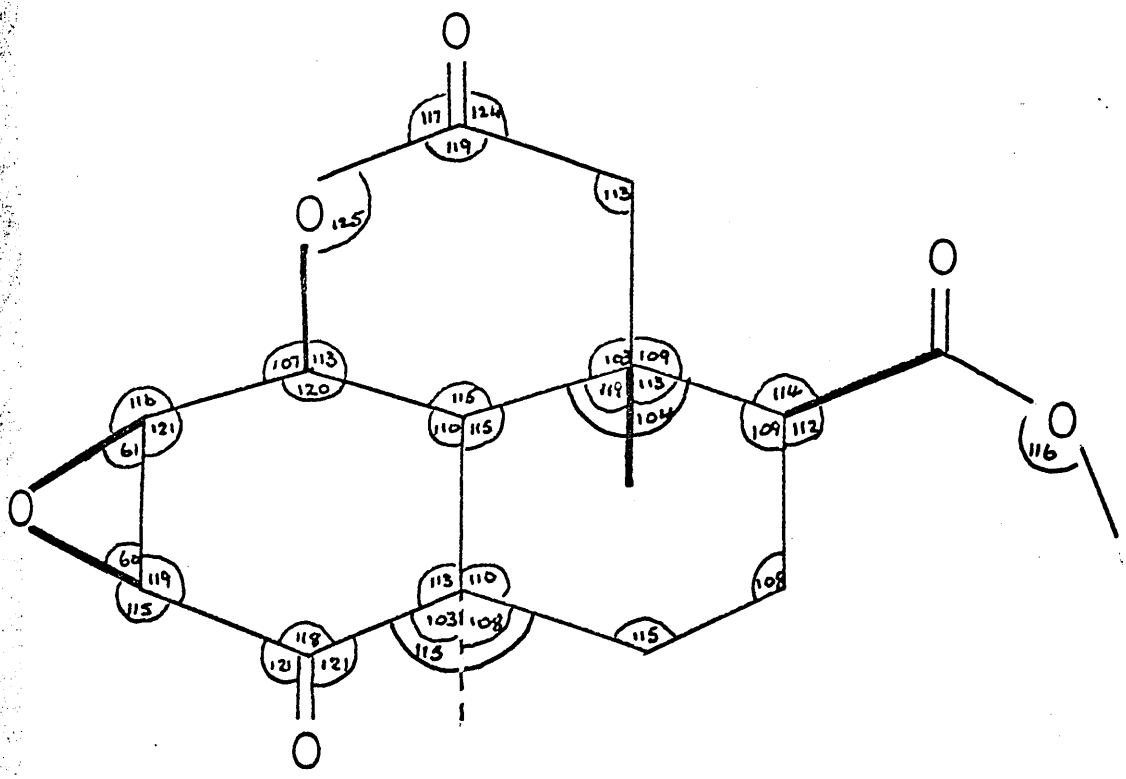
In a regular structure, it would be expected that the mean planes through rings A, B and C would be parallel. However, owing to the severe strain involved in the molecule, the mean plane through ring A makes angles of 55.5 and 51.7^{o} with the mean planes through rings B and C respectively. The mean planes through B and C have a dihedral angle of only 4^{o}, showing them to be almost planar.

BOND LENGTHS IN ÅNGSTROMS



(93)

BOND ANGLES IN DEGREES



$$C_{10}\hat{C}_5C_{13} = 107^\circ$$

$$C_{11}\hat{C}_9C_{14} = 108^\circ$$

EXPERIMENTAL.

Extraction of Palmarin.

10g. of colombo root bitter principles, rich in chasmanthin, were heated under nitrogen at 225°C for 10 min, to decarboxylate the columbin and iso-columbin present. After cooling under nitrogen, the resulting brown glass was heated with chloroform (60 ml.) and a residue of palmarin (20mg) was obtained. The chloroform solution was filtered through grade IV acid alumina (10g.), and the alumina column washed with 60 ml. chloroform. Crystallisation of the eluted material from ethyl acetate yielded 4.2 g. of a mixture of palmarin and iso-jateorin, which, on prolonged refluxing in ethyl acetate (30 ml.), dissolved the iso-jateorin, and some palmarin, and left a residue of palmarin (2.6 g.). mp. 254-6°C

Since palmarin is much less soluble in ethyl acetate than iso-jateorin, crystallisation of the ethyl acetate solution produced 730 mg. of iso-jateorin. mp. 164-166°C from the later crops.

Ozonolysis of Palmarin.

Palmarin (320 mg.) dissolved in ethyl acetate (130 ml.) was treated with ozone at -5°C for 1½ hrs. Water (4 ml.) was added and the mixture was allowed to stand overnight at room temperature. The solvent was removed at room temperature and the clear gum obtained crystallised after several hours in white rods. Recrystallisation from ethanol yielded tris-nor-palmarinic acid (210 mg.). mp. 269-272°C.

Lithium Aluminium Hydride Reduction of tris-nor-palmarinic acid mp. 269-272°C (**77**).

Tris-nor-palmarinic acid (620 mg.) in 50 ml. dry tetrahydro-furan was slowly added, over 2 hrs. to a stirred, refluxing suspension of lithium aluminium hydride (1.75g.) in 50 ml. dry tetrahydro-furan. The reaction mixture was refluxed a further two hours and, after cooling, the excess lithium aluminium hydride was destroyed by addition of ethyl acetate. A saturated solution of ammonium sulphate (5 ml.) was added and the mixture filtered. The precipitated material was washed with warm water (4 x 10 ml.) and the washings added to the filtrate and the reaction mixture freed of organic solvent. The remaining aqueous solution was saturated with ammonium sulphate and extracted with ether for four days, 580 mg. material was extracted. Crystallisation from methanol-benzene produced the epoxy-tetrol-hemiacetal (**78**) (315 mg.) as square plates mp. 210-213°C.

Attempted ortho-esterification of the epoxy-tetrol hemiacetal (**78**) mp. 210-213°C.

(a) Epoxy-tetrol-hemiacetal (10 mg.) was shaken at room temperature for 16 hours with ethyl ortho-formate (5 mg.) and one drop of boron trifluoride etherate in 3 ml. ethanol. Gradually the epoxy-tetrol-hemiacetal dissolved. The solvent was removed at 30°C under reduced pressure. The starting material was recovered unchanged.

(b) Epoxy-tetrol-hemiacetal (10 mg.) was refluxed 1½ hours with ethyl ortho-formate (5 mg.) and two drops boron trifluoride etherate in 3 ml. ethanol. The solvent was removed and the residue dissolved in acetone-benzene (1:4) and chromatographed over grade V neutral alumina. The material was eluted with benzene-ether mixtures. No crystalline material was produced.

Preparation of the acetonide of epoxy-tetrol-hemiacetal (78) mp. 210-213°C.

The epoxy-tetrol-hemiacetal (10 mg.) was shaken in AnalaR acetone (8 ml.) with a small amount of anhydrous copper sulphate for 16 hours at room temperature. The mixture was filtered and the solvent removed under reduced pressure at room temperature. Thin layer preparative chromatography, eluting with methanol-ethyl acetate (1:9), separated the acetonide from the other materials of the reaction mixture. The acetonide was non-crystalline.

Tosylation of the epoxy-tetrol-hemiacetal (78) mp. 210-213°C.

p-toluene sulphonyl chloride (213 mg.) was added in one portion to the epoxy-tetrol-hemiacetal (40 mg.) in analar pyridine (4 ml.) and the mixture was allowed to stand at room temperature 2½ hours. The reaction mixture was poured into sodium carbonate solution (10 ml.), left for 1 hour, then extracted with chloroform (3 x 20 ml.) and washed with water until the washings were neutral. The solvent was removed at room temperature under reduced pressure and the residue taken

up in benzene and chromatographed over 3g. grade IV neutral alumina eluting with benzene-ether mixtures. A non-crystalline tosylate was eluted with benzene-ether(1:1) (21mg.).

$$\gamma_{max}^{nujol} \quad 1600cm^{-1}, \quad 1190cm^{-1}$$

The n.m.r. spectrum integrated for 30 protons and had peaks at: 2.60τ(multiplet) (β-furyl protons), 3.52τ(α-furyl proton), 5.06τ(singlet) (hemiacetal proton), 7.93τ(tosylate methyl protons), 8.60τ(C_9 methyl protons), 9.01τ(C_5 methyl group).

Periodate oxidation of the epoxy-tetrol-hemiacetal (78).

The epoxy-tetrol-hemiacetal (165 mg.) dissolved in 5 ml. water was treated with sodium periodate (256 mg.) in 3 ml. water at room temperature for 30 min. After 15 min. crystals began to form in the reaction mixture. These were filtered off and recrystallised from acetone-petrol as white needles (124 mg.) mp. 199-202°C, of epoxy-hemiacetal keto-formate (81).

Oxidation of epoxy-hemiacetal-keto-formate (81).

The epoxy-hemiacetal-keto-formate (124 mg.) in AnalaR acetic acid (1.5 ml.) was treated with a 0.27 N solution of chromium trioxide in aqueous acetic acid (1:19) for 16 hours. Methanol (2 ml.) was added and the combined solvents were removed in vacuo. Water (2 ml.) was added to the residue and the white needles of the epoxy-keto-formate-lactone (82) which formed were recrystallised from aqueous ethanol to give white rods (98 mg.) mp. 233-236°C.

Hydrolysis of the lactone (82) with potassium carbonate.

The lactone (95 mg.) in methanol (4 ml.) was treated with potassium carbonate (515 mg.) in water (4 ml.) at room temperature for 16 hours. The methanol was removed in vacuo and the remaining aqueous solution saturated with dihydrogen sodium phosphate and extracted with chloroform (3 x 10 ml.). Removal of solvent and crystallisation from acetone-benzene furnished the hydroxy lactone (83b) (85 mg.) mp. 253-256°C ν_{max}^{nujol} 3580, 1723, 1707cm^{-1} for hydroxyl group, δ -lactone, carbonyl and ketone group respectively.

Acetylation of the hydroxy-lactone (84b).

The hydroxy-lactone (30 mg.) in dry pyridine (2 ml.) was treated with acetic anhydride (0.5 ml.) at room temperature for 16 hours. Crushed ice was added and the mixture was allowed to warm to room temperature then it was extracted with chloroform (3 x 5 ml.). Removal of solvent and crystallisation of the residue from aqueous ethanol furnished the lactone acetate (84 a) (26 mg.) mp. 196-198°C, resolidifies 213-215°.

Hydrogenation of iso-columbin.

Iso-columbin (600 mg.) dissolved in ethyl acetate (50 ml.) was hydrogenated over freshly prepared 1% Palladium on calcium carbonate (160 mg.) until 1 mole of hydrogen had been absorbed ca. 30 min. The catalyst was removed by filtration through Kieselguhr, and the solvent evaporated in vacuo. Recrystallisation of the residue from aqueous ethanol gave dihydro-isocolumbin (30) (560 mg.) as large needles mp. 233-236°C.

Ozonolysis of dihydro-isocolumbin.

Dihydro-isocolumbin (560 mg.) mp. 233-236°C was dissolved in ethyl acetate (60 ml.) and treated with ozone for 3 hours at -5°C. Water 5 ml. was added and the mixture allowed to stand at room temperature 16 hours. The solvent was removed at room temperature in vacuo and water (3 ml.) was added to the residual gum. The crystalline material obtained from this gum was filtered and recrystallised from aqueous ethanol to yield the tris-nor acid (77a) (365 mg.) mp. 251-253°C.

Lithium Aluminium Hydride Reduction of the tris-nor-acid (77a).

The tris-nor-acid (365 mg.) in dry tetrahydrofuran (30 ml.) was added slowly (over 2 hours) to a refluxing suspension of lithium aluminium hydride (1.1 g) in dry tetrahydrofuran (30 ml.). After addition of the tris-nor acid, the reaction mixture was refluxed 1 hour longer. On cooling, the excess lithium aluminium hydride was destroyed with ethyl acetate and a saturated solution of ammonium sulphate (5 ml.) was added. The precipitated salts were filtered and washed with warm water (3 x 5 ml.) and the washings combined with the filtrate. The tetrahydrofuran was removed *in vacuo* and the residual aqueous solution saturated with ammonium sulphate and extracted continuously with ether for 4 days. The resulting gum on crystallisation furnished the tetrol hemiacetal (78a) (80 mg.) as square plates, *mp.* 119-121°C.
(Found: C 61.39%, H 8.93%. $C_{17}H_{32}O_6$ requires C 61.44%, H 9.15%).
The mother liquors contained 260 mg. of a gum consisting mainly of the tetrolhemiacetal (78a).

Formation of the formate lactol (81a).

The tetrol hemiacetal (78a) (260 mg.) in water (8 ml.) was treated with sodium periodate (400 mg.) in water (5 ml.) at room temperature for 30 min. The solution was saturated with salt and extracted with chloroform (3 x 15 ml.) to furnish the formate lactol (81a) (210 mg.) as a gum.

The formate lactol gum (210 mg.) was dissolved in acetic acid (2 ml.) and oxidised with 0.27 N chromium trioxide in

aqueous acetic acid (1:19) (6.5 ml.) and left at room temperature for 16 hours. Removal of solvent at room temperature in vacuo and addition of water (3 ml.) deposited white needles of the formate lactone (**83**). Crystallisation from aqueous ethanol yielded white rods (165mg.) mp. 232-234°C. $[\alpha]_D$ = + 50.1° $\nu_{max}^{CHCl_3}$ 1700cm^{-1}(ketone), 1707cm^{-1} (formate) 1719cm^{-1} (δ -lactone). (Found: C 64.85%, H 7.47%. $C_{10}H_{24}O_5$ requires C 64.86%, H 7.53%).

Hydrolysis of the formate lactone (**83**) with potassium carbonate.

The formate lactone (**83**) (58mg.) in aqueous methanol (4 ml.) was treated with potassium carbonate (300mg.) in water (2 ml.) at room temperature for 16 hours. The methanol was removed in vacuo and the residual aqueous solution saturated with sodium dihydrogen phosphate and extracted with chloroform (3 x 8 ml.). The reaction product was crystallised three times from acetone-benzene to yield the hydroxy lactone (**85a**) (35mg.) mp. 233-235°C. (Found: C 67.34%, H 8.47%. $C_{15}H_{22}O_4$ requires C 67.64%, H 8.33%). $\nu_{max}^{CHCl_3}$ 3590cm^{-1}(OH) 3480cm^{-1} (bonded OH) 1714cm^{-1} (ketone) 1737cm^{-1} (δ -lactone) $[\alpha]_D$ = + 121°.

Acetylation of the hydroxy lactone (**85a**).

The hydroxy lactone (28mg.) was dissolved in pyridine (0.5 ml.) and treated with acetic anhydride (0.2 ml.) at room temperature for 16 hours. Crushed ice was added and when the mixture had warmed to room temperature it was extracted with chloroform (3 x 5 ml.). The residual gum (27mg.) was taken up in benzene and chromatographed over grade IV acid alumina,

the acetate being eluted with benzene-chloroform (**85b**) (50:1). Crystallisation from chloroform-ether furnished the lactone acetate (**85b**) (23 mg.) mp. 128-130°C.

Hydrolysis of the epoxy-formate lactol (**81**)[84].

The epoxy-formate lactol (182 mg.) in aqueous methanol (12 ml.) was treated with potassium carbonate (2.43 g.) at room temperature for 16 hours. The methanol was removed *in vacuo* and the residual aqueous solution was saturated with sodium dihydrogen phosphate and extracted with chloroform (3 x 10 ml.) to yield a solid (175 mg.). Crystallisation of this solid from ethyl acetate-petrol, furnished the lactol alcohol (**84c**) (156 mg.) mp. 176-178°C.

Oxidation of the lactol alcohol (**84c**)[84].

The lactol alcohol (140 mg.) dissolved in AnalaR acetic acid (16 ml.) was treated with a 0.27 N solution of chromium trioxide in aqueous acetic acid (1:19) (7.5 ml.) at room temperature for 3 hours. Methanol (5 ml.) was added and the solvents were removed *in vacuo*. Water (5 ml.) was added and the suspension was extracted with chloroform (3 x 10 ml.) and washed with sodium bicarbonate solution (2 x 10 ml.) and water (10 ml.). The chloroform solution yielded a neutral product (40 mg.) which crystallised as white rods from acetone-petrol mp. 219-221°C.

The sodium bicarbonate and water washings were acidified with 6N hydrochloric acid and extracted with chloroform (3x10 ml.)

to yield 98 mg. of acidic product. Crystallisation from ethanol gave the lactone acid (**86b**) mp. 268-270°C. Methylation of the acid with ethereal diazomethane furnished the methyl ester (**86a**) mp. 198-201°C white plates from ethyl acetate-petrol.

Attempted Chloracetylation of the lactone alcohol (**84b**).

a) The lactone alcohol (20 mg.) mp. 255-258°C was dissolved in dry tetrahydrofuran (2 ml.) and pyridine (4 drops), and treated with chloracetic anhydride (31 mg.) at room temperature for 16 hours. Crushed ice was added and on warming to room temperature was extracted with chloroform (3 x 5ml.)to yield 23 mg. solid. From a chromatoplate, only the starting material was present. The lactone alcohol was recovered by crystallisation of the reaction mixture from acetone-benzene. (18 mg.).

b) The lactone alcohol (20 mg.) was dissolved in benzene (1 ml.) and pyridine (4 drops) and treated with chloracetyl chloride (0.4 ml.) at room temperature for 16 hours. The solvents were removed in vacuo and the residue taken up in benzene and chromatographed over grade IV acid alumina (2g.). The chloroacetate (**84d**) was eluted with benzene and crystallised from chloroform-ether as white needles (15 mg.) mp. 168-170°C.

ν_{max}^{nujol} 1755cm^{-1} (chloracetate carbonyl) 1720cm^{-1} (δ-lactone) 1705cm^{-1} (ketone) 800cm^{-1} (C-Cl str.).

Iodoacetate of the lactone alcohol (**84b**).

The chloracetate (**84d**) (15 mg.) was refluxed in analaR acetone (4 ml.) with sodium iodide (30 mg.) for 3 hours under nitrogen. The solution was concentrated, water (5 ml.) added and the mixture extracted with chloroform (3 x 5 ml.) to give a residue (18 mg.). The residue was taken up in ethyl acetate-benzene (1:3) and filtered through a short column (1g.) of grade IV acid alumina. The gum obtained was crystallised from acetone-petrol as fine white needles mp. 148-150°C (15 mg.). The iodoacetate could not be obtained in a different crystalline form from any of the solvents or solvent mixtures tried. Some decomposition occurred during each crystallisation and the material had to be filtered through alumina several times.

X-RAY DERIVATIVES OF PALMARIN.

Cesium salt of tris-nor acid (77).

The acid (30 mg.) was dissolved in aqueous ethanol (3 ml.) and shaken with cesium carbonate (14 mg. = 0.5 mole) until solution was complete, ca. 2 hours. The solvents were removed *in vacuo* and the residue was crystallised from methanol as fine white needles *mp.* 223-227°C. This material could not be obtained in a different crystalline form from any solvent or solvent mixture tried.

Attempted preparation of p-iodo anilide of tris-nor acid (77).

The tris-nor acid (20 mg.) was neutralised with sodium bicarbonate solution (5 mg. in 2 ml. water). The water was removed and the sodium salt dried under reduced pressure for 1 hour on the steam bath. The sodium salt was mixed with excess oxalyl chloride and a trace of pyridine was added; the mixture was allowed to stand at room temperature 16 hours. The insoluble salt was filtered and the excess oxalyl chloride was removed *in vacuo*. The residue was dissolved in benzene, p-iodo aniline (26.4 mg.) was added and the mixture was allowed to stand at room temperature for 4 hours. The solvent was evaporated and the products separated on a 0.5 min. 20 x 20cm chromatoplate, eluant methanol-chloroform (3:97). Only amorphous material was isolated.

Preparation of p-bromo-S-benzyl thiuronium salt of the tris-nor acid (77).

The tris-nor acid (20 mg.) was dissolved in aqueous ethanol (3 ml.) and neutralised with sodium bicarbonate (5 ml.). A hot ethanolic solution of p-bromo S-benzyl thiuronium bromide (17.6 mg. (1 mole) in 1 ml. ethanol) was added to the sodium salt of the tris-nor acid. On cooling, the solvent was removed and the residue crystallised from aqueous ethanol as very fine white needles mp. 278-281°C.

This material could not be obtained in a different crystalline shape from any solvent or solvent mixture tried.

Formation of the p-iodo anilide of the acid (86b).

The acid (16 mg.) was dissolved in aqueous methanol (2 ml.) and treated with sodium bicarbonate (5 mg.) for 15 min. The solvents were removed *in vacuo* and the sodium salt dried under reduced pressure (0.01mm mercury) at room temperature for 5 hours. The sodium salt, suspended in dry benzene (2 ml.) was treated with oxalyl chloride (1 ml.) and pyridine (2 drops). The mixture was left at room temperature 16 hours, under anhydrous conditions after which it was filtered and the solvents removed *in vacuo*. The residue (16 mg.) was dissolved in dry benzene (2 ml.) and treated with p-iodo-aniline (36 mg.-3 mole) in dry benzene (1 ml.). After 5 hours, the reaction mixture was filtered and the solvent evaporated. The residue crystallised from ethanol as fine yellow plates mp. 297-299°C.

ν_{max}^{nujol} 3300cm^{-1} (N-H), 1730cm^{-1} (δ-lactone carbonyl), 1710 (Ketone) 1700cm^{-1}, 1680cm^{-1} (amide bands I and II respectively).

Formation of the p-bromophenacyl ester of acid (86 b).

The acid (30 mg.) in aqueous ethanol (3 ml.) was treated with sodium carbonate (5.25 mg.) and the solution was tested with BDH universal indicator paper to ensure that the pH was less than 10, p-bromo phenacyl bromide (57 mg.) was added and the solution was refluxed on the steam bath 2½ hours. The products of reaction were separated by thick layer preparative chromatography (0.8 mm. plate 20 x 20 cm, eluant methanol-chloroform (1.49)) and the p-bromophenacyl ester (24 mg.) was crystallised from chloroform-toluene as stout rods mp. 252-253°C.

ν_{max}^{nujol} 1745cm^{-1} (δ-lactone carbonyl), 1730cm^{-1} (ester carbonyl) 1725cm^{-1} (cyclo-hexanone), 1690cm^{-1} (carbonyl α to phenyl ring).

MW 491, mass spectrographic MW 491.

SECTION V

BIOGENESIS

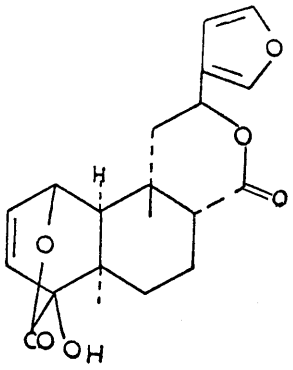
of the

COLOMBO ROOT BITTER PRINCIPLES.

Biogenesis of the Colombo Root Bitter Principles.

The main bitter principles isolated from the root extract of Jateorrhiza palmata is columbin, $C_{20}H_{22}O_6$ (95). Three other isomeric compounds (chasmanthin(96a), palmarin(96b) and jateorin (97))also occur in the root extract and are 2,3-epoxy columbins. Chasmanthin, and its $C_{(3)}$ epimer, palmarin are epimeric with jateorin at $C_{(12)}$ while jateorin has the same stereochemistry at $C_{(12)}$ as columbin. The small amounts of iso-columbin (8-epi-columbin) and palmarin (8-epi-chasmanthin) isolated from the root extract may be artefacts arising during the extraction process.

Barton et al(54) postulated a biogenetic route to columbin from (98) derived from the bicyclic labdane alcohol (99) obtained by normal cyclisation of geranyl geraniol(100). Rearrangement of (101) by 1,2 trans, anti-parallel shifts of hydride and methyl groups leading to (102) was postulated for columbin. However, if the configuration of the groups concerned is maintained, this leads to a trans A-B ring junction at C_5, C_{10} with a β-methyl group at $C_{(5)}$. Although this has since been proved erroneous(36), the determination of the structures of cascarillin (103)(37) and clerodin (104)(38) has shown that the migration of hydride and methyl groups in this fashion is possible and that the configuration at $C_{(5)}$ and $C_{(10)}$ in these compounds may represent a precursor of the columbin cis A-B ring fusion.



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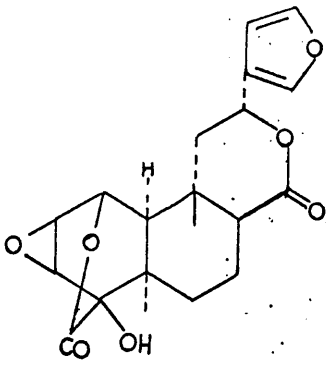
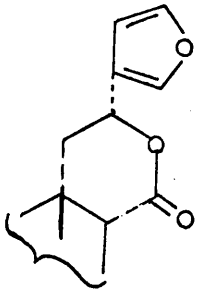
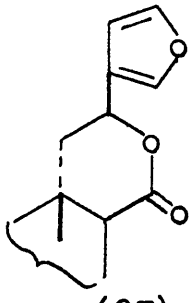


(96) b



(96) a



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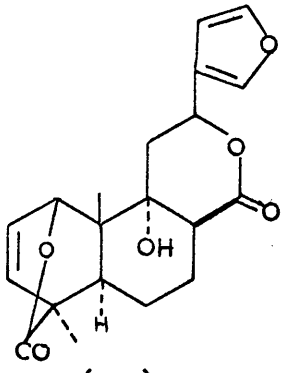

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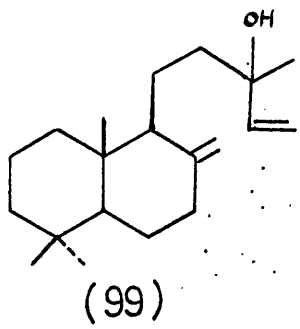

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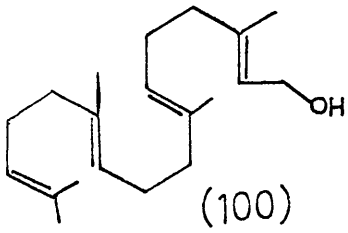
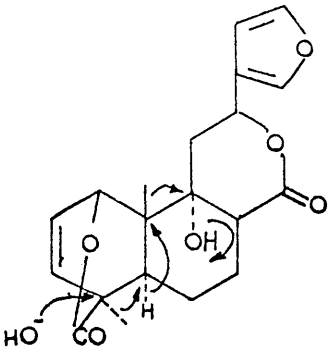


(100)

The problem of postulating a biogenetic route to columbin hinges on the mechanism or mechanisms required to introduce a cis fusion of rings A and B. The concerted 1,2 - shifts of methyl and hydride groups proposed by Barton[54] (101) give the correct configurations at $C_{(9)}$ and $C_{(10)}$ for columbin, but the wrong configuration at $C_{(5)}$. If these shifts are employed the configuration of $C_{(5)}$ could be altered by ring A cleavage to an exo-methylene group at $C_{(5)}$ followed by recyclisation, a procedure which, so far, is without precedent in the diterpenoid series. The migration of a methyl group to $C_{(5)}$ must thus be independent of the change of configuration at $C_{(9)}$ and $C_{(10)}$ from the configuration at these positions in the labdane bicyclic alcohol (99).

Determination of the structure of the diterpene alkaloid thelepogine (105)[57] has indicated a method of methyl migration which may have a bearing on the biogenesis of columbin. Thelepogine, which can be formally derived from manool, has a cis fused A-B ring junction indicating that methyl migration from $C_{(4)}$ to $C_{(5)}$ can occur without concomitant hydride shift from $C_{(5)}$ to $C_{(10)}$. A mechanism for this migration is indicated in (106).

If rings A and B are cis fused, with 5α, 6α hydrogen atoms (107) then elimination of the 6β-hydroxyl group and hydride shift as in (107) induces methyl shift from $C_{(4)}$ to $C_{(5)}$ as shown (108). The incipient carbonium ion on $C_{(4)}$ is neutralised by a solvent molecule to give an α-hydroxyl group.



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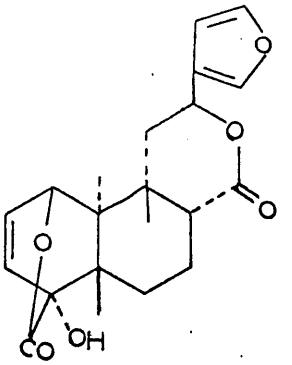

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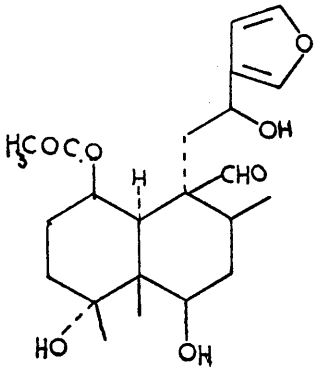

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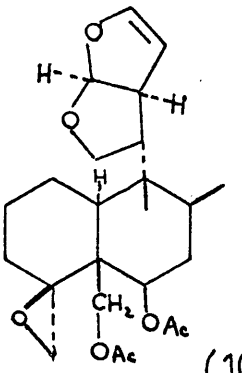

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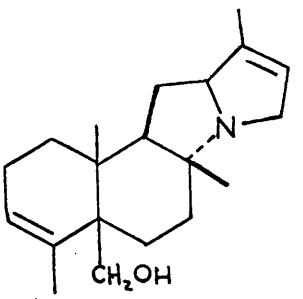
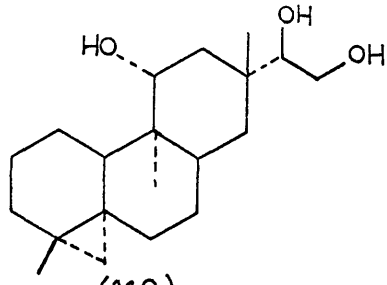


(105)

The methyl shift may proceed directly, via the methyl carbanion, or it may involve formation of a cyclopropane intermediate (109) which makes the methyl transfer a two step mechanism. Formation of this cyclopropane intermediate may be a general biogenetic pathway for methyl group migration since a variety of naturally occurring compounds are known containing cyclopropane rings in positions not only compatible, but co-occurring with methyl migrated products, eg. triol Q from Erythoxlon monogynum (110)[106], cimigenol (111)[107].

For such methyl migration to occur, rings A and B must be cis fused, since trans fusion of rings A, B gives rise to the situation encountered in thelepogine (5β, 10β methyl groups) on methyl migration. Before methyl migration, therefore, the trans A,B ring junction arising from the cyclisation of geranyl-geraniol, must be transformed to a cis ring junction without alteration of the $C_{(5)}$ α-hydrogen configuration. This can be achieved by protonation of the $C_{(8)}$ exo-methylene group, hydride shift from $C_{(9)}$ to $C_{(8)}$, methyl transfer from $C_{(10)}$ to $C_{(9)}$ and hydride shift from $C_{(9)}$ to $C_{(10)}$. The carbonium ion induced at $C_{(1)}$ can be neutralised either by solvent attack to yield a $C_{(1)}$ β-hydroxy compound (112) or by attack with the $C_{(4)}$ carboxylate anion (113). Methyl migration from $C_{(4)}$ to $C_{(5)}$ must be preceded by 1,4-δ-lactone bridge formation in order to "freeze" the $C_{(4)}$ stereochemistry. If the lactone bridge is not formed then inversion, at $C_{(4)}$ during methyl transfer, could occur to produce an α,δ-lactone bridge eventually.



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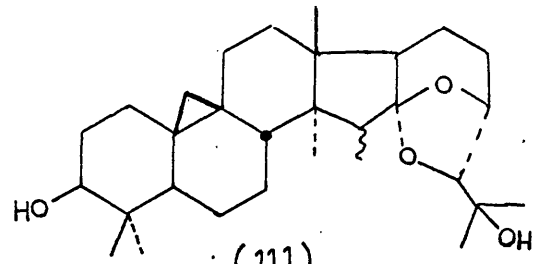

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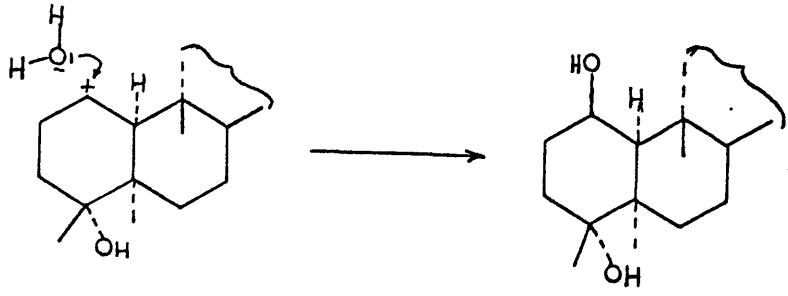

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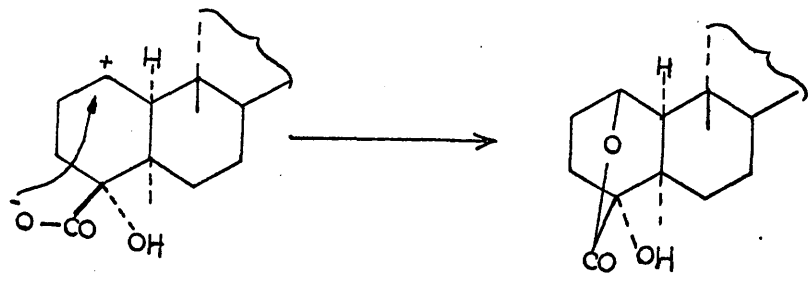
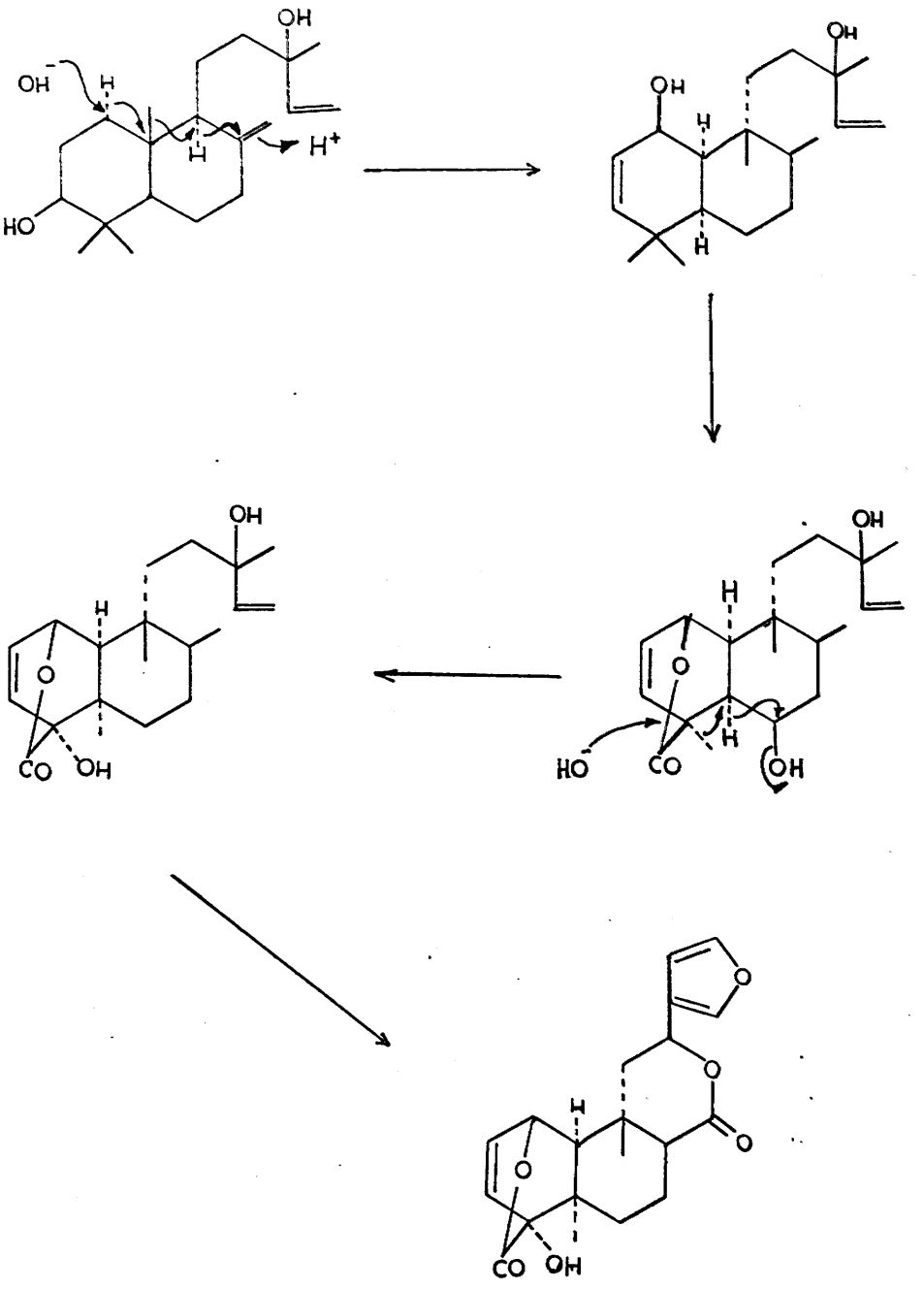


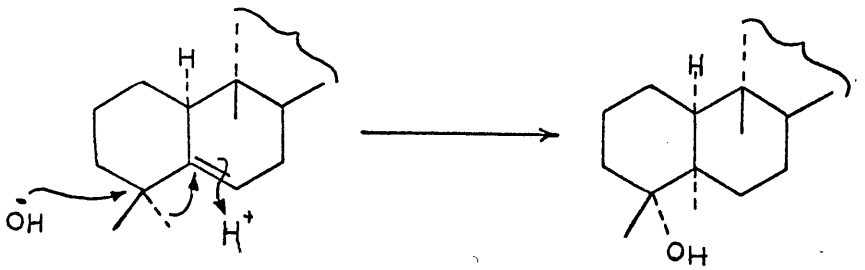
(113)

Although most cyclisations of geranyl-geraniol to produce the diterpenoids appear to be initiated by H^+ or a solvent molecule, the presence of a 2,3-epoxide in chasmanthin and its epimers, and a 2,3-double bond in columbin, may indicate that the initial cyclisation has been initiated by OH^+ or its equivalent, in order to account for the oxygenation at $C_{(3)}$ in the columbo root bitter principles. A scheme for the biogenesis of columbin incorporating the above proposals is given in Scheme 11.

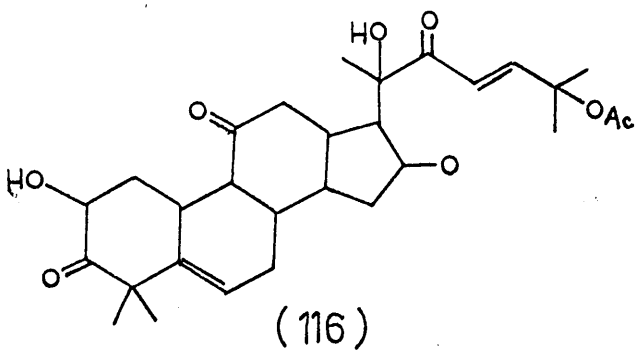
A further mechanism of methyl migration can arise from protonation of a $\Delta^{5,6}$ compound (114) and methyl migration to form the required 5 α-methyl group. The carbonium ion produced at $C_{(4)}$ is neutralised by a solvent molecule to produce a $C_{(4)}$ α-hydroxy compound (115). A double bond at $C_{(5)}C_{(6)}$ has a precedent in the cucurbitacins (116)[108] and probably arises by concerted 1,2-trans, auti-parallel shifts as in (117). The sequence of shifts is initiated by protonation of the $C_{(8)}$ exo-methylene group which causes hydride shift from $C_{(9)}$ to $C_{(8)}$, methyl transfer from $C_{(10)}$ to $C_{(9)}$ and hydride shift from $C_{(5)}$ to $C_{(10)}$. The carbonium ion induced at $C_{(5)}$ is neutralised by formation of a 5,6-double bond and loss of a proton from $C_{(6)}$. Since no oxidation at $C_{(1)}$ is introduced by this mechanism, in order to form the 1,4- δ-lactone bridge, oxidation at $C_{(1)}$ must be independent of the rearrangement of the configuration at $C_{(5)}$, $C_{(9)}$ and $C_{(10)}$. Simple oxidation at $C_{(4)}$ of a methyl group to a carboxyl group may occur. A scheme for the biogenesis of columbin, incorporating these suggestions is given in Scheme 12.

Scheme 11





(114) (115)



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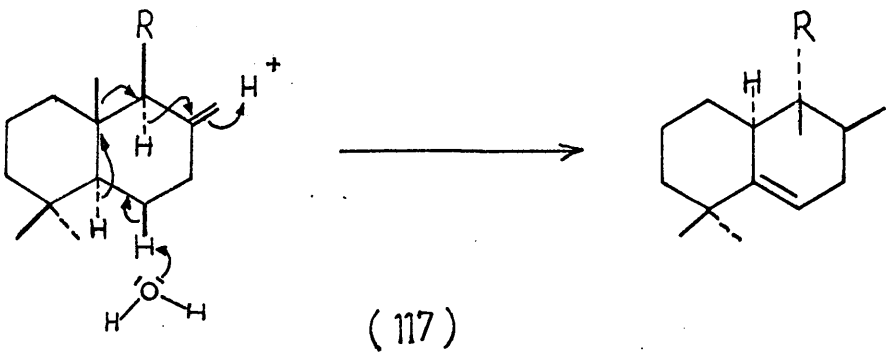
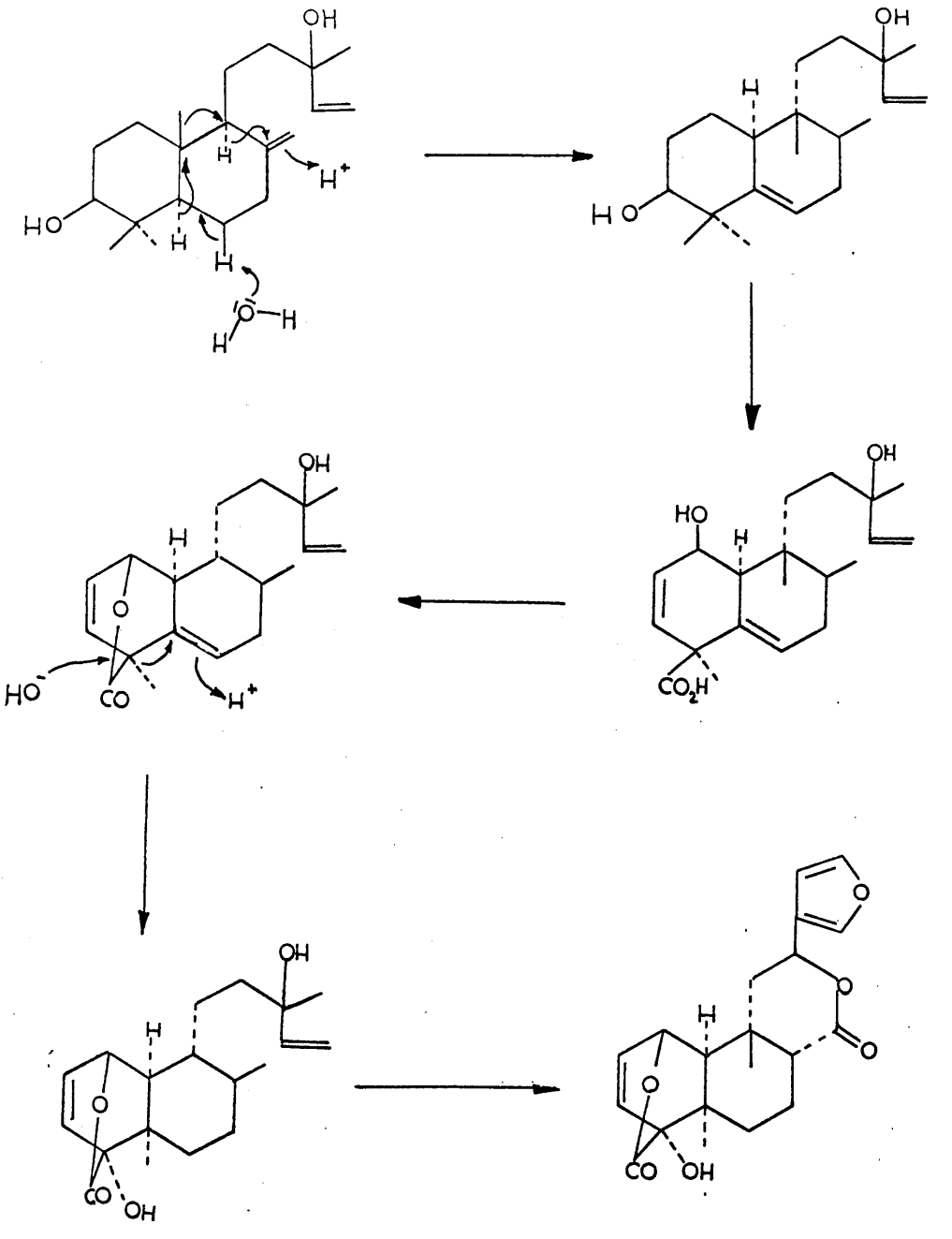


(117)

Scheme 12


OH
H
H+
HO
H
H
O
H
H
OH
H
HO
OH
HO
H
CO2H
OH
H
O
HO
CO
H+
OH
H
O
CO
OH
O
O
O
O
H
O
CO
OH

SECTION VI

DETERMINATION OF THE $C_{(13)}$ CONFIGURATION IN LABDANOLIC ACID.

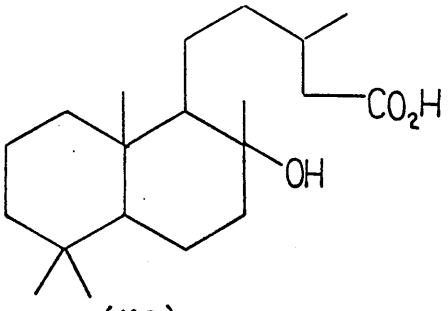
INTRODUCTION.

Labdanolic acid, a constituent of gum labdanum, has been shown, by Cocker and Halsall[92], to have the structure (118). The C_{17} acid (119) obtained by Barbier Wieland degradation was found to be identical with the C_{17} acid (119) obtained from the degradation of marrubiin[93]. This indicated that the rings in labdanolic acid were trans-fused and that the $C_{(10)}$ methyl group had the β-configuration.

The β-configuration of the $C_{(9)}$ side chain was established by comparison of the hydrogenation product of methyl dihydro-labdanolate (120) and methyl dihydro-cativate[95] (120), the two being identical. The $C_{(8)}$ configuration was established as shown in (121), by comparison of the molecular rotation differences between sclareol and manool, and between labdanolic acid and dehydro-labdanolic acid.[92] Thus, only the configuration at $C_{(13)}$ was undetermined.

Attempts to determine the $C_{(13)}$ configuration by indirect methods of configurational analysis have led to considerable confusion. Lederer and Bory[95] assigned the R-configuration to $C_{(13)}$ of methyl labdanolate on the basis of molecular rotation differences but this conclusion was invalidated by the discovery that the presence of hydrogen bonding could lead to anomalous results.

However, Bigley, Barltrop and Rogers[96] reinvestigated this work. They prepared methyl labdanolate and 13-epi-labdanolate



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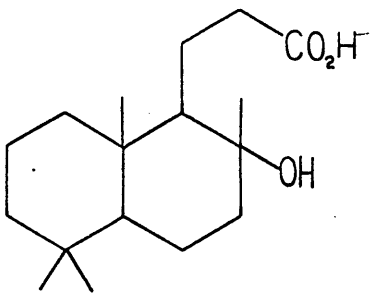

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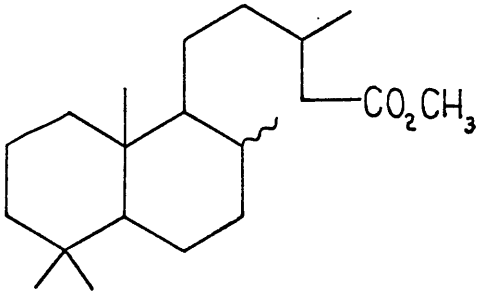
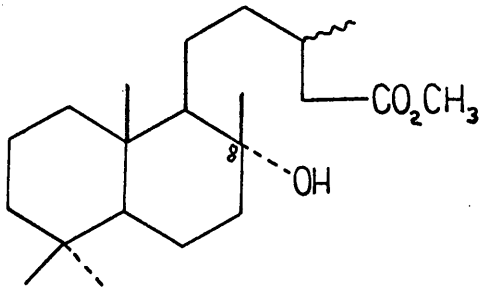


(120)

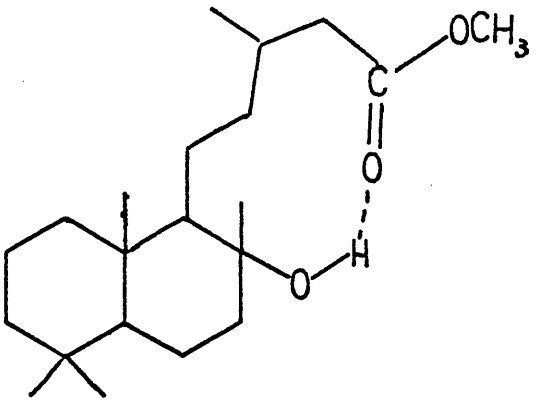


(121)

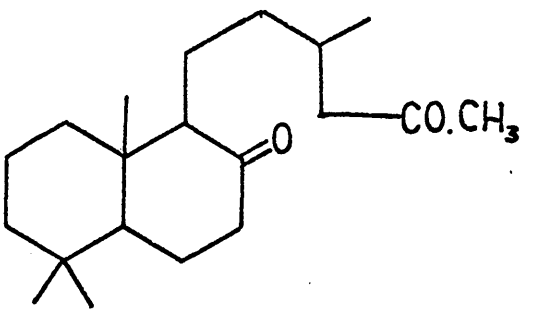
and claimed to find no evidence of hydrogen bonding in these compounds. In carbon disulphide solution they found only one sharp bond at $3540cm^{-1}$ and concluded that no hydrogen bonding existed in these compounds, although a value of $3540cm^{-1}$ is in the region normally associated with bonded hydroxyl ($3600-3650cm^{-1}$ - non-bonded hydroxyl). Hence they agreed with the conclusions of Lederer and Bory and assigned the R-configuration to $C_{(13)}$.

When, however, Raphael and his associates[97] conducted infra-red dilution studies on carbon disulphide and carbon tetrachloride solutions of methyl labdanolate, they discovered the existence of intramolecular hydrogen bonding involving a ten membered ring (12.1a) Thus the stereochemistry at $C_{(13)}$ in labdanolic acid was once more in doubt.

Renfrew and Overton[100] deduced the stereochemistry at $C_{(13)}$ in eperuic and labdanolic acids to be R by chemical and spectroscopic means. Their work involved studying the behaviour of the bicyclic diones(122), derived from eperuic and labdanolic acids, towards acid and base. This deduction was confirmed by the following X-ray analysis.



(121 a)



(122)

EXPERIMENTAL.

The molecular formula of p-bromophenacyl labdanolate is $C_{28}H_{41}O_4Br$ molecular weight 521. Found: C64.52% H8.25%, $C_{28}H_{41}O_4Br$ required C 64.49%, H 7.89%.

Rotation, oscillation, Weissenberg and precession photographs were taken using copper Kα radiation (λ = 1.542 Å). The cell dimensions were obtained from oscillation, Weissenberg and precession photographs, the following values were found,

$a = 11.55$ Å, $b = 6.05$ Å, $C = 19.94$ Å, $\beta = 99.02^{o}$

The volume of the unit cell was calculated from

$$V = abc \sin\beta$$

and found to be 1377 Å^3.

The density of the crystal, measured by flotation in potassium iodide solution, was found to be 1.276g/cm^3 and the number of molecules per unit cell (n) calculated from,

$$n = \frac{d\ V\ N_A}{M}$$

N_A Avogadro's number,

was 2. From this the calculated density of the crystal was 1.257g/cm^3.

From examination of the zero-layer Weissenberg photograph, the absent reflections were found to be OKO when K is odd, which indicates that the space group is either P_2, or P_{21}/m. The space group P_{21}/m has four equivalent positions, and with two molecules per unit cell this imposes the condition that the molecule has a mirror plane. However, labdanolic acid and hence p-bromo phenacyl labdanolate, is optically active and can have

no mirror plane, therefore the space group is $P2_1$.

The linear absorbtion coefficient (μ) for copper Kα radiation was found to be 33.3cm^{-1}. The total number of electrons per unit cell F(ooo) is 552.

The value of $\sum f^2$ for the "heavy" bromine atom is 1225 while that of $\sum f^2$ for the "light" atoms is 1264. Hence the value of $\underline{r}$ which can be found from the expression

$$\underline{r} = \left(\frac{\sum f_H^2}{\sum f_L^2}\right)^{\frac{1}{2}}$$ was found to be 0.98.

The compound crystallised in long needles, one of which was mounted along the needle axis but after seven to nine days irradiation, decomposition occurred. However, owing to the length of the needle, it was possible to move the crystal as desired, to irradiate a fresh portion.

Intensity data were recorded from equatorial and equi-inclination Weissenberg photographs, taken from a crystal rotated about the needle axis (b-crystal axis); in this way the reciprocal lattice nets hOl----h5l were recorded. The multiple film technique (Robertson 1943) was employed. The intensities were estimated visually by comparison with a calibrated film strip and were corrected for Lorentz, polarisation and Tunnel rotation factors. This procedure leads to a set of structure amplitudes on a relative scale; at a later stage the structure amplitudes were placed on an absolute scale.

The total number of structure amplitudes was 1672.

Since a thin needle crystal was used, no corrections for absorbtion were applied.

STRUCTURE DETERMINATION.

As the crystal had a short b-axis (6.05Å) it was hoped to be able to solve the structure in projection. Consequently, a two dimensional Patterson projection (P(uw)) was computed, using h0l data, while the remaining three dimensional data were being collected. However, since the bromine-bromine vector could not be distinguished from the peaks obtained, a sharpened Patterson projection was computed. One peak increased in height much more than the others in this sharpened Patterson Fig. 1 and was considered to be the bromine-bromine vector; the coordinates of the bromine atom were calculated from this projection as

$$x/a = 0.218, \qquad z/c = 0.184$$

Using these coordinates for the "heavy" atom and a temperature factor of U = 0.044 Å^2, structure factors were computed which gave a discrepancy factor (R) over all observed structure amplitudes of 60%. The discrepancy factor is given by

$$R = \frac{\Sigma(|Fo| - |Fc|)}{\Sigma|Fo|}.$$

Using the observed data and the phases calculated for the bromine atom, a two dimensional electron density distribution was computed for the (010) projection, and allowed the positions of all the atoms in the molecule to be located. Fig. 2. The The coordinates of the atoms were refined in projection by minimum residual calculations, three cycles of which, reduced the value of R to 0.23. Unfortunately, the two dimensional electron density distribution did not completely define the

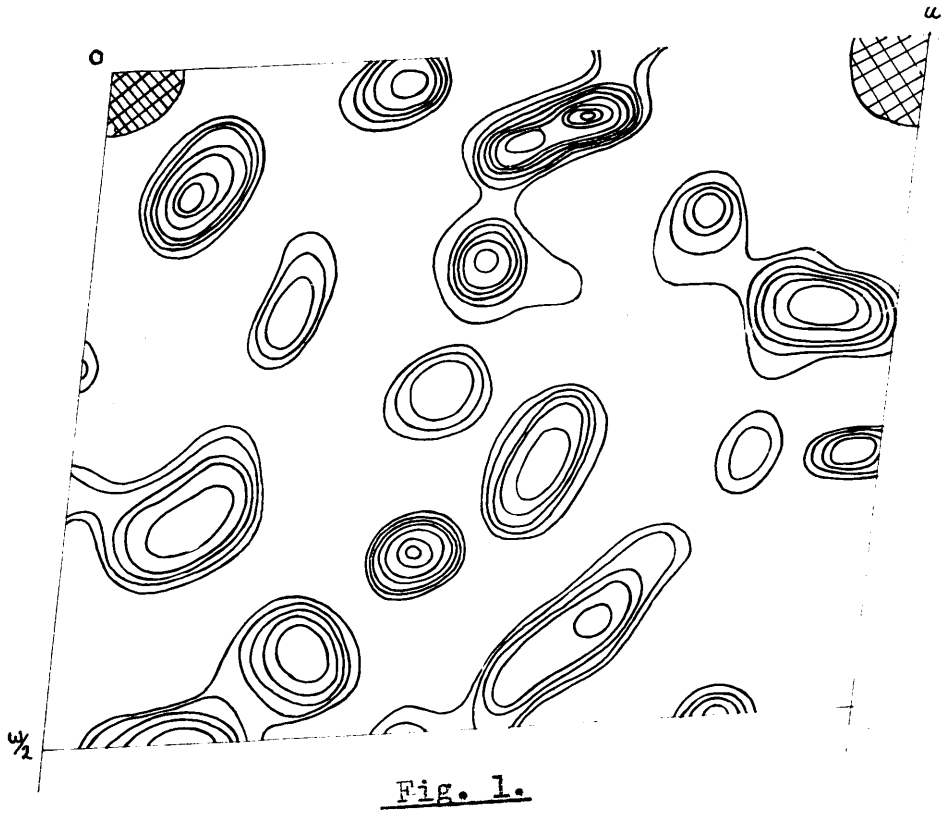


Fig. 1.

Patterson Diagram : arbitrary scale.

A= the Br-Br vector

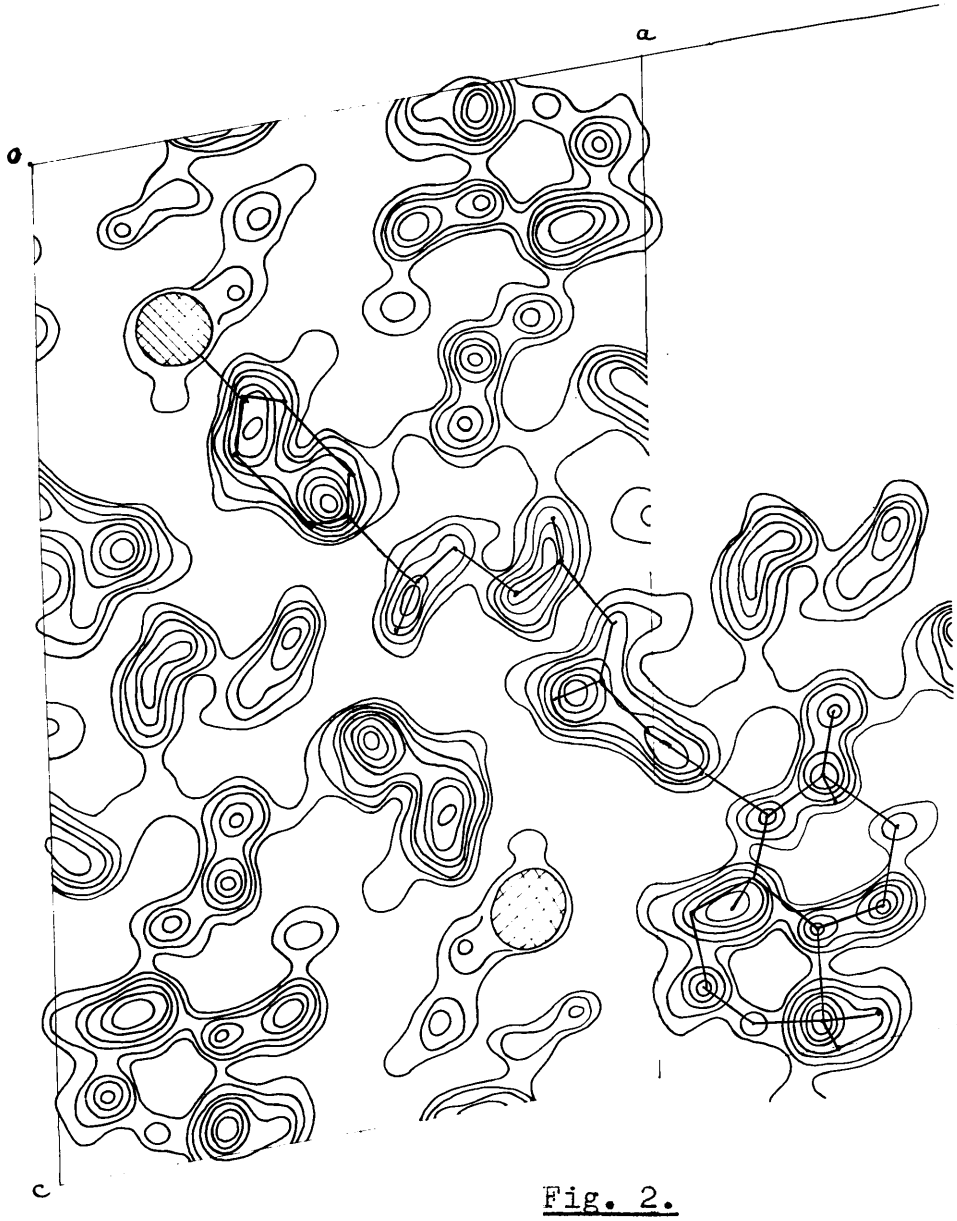


Fig. 2.

Two dimensional Fourier synthesis.

stereochemistry of the molecule, and hence a three-dimensional electron density distribution was computed, using the phases calculated for the bromine atom and the hkl observed data.

There being no fixed origin in the space group P_{21} in the y-direction, the y-coordinate of the bromine atom was arbitrarily assigned the value of 3/4. As the x and z coordinates of the bromine atom were available from the two dimensional refinement, the coordinates of the bromine atom used in the next calculation of structure amplitudes were

$$x/a = 0.21400, \quad y/b = 0.75000, \quad z/c = 0.18430.$$

Structure factors were computed using the above coordinates for the bromine atom which was given an isotropic temperature factor U = 0.075. After scaling, so that $\sum k|Fo| = \sum|Fc|$, for each layer, the overall discrepancy factor (R) was 0.49 at this stage.

Three-dimensional, electron-density calculations involving a crystal of space group P_{21}, with phases calculated for the heavy atom alone, involve pseudo mirror planes through the heavy atom i.e. at $y = \frac{1}{4}$, and also at $y = \frac{3}{4}$. Since the unit cell of p-promo phenacyl labdanolate has a short b-axis (6.05Å), the separation between the pseudo mirror planes is only 3.03Å. This short distance led to a merging of the electron densities of an atom and its pseudo mirror image, resulting in cylinders, instead of resolved spheres, of electron density, at or near $y = \frac{1}{4}$. From the first, three-dimensional, electron-density distribution, for which the observed structure amplitudes with phase constants,

appropriate to the bromine atom alone, were employed, approximate coordinates were assigned to C(6) and C(7) of the decalin system. The y-coordinates of these two atoms were far enough removed from the mirror plane to identify these atoms as belonging to one molecule (R = 0.49).

These two atoms were included along with the bromine atom in the next phasing calculations, each atom being given an isotropic temperature factor of U = 0.075$\AA^2$. A second electron-density distribution, for which the improved phase angles were used in conjunction with the observed structure amplitudes, improved the coordinates of the bromine atom, C(6) and C(7) and located the positions of a further three atoms.

As each atom was included in the calculations it was given an isotropic temperature factor of U = 0.063$\AA^2$. Six subsequent cycles of structure amplitude and electron-density distribution calculations located the positions of all thirty-three atoms Fig.3, and the value of the discrepancy factor (R) had been lowered to 0.34. The intensity data were revised and structure factors were calculated using the coordinates from the eighth electron-density distribution, isotropic temperature factors and the observed data, which reduced the value of the overall discrepancy factor to 0.27.

The progress of the structure determination is shown in Table 1.

STRUCTURE REFINEMENT.

Employing the phase constants calculated from the last structure amplitude calculation, three-dimensional Fo and Fc electron-density distributions were computed and corrections for termination of series errors were applied to the coordinates obtained from the Fo synthesis; the isotropic temperature factors were also adjusted. When structure factors were calculated, using these improved coordinates and temperature factors, the discrepancy factor was reduced to 0.28. A further cycle of coordinate, and temperature factor refinement was carried out, reducing the discrepancy factor to 0.266, which indicated that the atomic parameters were sufficiently accurate to allow refinement to continue by the method of least squares.

The block diagonal approximation was used, since a full matrix calculation with this size of molecule would have exceeded the computor storage capacity. In the initial cycles of calculation, each plane was given unit weight, only the atomic parameters, isotropic temperature factors and layer scale factors being refined. After six cycles of calculations the overall discrepancy had been reduced to 0.20 and the parameter shifts had become very small.

Before the atoms were allowed to refine anisotropically, the layer scale factors from the last cycle of isotropic least squares calculations, were used to convert all layers to one scale and the weighting scheme was altered.

The weighting function used was:

$$w = 1/(p_1 + |Fo| + p_2|Fo|^2 + p_3|Fo|^3)^{\frac{1}{2}}$$

where initially, $p_1 = 4.34$, $p_2 = 0.0184$, $p_3 = 0$.

Corrections for anomalous dispersion by the bromine atom were made at this stage. The wavelength of X-radiation used ($\lambda = 1.5422\text{Å}$) is just shorter than that of the absorbtion edge of bromine, which causes the amplitude and phase of the scattered radiation to be abnormal, and hence Friedal's law ($F(hkl) = F(\overline{hkl})$) no longer holds. For normal scattering, the structure amplitudes are real and positive; for anomalous dispersion they are complex. Hence, the structure factors were adjusted by using a bromine structure factor curve (f) corrected for real and imaginary parts in the structure factor calculation.

i.e. $f = fo + f' + i\,f''$ fo = original f_{Br} curve used

f' = correction for real part

f" = " " imaginary part.

The least squares refinement of the positional and anisotropic temperature factors was started and, after two cycles of calculations, the value of R had been reduced to 0.15. In order to downweight large Fc., the weighting scheme was altered, a value of 0.00004 for 3 being used. At the end of three further cycles of anisotropic least squares calculations, it was noticed that as |Fo| increased, $w\Delta^2$ also increased ($\Delta = |Fo| - |Fc|$), consequently the weighting scheme

was again adjusted. The new values of $p_1 = 4.0$, $p_2 = 0.05$, $p_3 = 0.005$ were used to downweight large Fo . The value of R' at this stage was 0.0249. R' is given by the expression:

$$R' = \frac{\sum w \Delta^2}{\sum w |Fo|^2}$$

After ten cycles of calculations the value of R' = 0.0241 and the refinement was concluded.

After the eighth cycle of calculations a difference Fourier synthesis was computed but, unfortunately, the positions of the hydrogen atoms could not be located, probably because of inaccuracies in the observed data. A final round of structure factor and electron-density distribution calculations were performed. The structure factors are listed in Table 2 and the final electron-density distribution is shown in Fig. 3.; the final value for the discrepancy factor R is 0.115.

Final coordinates are listed in Table 3, temperature factors are listed in Table 4. Bond lengths and bond angles with estimated standard deviations are listed in Table 5 and Table 6 respectively.

Mean planes calculations were computed for the atoms in rings A and B, the benzene ring and plane $O(4)\ C(21) C(22) C(23)$. The equations of the planes with the deviations of the atoms from the plane are listed as follows:

Ring A.

Equation of the plane : 0.1656 X - 0.9355 Y - 0.3122 Z + 7.1998=0

Deviations of atoms from the plane:

	Δ
C1	0.2464
C2	-0.2253
C3	0.2015
C4	-0.1917
C5	0.2102
C10	-0.2412

Ring B.

Equation of the plane : 0.1062 X - 0.9861 Y - 0.1276 Z + 5.5472=0

Deviations of atoms from the plane :

	Δ
C5	0.2750
C6	-0.2605
C7	0.2346
C8	-0.2293
C9	0.2324
C10	-0.2522

Benzene Ring.

Equation of the plane: 0.6939X - 0.3950Y - 0.6021Z + 2.1418 = 0

Deviations of atoms from the plane:

	Δ
C23	-0.0010
C24	0.0064
C25	-0.0023
C26	-0.0077
C27	0.0133
C28	-0.0086

Plane $O_{(4)}C_{(21)}C_{(22)}C_{(23)}C_{(24)}$.

Equation of the plane : 0.6108 X - 0.5708 Y - 0.5487 Z + 3.1189=0

Deviations of atoms from the plane :

	Δ
$O_{(4)}$	0.0065
$C_{(21)}$	0.0053
$C_{(22)}$	-0.0171
$C_{(23)}$	0.0054

Dihedral angles between planes.

Ring A and Ring B 11.50^{o}

Benzene ring and $O_{(4)}C_{(21)}C_{(22)}C_{(23)}$ plane 11.57^{o}

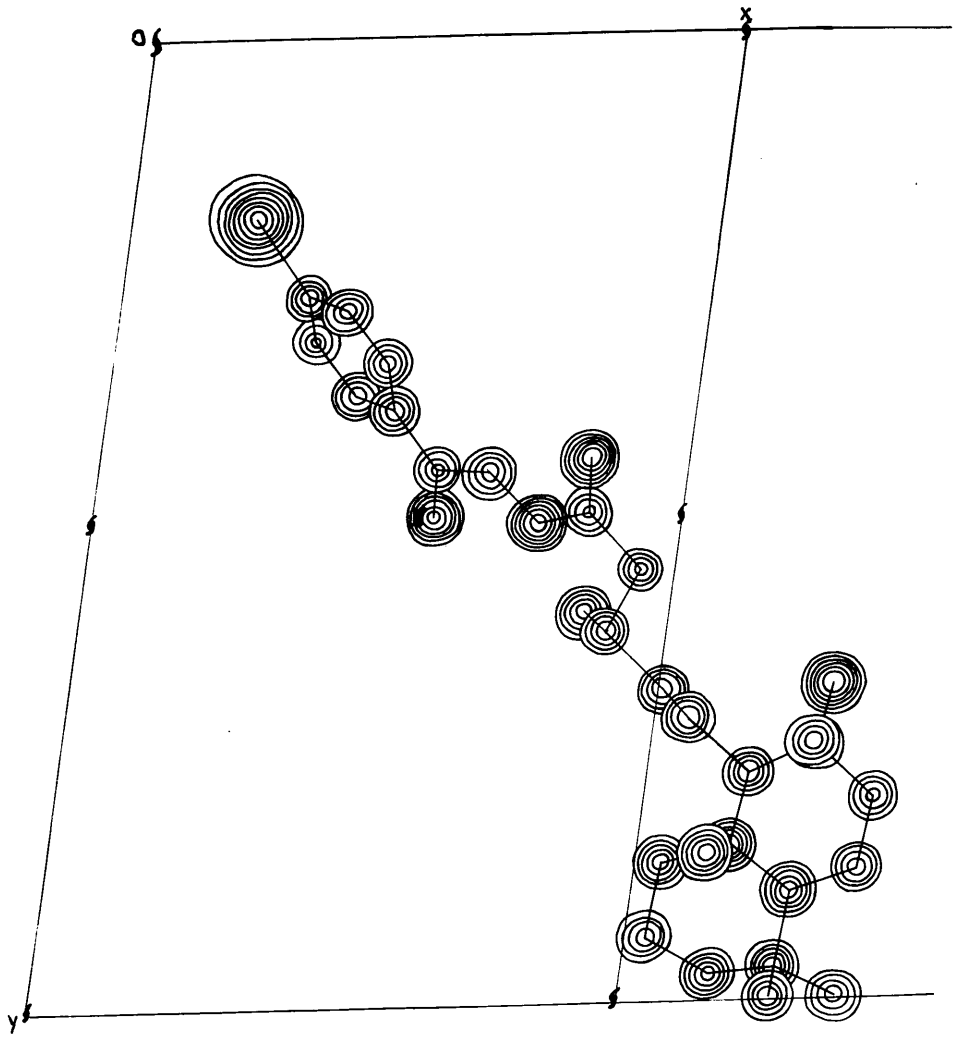


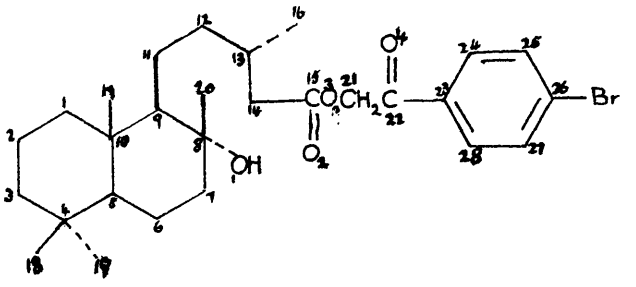
Fig. 3.

Three dimensional Fourier synthesis.

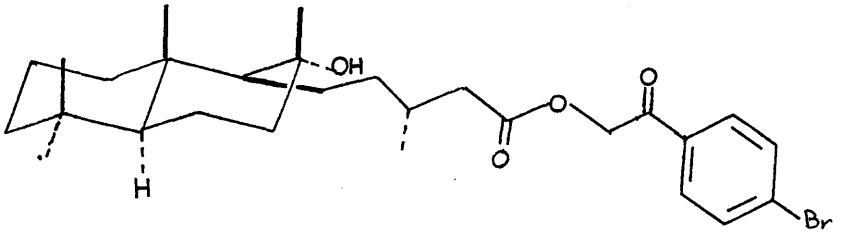
DISCUSSION OF STRUCTURE.

The configuration and stereochemistry of p-bromo phenacyl labdanolate has been shown to be as in I and II

I



II



This analysis confirms the structure of labdanolic acid and determines the configuration at C_{13} as R.

The molecule contains two cyclohexane rings A and B trans fused at $C_{(5)}$ and $C_{(10)}$. There are five methyl groups in the molecule : a gem dimethyl grouping at $C_{(4)}$, two axial methyl groups at $C_{(10)}$ and $C_{(8)}$ and an α-methyl group at $C_{(13)}$ in the side chain. This side chain consists of the carbon chain $C_{(11)}$, $C_{(12)}$, $C_{(13)}$, $C_{(16)}$ ($C_{(13)}$ α-methyl group), $C_{(14)}$ and p-bromo phenacyl ester grouping attached to $C_{(15)}$. The side chain is equatorial and virtually straight.

The hydroxyl group at $C_{(8)}$ is equatorial and there are 1,3 diaxial methyl groups at $C_{(4)}$ and $C_{(10)}$, $C_{(8)}$ and $C_{(10)}$.

Rings A and B of the decalin system have the chair conformation. The precise nature of the conformation adopted by this ring system is governed by the non-bonded 1,3 diaxial, dimethyl interactions present in the molecule. The theoretical distance between 1,3 diaxial substituents of an undistorted, cyclohexane chair is 2.53Å , however, in p-bromo phenacyl labdanolate, the distance between $C_{(17)}$ and $C_{(19)}$ is 3.31Å , which shows that there is a repulsion between these two methyl groups. Similarly, the non-bonded distance between $C_{(19)}$ and $C_{(20)}$ has been increased by repulsion to 3.122Å.

The slight, significant divergance of angles $C_{(2)}$ $C_{(3)}$ $C_{(4)}$ and $C_{(4)}$ $C_{(5)}$ $C_{(10)}$ (117.2° and 116.2° respectively) from the tetrahedral value of 109.5° could be due to a flattening of ring A by downward rotation of $C_{(4)}$ towards the plane of the atoms of ring A; this downward rotation being initiated by the repulsion between $C_{(17)}$ and $C_{(19)}$. Angle $C_{(5)}$ $C_{(4)}$ $C_{(17)}$ (114.9°) is slightly larger than the tetrahedral angle, and this slight distortion may indicate the direction of repulsion of $C_{(17)}$. The deviations of the other angles around $C_{(4)}$ from the tetrahedral value are not significant.

The repulsion of $C_{(20)}$ by $C_{(19)}$ is not reflected by distortion of the neighboring bond angles of ring B, only $C_{(20)}$ $C_{(8)}$ $C_{(9)}$ (116.1°) being greater than the tetrahedral angle, which possibly indicates the direction that repulsion of $C_{(20)}$ has taken.

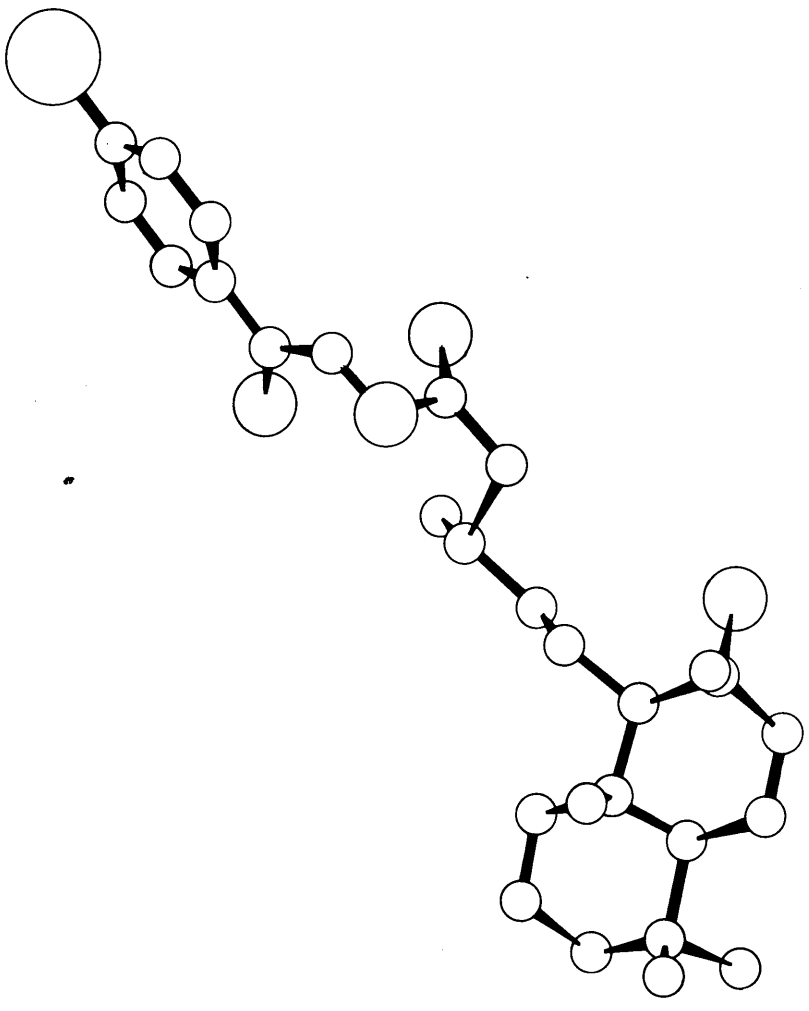


Fig. 4.

Repulsion of $C_{(19)}$ by both $C_{(17)}$ and $C_{(20)}$ does not noticeably distort the bond angles at $C_{(10)}$, although $C_{(5)}\ C_{(10)}\ C_{(19)}$ is increased to 114.7^{o}. The resultant of the repulsion forces from $C_{(17)}$ and $C_{(20)}$ with $C_{(19)}$ probably acts along the line of the bond $C_{(5)}$ - $C_{(10)}$.

Mean plane calculations, through rings A and B show that ring B approximates much more closely to the theoretical chair conformation than ring A. From the mean plane calculations $C_{(4)}$ is nearer the plane through ring A than it should be theoretically.

Ring A.	Δ	Ring B.	Δ
$C_{(1)}$	0.2464	$C_{(5)}$	0.2750
$C_{(2)}$	-0.2253	$C_{(6)}$	-0.2605
$C_{(3)}$	0.2015	$C_{(7)}$	0.2346
$C_{(4)}$	-0.1917	$C_{(8)}$	-0.2293
$C_{(5)}$	0.2102	$C_{(9)}$	0.2324
$C_{(10)}$	-0.2412	$C_{(10)}$	-0.2522

The side chain at $C_{(9)}$, as has been found for other long chain molecules[109], does not adopt a folded conformation; the chain is extended.

It was found that the $C_{(22)}$ carbonyl group and the benzene ring are non-planar, the dihedral angle between the plane of the benzene ring and the plane through $O_{(4)}\ C_{(21)}\ C_{(22)}\ C_{(23)}$ being 11.57^{o}. This rotation of the benzene ring in the crystal lattice is probably due to the close intermolecular approach of $O_{(1)}$ and $C_{(24)}$ 3.62Å Fig. 5, their interaction providing the driving force for ring rotation

about $C_{(22)}$-$C_{(23)}$ bond.

The hydroxyl group $O_{(1)}$, does not appear to be involved in hydrogen bonding; the shortest non-bonded inter-molecular distance being 3.25Å to O_2 of a neighbouring molecule. This distance is too long to involve a hydrogen bond and there are no atoms, in the one molecule, within 4Å of $O_{(1)}$ which could take part in an intramolecular hydrogen bond such as is found in labdanolic acid itself[97].

The average bond lengths C-C sp^3 and C-C sp^2 aromatic agree with the accepted literature value i.e. C-C sp^3: 1.55Å ±0.02, literature value 1.54Å; C-C sp^2: 1.39Å± 0.03, literature value 1.395Å. The C-O and C=O average bond lengths are also in agreement with the accepted values i.e. C-O 1.45Å ±0.02, 1.43Å (literature value); C=O : 1.23Å ± 0.02, literature value 1.23Å. The C-Br bond length is 1.91Å ± 0.01 agrees with the literature value of 1.85Å.

Packing Diagram

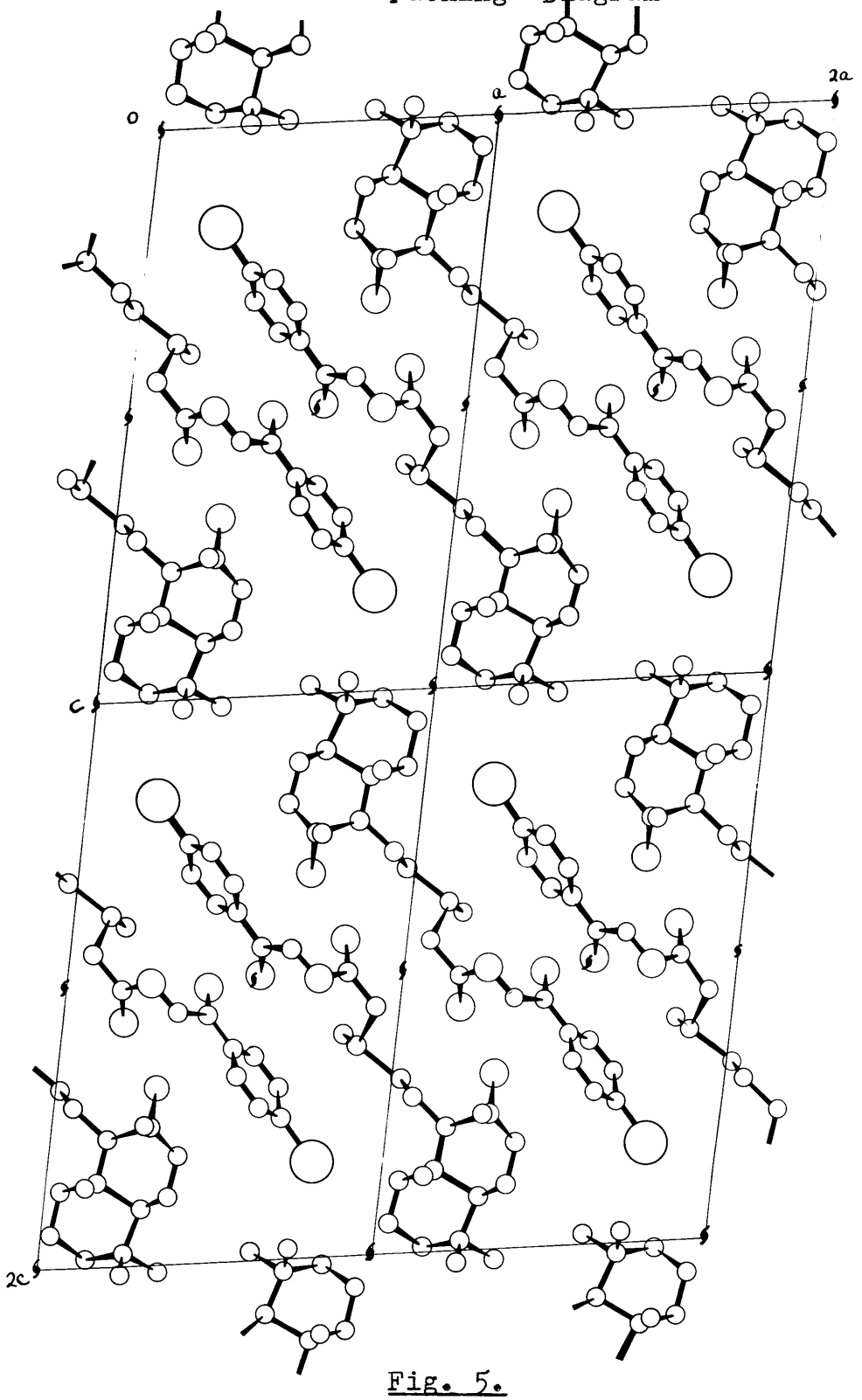


Fig. 5.

TABLE (1)

PROGRESS OF STRUCTURE ANALYSIS.

Patterson synthesis

Sharpened Patterson synthesis

1st S.F. calculation 60%
1st 2 dim. electron density distribution (e.d.d.)

3 cycles of minimum residual calculations (R = 23%)

2nd S.F. calculation (R = 49%)
1st 3 dim. electron density distribution

3rd S.F. calculation (R = 46.8%)
2nd 3 dim. e.d.d. - 3 atoms.

4th S.F. calculation (R = 45%)
3rd 3 dim e.d.d. - 12 atoms,

5th S.F. calculation (R = 43%)
4th 3 dim. e.d.d. - 18 atoms.

6th S.F. calculation (R = 39%)
5th 3 dim. e.d.d. - 25 atoms.

7th S.F. calculation (R = 37%)
6th 3 dim. e.d.d. - 28 atoms.

8th S.F. calculation (R = 36%)
7th 3 dim. e.d.d. - 29 atoms.

9th S.F. calculation including h41 and h51 (R = 34%)
8th 3 dim. e.d.d. - 33 atoms.

Data revised and 2 cycles of calculations to allow for termination of series errors and to adjust the individual temperature factors of the atoms (R = 27%)

6 cycles of isotropic structure factor least squares calculations R = 20%

10 cycles of anisotropic least squares refinement calculations and S.F. calculations R = 11.5%, R1 = 0.0241

Table 2.

H	K	L	Fo	Fc
0	0	2	23.7	24.7
0	0	3	38.6	47.5
0	0	4	39.4	35.1
0	0	5	22.5	20.1
0	0	6	82.9	77.3
0	0	7	21.6	19.7
0	0	8	13.3	14.7
0	0	9	22.6	24.4
0	0	10	6.5	7.9
0	0	11	20.8	20.3
0	0	12	12.6	15.5
0	0	13	14.5	13.5
0	0	14	12.1	12.5
0	0	16	19.5	23.0
0	0	17	10.1	12.5
0	0	18	9.3	10.4
0	0	19	9.5	9.0
0	0	22	6.6	4.9
1	0	0	17.8	10.9
2	0	0	89.6	83.0
3	0	0	8.4	14.7
4	0	0	11.5	6.4
5	0	0	41.8	34.2
7	0	0	6.3	5.1
9	0	0	11.6	10.5
1	0	1	39.8	30.8
1	0	2	13.6	4.4
1	0	3	3.8	2.5
1	0	4	19.7	21.6
1	0	5	44.3	62.0
1	0	6	45.2	48.1
1	0	8	43.9	38.6
1	0	9	32.2	34.6
1	0	10	47.9	45.0
1	0	11	15.3	20.4
1	0	12	10.3	10.3
1	0	13	7.4	7.6
1	0	14	8.4	9.0
1	0	17	7.8	7.6
1	0	18	13.6	11.7
1	0	20	7.4	5.6
1	0	21	7.0	6.2
1	0	23	8.9	8.0
1	0	24	6.7	5.2
2	0	1	42.0	39.1
2	0	2	28.2	22.1
2	0	3	23.6	34.0
2	0	4	3.8	3.8
2	0	5	31.6	29.2
2	0	6	50.8	48.4
2	0	7	11.8	15.3
2	0	8	24.9	33.3
2	0	9	26.6	32.0
2	0	10	14.3	14.5
2	0	11	39.1	37.3
2	0	12	23.5	24.0
2	0	13	26.7	28.4
2	0	14	28.5	31.9
2	0	15	13.1	14.1
2	0	16	19.5	20.7
2	0	17	8.6	8.8
2	0	18	8.6	6.7
2	0	19	9.0	5.0
2	0	22	8.1	6.1
2	0	23	7.2	4.6
3	0	1	39.2	29.7
3	0	2	68.1	60.3
3	0	3	41.5	46.1
3	0	4	42.1	43.0
3	0	5	80.1	82.6
3	0	6	17.7	15.0
3	0	7	79.1	70.3
3	0	10	22.6	29.6
3	0	13	16.3	17.0
3	0	15	15.6	17.3
3	0	16	11.9	11.0
3	0	18	8.4	7.8
3	0	19	7.4	8.6
3	0	23	5.7	4.8
4	0	1	63.9	64.8
4	0	3	7.3	8.7
4	0	5	54.7	43.4
4	0	6	43.3	39.7
4	0	8	12.3	10.8
4	0	9	31.1	29.3
4	0	10	7.8	9.6
4	0	11	14.2	14.7
4	0	12	19.3	19.2
4	0	13	8.9	6.7
4	0	14	26.0	27.8
4	0	16	9.3	7.6
4	0	17	10.1	10.5
4	0	18	7.5	7.9
4	0	20	6.5	6.6
4	0	22	6.4	6.8
5	0	1	5.0	4.2
5	0	2	9.7	11.0
5	0	3	69.9	61.5
5	0	4	67.7	56.0
5	0	5	29.4	31.4
5	0	6	15.0	15.4
5	0	7	38.8	36.6
5	0	8	17.1	15.8
5	0	9	8.0	9.8
5	0	10	18.8	19.6
5	0	11	17.9	18.3
5	0	13	15.9	17.1
5	0	14	7.8	7.7
5	0	16	15.3	14.1
5	0	19	14.6	12.2
6	0	1	33.6	26.6
6	0	2	35.6	30.5
6	0	3	39.5	28.0
6	0	4	23.5	20.5
6	0	6	13.7	15.3
6	0	7	20.9	22.5
6	0	8	14.4	16.2
6	0	9	25.0	21.0
6	0	12	14.1	15.2
6	0	15	18.4	15.0
7	0	1	13.0	12.4
7	0	2	9.6	8.0
7	0	3	25.7	24.3
7	0	5	12.0	13.1
7	0	6	16.6	16.5
7	0	8	18.7	18.1
7	0	10	14.9	16.9
7	0	11	19.2	18.6
7	0	14	10.7	13.5
7	0	16	13.6	10.8
7	0	19	9.4	8.6
8	0	1	51.3	44.4
8	0	2	17.3	16.1
8	0	4	15.3	14.6
8	0	6	9.9	10.8
8	0	7	17.5	16.1
8	0	8	14.6	16.3
8	0	12	7.7	6.6
8	0	13	8.5	10.4
9	0	2	11.5	11.2

H	K	L	Fo	Fc
9	0	4	8.5	9.0
9	0	6	14.9	16.0
9	0	11	7.4	8.8
9	0	12	8.4	8.4
9	0	14	10.9	10.1
10	0	1	12.1	12.3
10	0	2	19.7	18.0
10	0	5	7.8	8.1
10	0	13	5.8	6.3
11	0	3	8.4	6.2
11	0	9	7.6	9.1
11	0	12	6.1	6.7
1	0	-1	15.1	28.0
1	0	-2	16.1	7.1
1	0	-3	67.7	70.0
1	0	-4	69.6	60.7
1	0	-5	35.3	37.0
1	0	-6	50.2	51.8
1	0	-7	26.0	23.9
1	0	-8	31.0	25.7
1	0	-9	69.0	68.6
1	0	-10	65.4	52.1
1	0	-11	14.4	12.4
1	0	-12	21.6	23.2
1	0	-13	16.9	18.8
1	0	-14	12.8	15.9
1	0	-15	17.2	18.2
1	0	-16	9.8	8.0
1	0	-17	12.7	11.9
1	0	-19	7.8	6.9
2	0	-1	53.9	46.8
2	0	-2	20.9	28.9
2	0	-3	74.3	76.0
2	0	-4	105.6	114.2
2	0	-5	30.2	27.9
2	0	-6	11.1	9.8
2	0	-7	4.5	2.2
2	0	-8	18.4	14.6
2	0	-9	5.4	6.7
2	0	-10	13.9	14.4
2	0	-12	7.4	8.3
2	0	-13	30.4	32.9
2	0	-15	10.3	13.0
2	0	-18	8.6	8.1
2	0	-22	8.0	6.1
2	0	-25	4.6	1.9
3	0	-1	8.0	6.8
3	0	-2	106.2	85.7
3	0	-3	67.6	71.9
3	0	-4	83.4	81.3
3	0	-5	47.2	45.6
3	0	-6	21.2	21.0
3	0	-7	28.0	24.7
3	0	-8	5.0	4.7
3	0	-9	56.9	51.5
3	0	-10	6.2	4.7
3	0	-11	7.1	7.6
3	0	-13	7.4	6.8
3	0	-14	9.2	7.9
3	0	-15	11.4	10.2
3	0	-17	12.0	12.5
3	0	-19	11.6	11.7
4	0	-1	35.3	30.8
4	0	-2	38.1	40.0
4	0	-3	16.6	21.6
4	0	-4	19.3	10.7
4	0	-5	44.7	36.8
4	0	-6	4.9	4.3
4	0	-7	39.1	36.4
4	0	-8	23.9	20.2
4	0	-9	13.8	13.8
4	0	-10	25.2	23.3
4	0	-11	6.9	6.8
4	0	-13	22.5	21.0
4	0	-15	10.5	10.4
4	0	-21	9.3	6.9
5	0	-1	11.7	12.1
5	0	-3	45.8	41.5
5	0	-4	29.2	29.2
5	0	-5	14.0	11.7
5	0	-6	38.3	36.4
5	0	-7	6.6	5.6
5	0	-8	34.1	34.6
5	0	-10	11.8	11.6
5	0	-11	9.3	7.6
5	0	-12	6.9	7.6
5	0	-13	7.8	7.8
5	0	-14	14.0	14.7
5	0	-15	5.9	6.7
5	0	-17	8.2	6.0
6	0	-1	21.7	17.8
6	0	-2	8.3	6.7
6	0	-4	24.9	26.7
6	0	-7	31.1	27.0
6	0	-8	7.4	6.8
6	0	-10	19.9	18.3
6	0	-11	9.1	9.6
6	0	-12	27.2	26.0
6	0	-13	17.5	18.5
6	0	-14	10.7	11.8
6	0	-15	9.2	8.6
7	0	-1	6.8	7.1
7	0	-2	24.1	22.3
7	0	-3	13.8	11.6
7	0	-4	6.9	7.5
7	0	-5	23.2	20.5
7	0	-6	6.5	6.3
7	0	-8	14.5	13.7
7	0	-12	11.6	12.6
7	0	-14	8.5	6.6
8	0	-1	23.4	24.3
8	0	-3	9.7	8.2
8	0	-7	16.1	15.9
8	0	-8	12.6	12.6
8	0	-10	10.6	10.5
8	0	-13	11.1	9.7
8	0	-15	10.2	7.5
9	0	-1	12.9	11.3
9	0	-2	19.9	18.8
9	0	-5	8.2	8.6
9	0	-8	14.9	16.2
9	0	-10	9.2	8.5
10	0	-1	8.5	8.7
10	0	-3	7.8	6.1
10	0	-5	8.5	7.7
10	0	-7	8.6	9.2
10	0	-11	7.8	7.6
10	0	-14	8.1	6.3
11	0	-1	8.2	8.0
11	0	-3	8.6	7.0
12	0	-2	9.6	7.9
12	0	-3	8.8	2.3
13	0	-5	7.7	3.2
0	1	2	40.7	35.4
0	1	3	40.7	39.2
0	1	4	71.9	83.8
0	1	5	17.0	17.0
0	1	6	21.7	18.1
0	1	7	18.4	21.0
0	1	8	10.5	9.0

H	K	L	Fo	Fc
0	1	9	31.5	29.2
0	1	10	41.4	48.2
0	1	11	4.4	3.1
0	1	12	35.3	34.6
0	1	13	5.9	7.1
0	1	14	9.6	10.1
0	1	15	10.9	12.2
0	1	16	8.2	8.1
0	1	17	11.5	11.4
0	1	18	5.2	5.2
0	1	20	5.0	4.2
0	1	23	10.7	7.6
0	1	24	4.3	2.9
2	1	0	18.6	10.9
3	1	0	45.8	43.7
4	1	0	6.0	4.2
5	1	0	14.6	13.5
6	1	0	40.5	35.2
7	1	0	13.1	12.8
8	1	0	14.1	14.1
9	1	0	5.0	4.7
10	1	0	12.4	14.0
11	1	0	10.5	9.6
1	1	1	56.1	56.9
1	1	2	53.1	56.4
1	1	3	38.9	36.2
1	1	4	38.6	36.6
1	1	5	33.5	34.7
1	1	6	47.9	46.6
1	1	7	12.3	13.5
1	1	8	34.2	34.2
1	1	9	19.2	22.3
1	1	10	24.1	21.1
1	1	11	21.4	19.7
1	1	12	13.7	13.3
1	1	13	11.0	12.6
1	1	14	32.4	34.7
1	1	15	10.6	11.8
1	1	17	13.2	14.3
1	1	18	5.2	4.8
1	1	19	7.5	6.2
1	1	20	5.4	5.4
1	1	22	4.3	3.8
2	1	1	38.4	30.7
2	1	2	44.1	43.0
2	1	3	7.6	10.3
2	1	4	54.3	51.3
2	1	5	17.8	24.5
2	1	6	12.1	13.2
2	1	7	52.1	49.9
2	1	8	28.8	33.2
2	1	9	15.2	17.7
2	1	10	27.6	34.5
2	1	11	9.2	11.7
2	1	12	9.9	12.5
2	1	13	25.8	26.7
2	1	14	8.5	10.1
2	1	15	8.4	10.5
2	1	16	5.2	5.9
2	1	17	4.4	3.8
2	1	18	11.5	13.2
2	1	19	8.4	8.4
2	1	20	4.4	3.4
2	1	23	8.6	7.2
3	1	1	50.5	46.7
3	1	2	10.4	15.3
3	1	3	43.3	37.6
3	1	4	45.6	41.8
3	1	5	7.0	5.7
3	1	6	21.4	28.9
3	1	7	24.6	25.7
3	1	8	27.6	31.6
3	1	9	28.1	34.3
3	1	11	20.6	24.8
3	1	12	8.3	9.5
3	1	13	17.2	17.7
3	1	14	23.3	27.0
3	1	15	11.0	13.4
3	1	16	12.1	13.7
3	1	17	7.5	8.4
3	1	18	5.6	5.7
3	1	20	8.8	8.0
3	1	22	4.4	4.3
4	1	1	17.3	14.5
4	1	2	48.4	46.9
4	1	3	19.4	14.6
4	1	4	24.0	20.0
4	1	5	75.2	73.5
4	1	6	20.3	17.0
4	1	7	14.5	16.4
4	1	8	16.5	21.7
4	1	9	5.6	5.4
4	1	10	27.2	28.4
4	1	11	14.4	15.5
4	1	12	14.0	15.6
4	1	13	11.7	12.3
4	1	14	15.0	18.0
4	1	15	6.3	6.2
4	1	16	5.7	5.8
4	1	18	10.2	11.1
4	1	19	4.3	6.3
4	1	21	7.8	6.2
5	1	1	37.0	32.3
5	1	2	10.8	9.7
5	1	3	15.4	17.5
5	1	4	63.0	58.1
5	1	5	9.5	10.0
5	1	6	16.6	21.9
5	1	7	22.7	26.5
5	1	8	10.2	15.2
5	1	9	21.6	26.2
5	1	10	9.2	11.3
5	1	11	6.5	6.9
5	1	12	15.5	16.8
5	1	14	6.3	5.7
5	1	15	11.5	16.7
5	1	17	6.5	7.6
6	1	1	24.5	24.3
6	1	2	32.1	30.7
6	1	3	18.2	17.4
6	1	4	3.5	2.0
6	1	5	24.8	25.0
6	1	6	14.9	16.2
6	1	7	12.5	11.6
6	1	8	13.7	13.8
6	1	9	13.0	13.0
6	1	10	13.1	12.8
6	1	11	20.8	21.0
6	1	12	8.4	9.6
6	1	13	10.7	9.5
6	1	16	10.4	10.7
6	1	17	4.8	4.9
6	1	18	8.8	8.1
6	1	19	6.0	5.2
7	1	1	23.2	21.2
7	1	2	31.7	29.1
7	1	3	12.6	12.2
7	1	5	11.1	12.4
7	1	6	14.7	16.3
7	1	7	19.0	19.5

H	K	L	Fo	Fc
7	1	9	8.3	7.6
7	1	10	9.2	10.0
7	1	11	5.0	4.4
7	1	12	5.8	5.6
7	1	13	7.4	9.7
7	1	14	4.8	5.5
7	1	15	18.5	13.2
7	1	17	7.1	6.4
7	1	18	7.2	5.5
7	1	19	3.8	2.8
8	1	1	4.0	3.8
8	1	2	16.4	16.4
8	1	3	18.6	19.2
8	1	4	18.4	18.2
8	1	5	7.3	8.6
8	1	6	12.7	12.6
8	1	8	13.1	13.9
8	1	9	7.0	7.7
8	1	10	6.7	6.9
8	1	11	15.4	14.7
8	1	12	12.3	11.4
8	1	13	10.2	8.3
8	1	14	10.5	9.9
8	1	16	9.5	8.4
8	1	17	4.4	2.9
9	1	1	16.6	16.6
9	1	2	28.3	26.7
9	1	4	12.2	14.4
9	1	5	9.0	8.3
9	1	6	4.4	5.2
9	1	7	12.8	13.0
9	1	8	8.1	8.0
9	1	10	6.0	6.8
9	1	13	9.3	7.9
9	1	15	5.9	5.0
9	1	16	4.0	3.1
10	1	1	9.9	9.6
10	1	2	6.9	6.4
10	1	4	7.9	6.8
10	1	7	5.1	5.9
10	1	8	4.2	4.1
10	1	9	5.9	5.8
10	1	10	4.9	4.9
10	1	11	7.9	7.1
11	1	1	7.9	8.5
11	1	3	10.0	5.7
11	1	5	7.7	6.9
11	1	8	9.2	6.6
11	1	10	7.1	5.7
11	1	13	5.9	5.3
12	1	3	3.9	3.3
12	1	6	6.0	5.2
12	1	7	5.1	4.9
12	1	9	7.2	6.8
13	1	3	4.3	4.0
13	1	4	5.4	5.6
13	1	7	4.0	2.9
1	1	-3	19.8	20.1
1	1	-4	21.3	20.3
1	1	-5	66.1	60.5
1	1	-6	47.4	46.3
1	1	-7	32.4	32.9
1	1	-8	28.0	28.2
1	1	-9	9.7	7.2
1	1	-10	11.8	12.8
1	1	-11	12.2	14.4
1	1	-12	14.5	12.9
1	1	-13	17.2	17.0
1	1	-14	14.0	13.1
1	1	-15	10.5	11.4
1	1	-16	10.4	12.5
1	1	-17	9.0	9.6
1	1	-18	9.5	9.7
1	1	-19	4.8	3.9
1	1	-20	7.2	5.9
1	1	-21	7.0	5.8
2	1	-2	26.0	21.3
2	1	-3	56.2	60.5
2	1	-4	48.8	53.4
2	1	-5	39.7	34.4
2	1	-6	8.9	10.0
2	1	-7	25.2	24.5
2	1	-8	11.6	11.9
2	1	-9	46.6	50.8
2	1	-10	25.7	25.1
2	1	-11	17.8	18.9
2	1	-12	25.3	22.5
2	1	-13	5.3	5.9
2	1	-14	12.0	12.4
2	1	-15	9.9	9.6
2	1	-17	15.8	15.6
2	1	-19	8.3	8.1
2	1	-20	5.6	5.2
3	1	-1	53.5	51.6
3	1	-2	34.4	24.1
3	1	-3	89.6	81.9
3	1	-4	36.6	35.8
3	1	-5	51.4	47.6
3	1	-6	26.8	27.3
3	1	-7	49.5	43.8
3	1	-8	13.7	13.0
3	1	-9	30.4	33.3
3	1	-10	24.7	23.0
3	1	-11	9.3	9.8
3	1	-12	4.2	4.6
3	1	-13	12.4	12.0
3	1	-14	16.1	14.5
3	1	-15	7.1	6.3
3	1	-16	6.5	5.2
3	1	-19	11.7	10.7
3	1	-20	5.5	5.2
4	1	-1	41.9	36.0
4	1	-2	24.6	20.3
4	1	-3	43.7	40.9
4	1	-4	40.1	39.2
4	1	-5	48.5	48.1
4	1	-6	36.9	38.0
4	1	-7	16.5	14.7
4	1	-8	23.7	23.0
4	1	-9	17.9	18.6
4	1	-10	9.2	9.4
4	1	-11	28.7	30.1
4	1	-12	11.0	10.4
4	1	-14	12.1	10.7
4	1	-15	4.6	5.7
4	1	-17	8.8	7.6
4	1	-20	5.0	4.1
4	1	-24	4.8	3.3
5	1	-1	7.1	9.1
5	1	-2	9.9	9.0
5	1	-3	60.5	48.1
5	1	-5	14.1	12.6
5	1	-6	13.0	14.5
5	1	-7	33.7	32.9
5	1	-8	13.4	12.8
5	1	-9	19.1	16.9
5	1	-10	12.8	11.4
5	1	-11	9.4	10.0
5	1	-12	18.7	17.0
5	1	-13	7.6	6.9

H	K	L	Fo	Fc
5	1	-15	10.6	9.0
5	1	-16	7.6	7.5
5	1	-20	4.5	4.0
5	1	-21	4.4	3.3
6	1	-1	16.2	17.6
6	1	-2	14.6	14.2
6	1	-3	37.4	39.1
6	1	-4	6.1	5.6
6	1	-5	12.4	11.4
6	1	-6	25.8	23.9
6	1	-7	5.2	6.3
6	1	-8	17.3	13.4
6	1	-9	15.2	13.4
6	1	-10	9.4	10.0
6	1	-11	5.6	5.1
6	1	-12	17.9	16.3
6	1	-13	6.5	6.1
6	1	-14	10.7	10.6
6	1	-15	6.2	6.8
6	1	-22	3.7	2.6
7	1	-1	18.9	16.3
7	1	-2	5.6	4.4
7	1	-3	7.3	7.9
7	1	-5	18.8	18.3
7	1	-6	19.0	21.7
7	1	-7	17.5	15.3
7	1	-8	5.5	3.7
7	1	-9	24.0	21.8
7	1	-10	6.3	6.1
7	1	-11	6.2	5.6
7	1	-12	15.3	14.0
7	1	-13	19.5	17.2
7	1	-14	6.8	5.4
7	1	-15	8.3	6.8
7	1	-23	6.4	3.6
8	1	-1	13.6	13.5
8	1	-2	12.7	11.2
8	1	-3	15.0	15.9
8	1	-4	14.2	11.5
8	1	-5	6.2	4.9
8	1	-7	10.2	8.6
8	1	-8	5.4	4.6
8	1	-11	4.6	4.9
8	1	-12	5.7	5.0
8	1	-13	8.4	6.4
8	1	-14	4.9	4.0
8	1	-16	7.5	6.1
8	1	-22	6.8	4.0
9	1	-1	17.2	16.0
9	1	-3	8.9	7.8
9	1	-4	7.7	6.0
9	1	-6	4.7	3.5
9	1	-7	6.4	6.6
9	1	-8	4.8	4.2
9	1	-10	7.0	7.4
9	1	-11	3.1	3.8
9	1	-12	6.3	5.2
9	1	-13	5.9	5.1
9	1	-16	4.1	3.4
10	1	-1	8.8	9.0
10	1	-2	13.5	12.8
10	1	-4	6.4	5.8
10	1	-5	4.4	3.7
10	1	-7	5.0	4.3
10	1	-8	10.7	11.2
10	1	-10	6.2	4.9
10	1	-11	5.4	5.3
11	1	-3	6.1	5.1
11	1	-6	4.8	4.5
11	1	-7	9.2	7.2
11	1	-8	4.7	4.5
11	1	-9	4.7	4.0
11	1	-11	6.0	5.0
11	1	-17	4.5	3.0
12	1	-2	10.3	7.5
12	1	-3	6.4	4.9
12	1	-5	5.8	4.9
0	2	1	30.1	23.9
0	2	2	32.4	28.4
0	2	3	55.4	49.9
0	2	4	9.2	9.1
0	2	5	34.5	32.4
0	2	6	25.5	20.0
0	2	7	9.0	7.3
0	2	8	31.8	29.0
0	2	9	15.7	17.0
0	2	11	21.4	20.3
0	2	12	4.5	3.9
0	2	13	12.8	12.6
0	2	14	11.8	12.9
0	2	15	5.1	5.1
0	2	16	12.4	12.2
0	2	19	7.7	6.7
0	2	20	4.4	4.0
0	2	24	2.7	4.0
1	2	0	53.0	41.3
2	2	0	22.1	19.2
3	2	0	39.3	26.0
4	2	0	19.6	18.2
5	2	0	23.8	23.8
6	2	0	6.6	5.4
7	2	0	6.9	7.4
8	2	0	14.4	12.3
9	2	0	13.4	15.8
10	2	0	4.0	3.6
1	2	1	26.0	27.0
1	2	2	39.4	39.7
1	2	3	25.5	24.6
1	2	4	44.2	41.9
1	2	5	37.1	32.3
1	2	6	20.1	13.3
1	2	7	16.7	15.5
1	2	8	20.5	19.9
1	2	9	28.2	26.3
1	2	10	20.0	22.3
1	2	11	5.6	5.8
1	2	12	18.9	16.4
1	2	13	5.9	4.8
1	2	14	7.0	6.3
1	2	15	21.5	21.2
1	2	17	9.5	11.4
1	2	18	8.1	7.3
1	2	21	5.2	5.1
1	2	23	4.3	4.2
2	2	1	42.4	38.8
2	2	2	31.7	40.5
2	2	3	38.9	36.4
2	2	4	16.5	13.5
2	2	5	29.7	21.5
2	2	6	29.1	24.4
2	2	7	19.1	13.2
2	2	8	35.9	31.1
2	2	9	18.7	17.9
2	2	10	21.7	16.8
2	2	11	25.8	26.2
2	2	12	12.8	12.5
2	2	13	20.3	22.3
2	2	14	19.3	18.4
2	2	15	6.9	7.3
2	2	16	7.6	8.1

H	K	L	Fo	Fc
2	2	17	7.0	7.0
2	2	19	3.2	2.1
2	2	20	8.0	7.1
2	2	22	3.4	3.3
3	2	1	32.8	31.5
3	2	2	14.6	22.0
3	2	3	9.8	11.7
3	2	4	30.7	28.0
3	2	5	36.8	33.3
3	2	6	4.9	4.1
3	2	7	16.5	19.7
3	2	8	19.5	18.4
3	2	9	14.0	14.6
3	2	10	27.3	24.7
3	2	11	22.8	23.4
3	2	12	18.9	20.4
3	2	13	19.5	18.7
3	2	14	7.6	8.4
3	2	15	4.4	4.3
3	2	16	11.0	10.2
3	2	17	3.9	3.2
3	2	18	7.9	6.1
3	2	19	7.6	6.2
3	2	21	4.3	4.3
4	2	1	32.0	31.1
4	2	2	25.3	19.1
4	2	3	31.9	27.6
4	2	4	38.1	33.5
4	2	5	21.3	22.9
4	2	6	21.4	18.4
4	2	7	23.2	22.3
4	2	8	21.1	20.3
4	2	9	28.5	27.8
4	2	10	14.0	13.7
4	2	11	13.1	14.0
4	2	12	7.1	7.9
4	2	13	5.2	5.5
4	2	14	16.9	16.3
4	2	16	4.7	5.9
4	2	17	11.4	9.6
4	2	19	5.0	4.4
4	2	20	5.4	5.7
5	2	1	15.4	13.8
5	2	2	10.6	10.3
5	2	3	21.1	20.1
5	2	4	21.9	21.8
5	2	5	20.8	18.0
5	2	6	13.0	12.5
5	2	7	9.2	9.9
5	2	8	27.8	28.7
5	2	9	17.7	16.1
5	2	10	8.9	9.5
5	2	11	12.9	11.9
5	2	12	7.5	7.1
5	2	13	10.0	9.5
5	2	14	10.5	9.3
5	2	15	5.2	4.9
5	2	16	13.5	11.8
5	2	18	6.1	5.4
5	2	19	6.9	6.2
5	2	20	3.6	3.0
6	2	1	16.8	15.9
6	2	2	14.2	13.8
6	2	3	18.8	16.0
6	2	4	31.2	31.5
6	2	5	11.9	12.9
6	2	6	10.2	12.5
6	2	7	26.0	25.0
6	2	8	15.8	17.2
6	2	9	15.9	17.2
6	2	10	6.2	6.1
6	2	11	6.5	7.3
6	2	12	6.7	7.2
6	2	13	5.9	7.1
6	2	14	11.3	11.0
6	2	15	12.0	9.9
6	2	16	7.4	6.2
6	2	17	6.6	6.0
6	2	18	7.8	5.7
6	2	19	3.9	3.8
6	2	20	3.1	3.0
7	2	1	11.5	10.1
7	2	2	13.7	14.4
7	2	3	14.5	16.9
7	2	4	12.1	11.7
7	2	5	28.6	30.5
7	2	6	9.0	8.3
7	2	8	8.1	8.0
7	2	9	8.5	7.2
7	2	11	20.0	19.1
7	2	12	11.6	10.6
7	2	14	12.1	10.2
7	2	15	7.5	5.8
7	2	16	5.2	4.8
7	2	17	4.7	2.8
7	2	18	3.7	3.9
8	2	1	16.8	19.3
8	2	2	11.6	12.7
8	2	3	14.6	16.2
8	2	4	16.2	19.9
8	2	6	5.2	6.1
8	2	7	4.9	6.1
8	2	8	7.7	8.6
8	2	10	6.9	8.2
8	2	11	8.9	8.5
8	2	12	6.6	6.3
8	2	13	4.5	4.9
8	2	14	6.1	6.4
8	2	15	8.4	7.9
8	2	16	5.3	4.1
8	2	17	3.6	4.2
9	2	1	12.9	13.8
9	2	2	18.6	21.4
9	2	5	4.8	6.0
9	2	6	7.5	8.4
9	2	7	5.6	5.6
9	2	8	3.3	3.8
9	2	9	9.9	9.9
9	2	10	3.7	3.8
9	2	11	8.0	7.6
9	2	12	7.4	6.7
9	2	14	7.9	7.4
10	2	1	5.9	7.3
10	2	2	6.7	7.7
10	2	5	8.2	6.2
10	2	6	4.6	4.4
10	2	7	7.5	8.1
10	2	8	6.6	5.6
10	2	10	8.3	8.5
10	2	11	4.8	4.3
10	2	12	5.7	5.6
10	2	13	3.2	4.1
11	2	1	5.7	6.0
11	2	4	6.8	6.1
11	2	6	6.8	6.5
11	2	7	5.6	5.4
11	2	10	3.7	2.8
11	2	11	4.8	4.9
12	2	3	3.7	4.8
12	2	4	3.9	3.9

H	K L	Fo	Fc
12	2 5	2.8	3.7
12	2 6	3.7	4.6
12	2 7	3.1	4.5
12	2 8	3.3	4.0
12	2 9	2.6	2.6
1	2 -1	53.1	50.0
1	2 -2	17.4	16.7
1	2 -3	35.4	34.1
1	2 -4	49.1	50.0
1	2 -5	26.5	28.9
1	2 -6	28.3	26.8
1	2 -7	17.9	20.9
1	2 -8	23.6	22.8
1	2 -9	8.7	12.1
1	2-10	38.3	35.3
1	2-11	6.7	6.9
1	2-12	19.7	19.3
1	2-13	8.3	6.6
1	2-14	3.1	3.1
1	2-15	7.6	9.0
1	2-16	7.6	7.9
1	2-17	8.4	9.6
1	2-18	7.1	7.0
1	2-19	6.2	6.2
1	2-24	4.1	3.2
2	2 -1	36.2	24.8
2	2 -2	54.8	46.9
2	2 -3	21.4	20.7
2	2 -4	8.5	7.2
2	2 -5	36.6	37.9
2	2 -6	10.9	10.9
2	2 -7	37.9	36.2
2	2 -8	22.7	29.7
2	2 -9	11.9	12.0
2	2-10	22.1	21.8
2	2-11	12.0	14.1
2	2-12	13.0	14.1
2	2-13	25.1	24.0
2	2-14	11.4	13.0
2	2-15	6.9	7.2
2	2-16	6.4	6.8
2	2-17	7.6	7.0
2	2-18	6.3	6.0
2	2-19	6.9	6.8
2	2-20	4.1	4.6
2	2-21	4.2	4.6
2	2-23	4.1	3.3
2	2-24	3.3	3.4
3	2 -1	20.0	22.1
3	2 -2	20.0	19.3
3	2 -3	45.4	44.7
3	2 -4	12.5	10.2
3	2 -5	14.0	14.2
3	2 -6	16.7	20.3
3	2 -7	24.6	26.6
3	2 -8	6.6	7.0
3	2 -9	18.1	18.8
3	2-10	8.4	9.3
3	2-11	7.2	7.6
3	2-12	8.1	10.1
3	2-13	7.7	6.4
3	2-14	7.1	8.2
3	2-15	5.7	6.7
3	2-16	9.3	10.2
3	2-17	6.0	6.9
3	2-19	5.3	5.4
3	2-22	3.9	2.5
3	2-23	3.7	2.3
4	2 -1	13.9	12.8
4	2 -2	84.8	73.4
4	2 -3	24.1	19.4
4	2 -4	30.7	28.3
4	2 -5	38.8	39.4
4	2 -6	25.1	28.7
4	2 -7	11.7	11.3
4	2 -8	23.8	28.0
4	2 -9	11.5	10.9
4	2-10	6.3	8.7
4	2-11	11.6	11.0
4	2-12	8.2	10.2
4	2-13	5.3	6.7
4	2-14	4.8	4.4
4	2-15	5.1	5.7
4	2-17	4.0	3.4
4	2-18	5.0	4.9
4	2-20	6.0	4.3
4	2-21	4.6	3.2
5	2 -1	8.8	8.6
5	2 -2	18.3	17.0
5	2 -3	39.3	36.0
5	2 -4	10.4	8.4
5	2 -5	15.7	19.2
5	2 -6	30.7	29.5
5	2 -7	4.0	3.4
5	2 -8	19.3	21.0
5	2 -9	4.8	5.0
5	2-11	10.2	12.7
5	2-12	16.2	21.1
5	2-13	3.2	3.7
5	2-14	3.6	4.2
5	2-15	3.4	3.0
5	2-17	4.8	5.1
5	2-18	3.3	3.7
6	2 -1	10.7	12.2
6	2 -2	14.5	15.8
6	2 -3	9.8	7.0
6	2 -4	8.2	7.9
6	2 -5	14.4	17.1
6	2 -6	8.7	7.3
6	2 -7	13.8	15.0
6	2 -8	7.0	7.4
6	2 -9	16.9	18.0
6	2-10	4.1	5.3
6	2-11	4.2	4.1
6	2-12	15.4	17.7
6	2-13	7.1	7.2
6	2-15	7.3	8.5
6	2-18	3.2	3.3
7	2 -1	12.8	12.8
7	2 -2	14.0	14.7
7	2 -3	5.2	5.4
7	2 -4	8.6	9.5
7	2 -5	13.1	14.1
7	2 -6	8.8	10.0
7	2 -8	8.2	9.9
7	2 -9	7.8	8.8
7	2-10	6.3	7.6
7	2-11	5.0	5.0
7	2-12	12.1	14.0
7	2-14	4.0	5.0
7	2-15	6.1	6.3
7	2-16	4.4	5.8
7	2-17	4.6	4.9
7	2-21	4.3	3.2
8	2 -1	14.0	14.8
8	2 -2	4.5	6.0
8	2 -3	4.8	4.9
8	2 -4	5.6	7.4
8	2 -6	6.9	7.4
8	2 -7	6.0	7.5
8	2 -8	9.1	11.1
8	2 -9	5.8	7.1
8	2-10	4.7	7.2
8	2-12	6.4	7.0
8	2-13	4.0	2.5
8	2-16	3.2	2.4
8	2-21	2.8	2.4
9	2 -2	12.9	15.8
9	2 -3	4.7	5.7
9	2 -4	7.2	7.2
9	2 -5	4.9	6.4
9	2 -7	3.4	4.2
9	2-10	4.2	5.5
9	2-11	4.4	5.3
9	2-13	5.1	6.0
10	2 -1	4.8	5.2
10	2 -3	3.9	3.7
10	2 -5	5.2	6.1
10	2 -6	3.4	4.3
10	2 -9	4.0	5.1
10	2-10	5.7	6.1
10	2-12	5.6	6.9
10	2-14	4.7	5.4
10	2-15	5.0	4.3
11	2 -1	7.0	8.6
11	2 -2	4.7	5.6
11	2 -3	4.3	5.4
11	2 -4	3.3	4.5
11	2-15	3.0	2.8
12	2 -1	3.3	3.6
12	2 -2	3.6	4.4
12	2 -3	4.3	4.7
12	2 -7	3.5	3.8
12	2 -8	2.9	3.7
13	2 -4	2.6	3.0
0	3 1	34.2	38.2
0	3 2	12.2	11.0
0	3 3	38.5	27.2
0	3 4	27.2	30.1
0	3 6	18.4	17.9
0	3 7	19.9	18.4
0	3 9	11.7	10.2
0	3 10	12.3	11.3
0	3 11	7.7	7.9
0	3 12	15.1	14.9
0	3 13	6.7	6.9
0	3 14	6.2	6.6
0	3 15	5.6	5.6
0	3 16	6.0	6.2
0	3 17	7.6	8.5
0	3 19	3.3	3.9
0	3 20	4.2	3.9
1	3 0	20.2	18.0
2	3 0	26.0	20.1
3	3 0	44.9	33.9
4	3 0	6.7	5.7
5	3 0	21.8	21.8
6	3 0	14.8	14.3
8	3 0	11.5	13.1
9	3 0	5.4	5.5
10	3 0	7.9	7.6
11	3 0	5.8	3.7
1	3 1	33.2	27.0
1	3 2	22.8	15.0
1	3 3	46.7	42.8
1	3 4	22.0	14.8
1	3 5	31.6	28.5
1	3 6	17.8	17.5
1	3 8	14.8	13.8
1	3 9	18.5	17.2
1	3 10	6.4	6.8
1	3 11	13.6	13.9
1	3 12	10.1	8.6
1	3 13	5.4	5.8
1	3 14	13.6	13.5
1	3 16	4.3	4.8
1	3 17	7.6	6.7
1	3 21	2.9	3.8
2	3 1	42.4	29.4
2	3 2	42.4	32.8
2	3 3	23.0	17.5
2	3 4	24.5	19.4
2	3 5	11.8	7.8
2	3 6	18.8	12.8
2	3 7	26.2	23.9
2	3 8	4.9	2.9
2	3 10	18.3	17.9
2	3 11	12.1	12.5
2	3 12	7.9	8.6
2	3 13	14.0	13.1
2	3 14	7.3	8.0
2	3 15	13.9	13.2
2	3 16	4.7	4.9
2	3 17	7.5	5.7
2	3 18	4.4	4.4
2	3 19	3.7	3.7
2	3 21	3.3	3.1
3	3 1	14.4	11.7
3	3 2	22.3	16.6
3	3 3	16.5	13.9
3	3 4	18.9	13.2
3	3 5	12.3	11.4
3	3 6	22.5	19.2
3	3 7	13.9	14.1
3	3 8	6.7	6.4
3	3 9	17.8	16.9
3	3 10	13.3	12.7
3	3 11	13.5	12.0
3	3 13	12.4	13.8
3	3 15	9.7	8.8
3	3 17	5.9	6.2
3	3 18	3.2	2.8
3	3 19	3.0	3.6
3	3 20	4.2	3.5
4	3 1	10.0	8.6
4	3 2	9.5	10.0
4	3 3	14.9	13.4
4	3 4	16.2	15.7
4	3 5	12.6	11.3
4	3 6	11.7	8.3
4	3 8	20.3	16.8
4	3 9	5.8	6.7
4	3 10	23.6	22.8
4	3 11	8.3	7.3
4	3 12	13.6	12.9
4	3 13	9.3	11.5
4	3 14	5.6	5.8
4	3 15	6.0	7.3
4	3 17	4.3	4.0
4	3 17	3.6	4.0
4	3 18	3.0	3.2
4	3 19	4.1	3.2
4	3 20	3.7	1.3
5	3 1	19.2	19.0
5	3 2	35.9	27.6
5	3 3	14.4	14.9
5	3 4	34.0	29.5
5	3 5	20.3	18.2
5	3 6	16.3	17.5
5	3 7	20.0	18.6
5	3 8	9.5	9.9
5	3 9	18.5	17.2
5	3 10	22.8	21.9
5	3 11	16.4	16.4
5	3 12	6.4	6.7
5	3 13	5.7	5.8
5	3 15	8.7	6.7
5	3 17	4.3	3.4
5	3 18	3.6	3.8
6	3 1	6.8	5.4
6	3 2	13.4	11.3
6	3 3	5.6	5.3
6	3 4	15.1	14.4
6	3 5	11.4	12.5
6	3 6	9.8	10.2
6	3 7	7.4	7.0
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6	3 11	7.3	7.3
6	3 12	15.6	13.0
6	3 13	6.2	5.3
6	3 14	7.5	6.2
6	3 16	5.7	4.5
6	3 17	3.8	3.0
6	3 18	4.5	4.8
7	3 2	16.7	18.3
7	3 3	5.0	4.8
7	3 4	7.5	8.0
7	3 5	18.9	17.0
7	3 6	14.7	15.8
7	3 7	14.9	16.0
7	3 8	8.4	8.2
7	3 10	7.4	4.9
7	3 11	12.3	10.6
7	3 12	7.4	7.5
7	3 14	5.9	5.5
7	3 15	9.6	8.6
7	3 16	6.8	4.8
7	3 17	4.6	5.6
8	3 1	3.9	3.8
8	3 2	20.0	21.8
8	3 3	12.1	12.1
8	3 4	4.0	4.8
8	3 5	9.0	10.4
8	3 6	5.2	5.3
8	3 7	4.0	3.7
8	3 8	9.6	7.7
8	3 9	15.0	11.5
8	3 11	11.1	8.0
8	3 12	9.2	7.9
8	3 13	4.0	3.8
8	3 14	5.0	4.6
8	3 15	4.2	3.5
9	3 1	3.8	3.8
9	3 2	9.5	11.0
9	3 3	6.4	6.1
9	3 6	8.8	7.2
9	3 8	3.4	4.2
9	3 10	5.9	5.6
9	3 12	5.3	5.2
9	3 13	3.9	3.8
10	3 1	7.9	7.2
10	3 2	3.9	2.7
10	3 5	5.9	5.1
10	3 6	6.8	4.8
10	3 7	6.5	5.4
10	3 8	6.6	6.1
10	3 9	6.3	5.0
10	3 12	5.2	4.4
11	3 3	3.2	3.3
11	3 4	4.8	4.5
11	3 5	4.0	3.4
11	3 8	4.8	5.3
11	3 9	3.3	3.3
13	3 2	2.6	3.8
13	3 3	2.1	3.2
1	3 -1	28.3	31.3
1	3 -2	15.7	13.0
1	3 -3	19.6	20.3
1	3 -4	29.6	23.2
1	3 -5	17.4	18.4
1	3 -6	20.3	20.7
1	3 -7	28.7	27.6
1	3 -8	11.5	10.6
1	3-10	13.4	14.3
1	3-11	15.0	14.8
1	3-12	7.6	7.4
1	3-13	12.2	13.7
1	3-14	7.3	7.7
1	3-15	5.6	6.4
1	3-16	12.3	10.2
1	3-17	7.0	6.8
1	3-18	5.5	6.4
1	3-19	5.9	4.9
1	3-21	4.9	4.1
1	3-22	2.9	2.8
2	3 -1	46.3	42.4
2	3 -2	16.7	16.2
2	3 -3	21.1	17.7
2	3 -4	23.2	25.3
2	3 -5	15.4	17.5
2	3 -6	15.0	16.9
2	3 -7	10.3	13.8
2	3 -9	4.9	5.2
2	3-10	14.5	16.8
2	3-11	16.4	16.1
2	3-12	6.1	7.2
2	3-13	7.6	9.3
2	3-15	8.0	7.3
2	3-16	5.9	6.7
2	3-17	6.3	6.3
2	3-18	3.6	3.8
2	3-19	3.7	3.3
2	3-21	8.2	5.8
2	3-22	3.4	2.7
3	3 -1	16.4	13.2
3	3 -2	19.7	18.8
3	3 -3	19.5	20.6
3	3 -4	19.9	18.1
3	3 -5	23.7	22.5
3	3 -6	10.4	10.2
3	3 -7	23.0	22.4
3	3 -8	7.0	6.3
3	3 -9	13.6	12.7
3	3-10	16.9	21.1
3	3-11	18.2	19.1
3	3-12	11.1	11.9
3	3-13	9.0	9.7
3	3-14	6.0	7.1
3	3-16	9.0	8.7
3	3-17	4.3	3.4
3	3-19	7.2	6.1
3	3-22	4.9	4.1
3	3-23	2.2	1.7
4	3 -1	14.0	12.1
4	3 -2	33.5	28.5
4	3 -3	24.8	23.3
4	3 -4	3.9	3.7
4	3 -5	5.4	4.4
4	3 -6	12.1	13.5
4	3 -7	8.7	3.2
4	3 -8	8.9	8.6
4	3 -9	3.1	4.1
4	3-10	4.2	5.2
4	3-11	8.2	8.6
4	3-14	10.7	13.0
4	3-16	8.3	6.1
4	3-17	3.9	3.8
4	3-19	5.7	2.5
5	3 -1	3.2	1.8
5	3 -2	9.1	13.0
5	3 -3	16.7	16.5
5	3 -4	14.2	13.6
5	3 -5	13.3	14.6
5	3 -6	12.1	12.2
5	3 -7	19.7	23.7
5	3 -8	18.5	18.2
5	3 -9	8.1	8.2
5	3-10	5.0	5.3
5	3-11	11.4	11.5
5	3-12	5.1	5.1
5	3-13	9.3	10.2
5	3-14	4.0	4.3
5	3-16	4.3	4.9
5	3-20	3.0	2.3
6	3 -1	10.6	10.0
6	3 -2	9.4	9.7
6	3 -3	14.8	16.5
6	3 -4	4.4	3.8
6	3 -5	8.7	10.2
6	3 -6	6.8	8.6
6	3 -7	12.3	11.7
6	3 -8	9.0	10.0
6	3 -9	12.7	15.0
6	3-10	6.7	6.2
6	3-11	10.5	12.2
6	3-12	6.7	7.2
6	3-13	5.3	5.4
6	3-14	8.3	9.5
6	3-19	3.5	3.4
7	3 -1	12.6	11.9
7	3 -2	5.1	4.9
7	3 -3	3.5	3.0
7	3 -4	8.4	10.1
7	3 -6	4.0	3.6
7	3 -8	5.0	5.1
7	3 -9	9.0	10.8
7	3-10	6.4	6.2
7	3-11	14.2	15.9
7	3-12	4.8	4.2
7	3-13	4.3	4.4
7	3-15	3.9	4.1
7	3-17	3.3	2.9
8	3 -1	9.8	9.6
8	3 -2	6.8	6.9
8	3 -3	7.4	8.9
8	3 -4	5.7	5.1
8	3 -5	6.6	6.0
8	3 -6	4.7	5.2
8	3-10	4.0	2.9
8	3-11	4.6	6.1
8	3-12	4.9	4.4
8	3-13	6.6	7.2
8	3-14	4.1	4.0
8	3-16	3.6	3.7
9	3 -1	4.0	4.2
9	3 -2	5.2	5.7
9	3 -4	4.2	5.3
9	3 -5	6.1	5.2
9	3 -6	4.0	4.1
9	3 -7	4.3	6.7
9	3 -8	4.0	4.1
9	3-10	6.3	5.9
9	3-12	5.3	4.9
9	3-14	3.9	3.1
10	3 -1	6.7	6.2
10	3 -2	8.9	9.7
10	3 -3	4.0	5.7
10	3 -4	7.7	7.5
10	3-15	7.1	6.3
11	3 -3	3.4	3.3
11	3 -5	7.3	6.1
11	3 -8	4.7	4.3
11	3-10	3.8	3.2
11	3-11	4.3	4.1
11	3-15	3.6	3.1
12	3 -8	2.8	2.8
0	4 1	2.9	3.5
0	4 2	12.6	13.0
0	4 3	14.2	14.1
0	4 4	13.0	9.6
0	4 5	5.5	5.2
0	4 6	9.8	8.7
0	4 7	5.1	4.3
0	4 8	6.2	5.3
0	4 9	12.1	12.7
0	4 11	9.7	10.4
0	4 12	8.5	9.7
0	4 13	12.5	11.6
0	4 14	4.0	4.1
0	4 15	7.0	7.2
0	4 16	5.1	5.8
0	4 19	3.5	3.9
1	4 0	6.5	4.4
2	4 0	16.3	15.4
3	4 0	7.4	4.7
4	4 0	34.0	24.7
5	4 0	9.2	7.0
6	4 0	5.7	5.8
7	4 0	8.5	8.1
8	4 0	7.1	7.6
1	4 1	15.7	16.2
1	4 2	4.9	4.2
1	4 3	4.1	3.6
1	4 4	20.0	16.5
1	4 5	23.6	20.3
1	4 6	13.9	13.9
1	4 7	18.8	16.5
1	4 8	5.1	5.3
1	4 9	6.3	6.9
1	4 10	5.5	6.6
1	4 11	6.2	7.9
1	4 13	6.9	7.7
1	4 14	5.8	5.0
1	4 16	6.0	6.5
1	4 18	4.1	4.3
2	4 1	14.8	11.9
2	4 2	11.0	7.9
2	4 3	20.0	14.7
2	4 4	8.1	7.8
2	4 6	6.6	6.1
2	4 7	12.6	13.0
2	4 8	8.0	6.1
2	4 9	6.4	5.7
2	4 10	6.1	6.6
2	4 11	14.2	14.7
2	4 12	8.9	3.9
2	4 13	7.3	6.8
2	4 14	6.3	5.0
2	4 15	4.1	3.7
3	4 1	10.8	9.7
3	4 2	12.7	9.0
3	4 3	10.7	8.7
3	4 4	5.6	5.4
3	4 5	13.6	11.7
3	4 7	8.7	7.8
3	4 8	8.1	7.9
3	4 9	9.9	10.7
3	4 10	20.9	18.2
3	4 11	4.4	4.5
3	4 13	7.7	8.1
3	4 15	6.7	6.7
3	4 16	4.3	4.6
4	4 1	28.5	23.6
4	4 2	25.5	18.8
4	4 3	15.8	14.5
4	4 4	15.9	13.7
4	4 5	7.9	8.0
4	4 6	17.5	18.0
4	4 7	11.6	12.0
4	4 8	10.2	10.4
4	4 9	5.1	5.1
4	4 10	5.8	4.8
4	4 12	8.4	8.1
4	4 13	6.2	6.0
5	4 1	10.6	6.6
5	4 2	11.8	8.2
5	4 3	12.5	8.6
5	4 4	13.6	6.0
5	4 6	16.0	13.6
5	4 7	8.3	9.9
5	4 10	4.4	3.7
5	4 11	6.9	6.1
5	4 12	4.9	5.0
5	4 18	2.7	3.8
6	4 1	13.1	13.9
6	4 2	8.8	8.2
6	4 3	15.2	16.6
6	4 4	7.9	7.9
6	4 5	5.1	5.1
6	4 6	11.5	11.2
6	4 7	12.8	10.4
6	4 8	11.9	10.1
6	4 9	13.2	12.2
6	4 12	14.8	14.0
6	4 15	5.2	5.8
7	4 2	6.9	9.5
7	4 3	15.0	16.4
7	4 6	12.4	12.3
7	4 7	4.9	6.5
7	4 8	3.9	4.5
7	4 9	4.2	5.1
7	4 10	5.7	4.6
7	4 11	4.1	4.8
7	4 12	7.1	8.0
7	4 13	3.8	2.7
7	4 14	4.2	5.7
7	4 15	3.3	4.5
8	4 2	4.2	6.3
8	4 3	9.1	8.7
8	4 5	6.9	6.4
8	4 7	4.2	4.1
8	4 8	3.7	3.5
8	4 12	4.0	4.3
9	4 3	5.3	5.4
9	4 7	6.7	6.8
9	4 11	2.9	3.7
10	4 1	4.0	4.6
10	4 2	6.0	6.6
10	4 4	6.8	8.2
10	4 5	3.4	4.0
10	4 8	3.3	4.2
1	4 -1	21.4	19.8
1	4 -2	14.0	17.1
1	4 -3	23.8	23.4
1	4 -4	19.8	19.4
1	4 -5	7.6	6.2
1	4 -6	2.8	3.1
1	4 -7	5.3	5.6
1	4 -8	7.9	8.5
1	4-11	6.3	6.0
1	4-12	7.7	7.7
1	4-13	12.9	10.9
1	4-14	7.1	6.8
1	4-15	4.0	4.3
1	4-16	6.9	7.0
1	4-17	4.1	4.1
1	4-20	4.8	4.2
2	4 -1	19.2	18.5
2	4 -2	5.1	5.8
2	4 -3	4.5	6.1
2	4 -4	20.4	17.1
2	4 -5	16.2	17.2
2	4 -6	10.9	10.6
2	4 -7	15.6	17.2
2	4 -8	7.2	7.9
2	4 -9	13.1	12.0
2	4-10	13.2	13.3
2	4-11	9.8	10.9
2	4-12	9.2	9.7
2	4-13	8.3	10.1
2	4-14	7.6	8.9
2	4-16	7.9	8.6
2	4-17	5.8	6.6
2	4-19	3.3	2.8
3	4 -1	17.5	10.7
3	4 -2	32.4	21.9
3	4 -3	19.9	14.1
3	4 -4	12.7	9.1
3	4 -5	7.7	9.1
3	4 -6	16.8	16.1
3	4 -7	16.2	16.5
3	4 -8	18.1	18.0
3	4 -9	5.6	6.3
3	4-10	10.1	3.6
3	4-11	6.8	7.4
3	4-13	4.0	3.6
3	4-16	8.0	8.7
3	4-17	5.8	4.7
4	4 -1	19.9	14.4
4	4 -2	20.7	14.5
4	4 -3	12.8	8.9
4	4 -4	19.2	17.2
4	4 -5	21.1	18.3
4	4 -6	10.6	11.1
4	4 -7	7.2	7.2
4	4 -8	6.3	6.7
4	4 -9	5.2	6.6
4	4-10	7.5	4.6
4	4-11	7.6	8.1
4	4-12	8.9	10.7
4	4-13	4.0	3.9
5	4 -1	6.2	[illegible]
5	4 -2	27.3	23.2
5	4 -3	10.8	10.2
5	4 -4	5.3	6.0
5	4 -5	10.6	9.9
5	4 -6	6.3	7.2
5	4 -7	12.2	12.2
5	4 -8	14.8	16.3
5	4 -9	14.3	15.6
5	4-10	9.0	9.9
5	4-12	7.1	6.3
5	4-15	5.5	5.4
6	4 -1	4.0	5.7
6	4 -2	7.7	7.4
6	4 -3	9.3	9.0
6	4 -4	13.9	12.1
6	4 -5	10.3	10.1
6	4 -7	5.7	5.7
6	4 -8	5.5	5.5
6	4 -9	13.7	14.5
6	4-10	12.7	13.3
6	4-11	11.3	11.5
6	4-16	4.3	4.5
7	4 -1	10.2	11.5
7	4 -4	9.2	10.6
7	4 -6	4.5	6.1
7	4 -8	5.5	6.0
7	4-10	4.7	4.7
7	4-13	4.2	4.5
7	4-14	3.7	4.1
7	4-15	5.1	4.5
8	4 -1	5.2	5.9
8	4 -3	7.2	6.8
8	4 -4	4.4	5.4
8	4 -5	4.0	3.2
8	4 -8	4.4	4.0
8	4-10	6.8	6.8
8	4-12	3.9	3.7
9	4 -2	6.4	6.3
9	4 -4	3.9	5.2
9	4 -7	5.9	6.4
9	4-10	4.1	3.3
9	4-14	5.7	4.8
10	4 -8	3.7	2.9
10	4-13	3.4	2.6
10	4-14	3.5	2.5
11	4 -1	4.4	5.5
0	5 1	10.8	14.7
0	5 2	7.3	10.6
0	5 3	5.3	6.0
0	5 4	3.8	3.4
0	5 5	9.3	12.2
0	5 6	6.3	9.0
0	5 7	5.2	6.3
0	5 9	6.2	8.7
0	5 10	7.3	9.0
2	5 0	6.0	7.1
3	5 0	17.0	12.4
4	5 0	5.2	3.2
5	5 0	7.1	5.4
6	5 0	7.3	7.9
1	5 1	4.1	5.4
1	5 2	15.5	16.9
1	5 3	9.9	10.2
1	5 4	9.0	9.6
1	5 6	6.8	8.1
1	5 8	10.0	12.5
1	5 9	7.9	8.3
1	5 11	8.2	7.6
2	5 1	8.5	7.5
2	5 2	5.3	6.0
2	5 3	7.8	6.9
2	5 4	4.7	4.5
2	5 5	10.8	12.3
2	5 6	7.0	6.8
2	5 7	5.6	4.2
2	5 9	9.2	11.0
2	5 10	5.5	6.0
3	5 1	17.5	16.3
3	5 2	8.2	6.3
3	5 3	8.9	7.3
3	5 5	10.7	12.1
3	5 7	9.2	11.0
3	5 9	8.7	10.1
3	5 10	8.0	7.3
4	5 1	5.9	4.3
4	5 2	10.9	10.6
4	5 4	6.1	5.9
4	5 5	9.1	8.6
4	5 6	11.4	11.1
4	5 7	5.5	5.5
4	5 9	7.2	6.9
5	5 1	15.5	14.8
5	5 2	7.8	8.6
5	5 3	7.9	6.6
5	5 5	6.0	7.1
5	5 8	6.3	7.3
6	5 1	9.5	9.0
6	5 4	5.6	5.1
7	5 10	5.2	7.7
9	5 3	5.8	6.2
1	5 -1	7.3	6.4
1	5 -2	8.5	8.0
1	5 -3	14.0	16.3
1	5 -4	5.5	5.1
1	5 -5	4.1	4.3
1	5 -6	10.8	9.6
1	5 -9	7.4	9.1
1	5-10	6.4	6.5
1	5-13	6.1	7.9
2	5 -1	9.5	12.1
2	5 -2	8.8	8.5
2	5 -3	5.6	4.9
2	5 -4	16.0	16.1
2	5 -5	11.7	9.5
2	5 -6	13.4	13.2
2	5 -8	5.6	5.6
2	5 -9	5.8	6.7
3	5 -1	13.7	11.0
3	5 -2	13.3	12.1
3	5 -4	14.2	12.4
3	5 -6	13.5	11.7
3	5 -7	15.0	14.3
3	5 -8	9.7	9.0
3	5-11	5.5	6.6
4	5 -1	16.2	16.5
4	5 -2	12.1	8.4
4	5 -5	12.2	11.3
4	5 -7	6.2	6.8
4	5 -8	14.5	14.2
4	5-10	7.2	8.5
5	5 -1	5.9	6.7
5	5 -3	7.9	7.7
5	5 -6	6.4	6.6
5	5 -8	8.7	10.5
6	5 -1	6.0	7.4
6	5 -7	5.5	5.2
7	5 -2	5.6	5.6
7	5 -3	6.6	6.0
7	5 -7	5.8	6.2
7	5 -2	5.4	6.1

TABLE 3.1

FRACTIONAL COORDINATES AND E.S.D.S

ATOM	X	Y	Z
Br(1)	0.2116 ± 2	0.7500 ± 0	0.1843 ± 1
O(1)	1.2969 ± 11	0.8726 ± 21	0.6726 ± 5
O(2)	0.8295 ± 9	0.7174 ± 22	0.4405 ± 5
O(3)	0.7617 ± 9	0.9743 ± 18	0.5055 ± 5
O(4)	0.5859 ± 11	0.6923 ± 24	0.4924 ± 7
C(1)	1.0488 ± 10	0.7467 ± 27	0.8517 ± 6
C(2)	1.0354 ± 13	0.7394 ± 31	0.9288 ± 7
C(3)	1.1531 ± 16	0.6681 ± 25	0.9715 ± 7
C(4)	1.2616 ± 12	0.7959 ± 22	0.9612 ± 6
C(5)	1.2660 ± 11	0.8136 ± 18	0.8823 ± 6
C(6)	1.3734 ± 11	0.9361 ± 27	0.8664 ± 7
C(7)	1.3861 ± 13	0.8894 ± 29	0.7886 ± 7
C(8)	1.2807 ± 12	0.9624 ± 21	0.7400 ± 7
C(9)	1.1698 ± 12	0.8495 ± 21	0.7604 ± 6
C(10)	1.1511 ± 11	0.8936 ± 20	0.8368 ± 6
C(11)	1.0569 ± 13	0.8860 ± 25	0.7045 ± 7
C(12)	1.0101 ± 13	0.6687 ± 23	0.6742 ± 7
C(13)	0.9022 ± 12	0.6876 ± 26	0.6159 ± 7
C(14)	0.9411 ± 12	0.8083 ± 27	0.5516 ± 7
C(15)	0.8443 ± 13	0.8170 ± 30	0.4929 ± 7

C(16)	0.8517 ± 16	0.4626 ± 28	0.5959 ± 8
C(17)	1.1205 ± 16	1.1376 ± 27	0.8460 ± 8
C(18)	1.2651 ± 16	1.0182 ± 26	0.9986 ± 8
C(19)	1.3737 ± 14	0.6694 ± 22	0.9967 ± 7
C(20)	1.2761 ± 15	1.2104 ± 33	0.7333 ± 8
C(21)	0.6679 ± 14	1.0113 ± 30	0.4514 ± 7
C(22)	0.5798 ± 12	0.8318 ± 26	0.4460 ± 7
C(23)	0.4892 ± 11	0.8157 ± 27	0.3831 ± 7
C(24)	0.4193 ± 15	0.6213 ± 33	0.3688 ± 8
C(25)	0.3393 ± 16	0.6043 ± 30	0.3128 ± 8
C(26)	0.3235 ± 11	0.7879 ± 37	0.2649 ± 6
C(27)	0.3891 ± 13	0.9662 ± 36	0.2769 ± 8
C(28)	0.4699 ± 13	0.9891 ± 31	0.3347 ± 8

TABLE 3.2

ORTHOGONAL COORDINATES AND E.S.D.S
IN ANGSTROMS

ATOM	X	Y	Z
Br(1)	2.414 ± 2	4.538 ± 0	3.291 ± 2
O(1)	14.794 ± 13	5.279 ± 13	11.064 ± 10
O(2)	9.462 ± 11	4.340 ± 13	7.281 ± 10
O(3)	8.689 ± 10	5.894 ± 11	8.700 ± 10
O(4)	6.684 ± 13	4.188 ± 14	8.757 ± 13
C(1)	11.963 ± 12	4.517 ± 16	15.084 ± 12
C(2)	11.811 ± 15	4.474 ± 19	16.645 ± 14
C(3)	13.154 ± 18	4.042 ± 15	17.284 ± 14
C(4)	14.391 ± 14	4.815 ± 13	16.882 ± 13
C(5)	14.442 ± 12	4.922 ± 11	15.301 ± 12
C(6)	15.667 ± 13	5.664 ± 17	14.789 ± 15
C(7)	15.811 ± 14	5.381 ± 18	13.215 ± 15
C(8)	14.610 ± 14	5.822 ± 13	12.437 ± 14
C(9)	13.344 ± 14	5.139 ± 13	13.045 ± 13
C(10)	13.130 ± 12	5.406 ± 12	14.601 ± 12
C(11)	12.056 ± 15	5.360 ± 15	12.135 ± 14
C(12)	11.522 ± 15	4.046 ± 14	11.615 ± 14
C(13)	10.292 ± 14	4.160 ± 16	10.647 ± 13
C(14)	10.735 ± 13	4.890 ± 16	9.294 ± 14
C(15)	9.631 ± 15	4.943 ± 18	8.300 ± 14

C(16)	9.715 ± 19	2.799 ± 17	10.341 ± 16
C(17)	12.782 ± 18	6.883 ± 16	14.841 ± 15
C(18)	14.432 ± 19	6.160 ± 16	17.621 ± 16
C(19)	15.670 ± 16	4.050 ± 13	17.387 ± 13
C(20)	14.557 ± 17	7.323 ± 20	12.310 ± 16
C(21)	7.619 ± 16	6.119 ± 18	7.792 ± 15
C(22)	6.613 ± 14	5.032 ± 16	7.843 ± 14
C(23)	5.581 ± 13	4.935 ± 16	6.754 ± 13
C(24)	4.783 ± 17	3.759 ± 20	6.594 ± 16
C(25)	3.870 ± 18	3.656 ± 18	5.623 ± 17
C(26)	3.690 ± 12	4.767 ± 23	4.696 ± 13
C(27)	4.438 ± 15	5.846 ± 22	4.816 ± 17
C(28)	5.360 ± 15	5.984 ± 19	5.824 ± 16

TABLE 4.

ANISOTROPIC TEMPERATURE PARAMETERS AND E.S.D.S

ATOM	U11	U22	U33	2U23	2U31	2U12
Br(1)	0.1071 15	0.1667 28	0.0871 12	0.0213 34	-0.0555 22	-0.0988 37
O(1)	0.1101 91	0.0601 78	0.0597 59	0.0002 107	0.0397 122	-0.0015 134
O(2)	0.0790 66	0.0662 77	0.0614 53	-0.0344 113	-0.0227 94	0.0353 124
O(3)	0.0773 68	0.0415 66	0.0628 56	-0.0321 97	-0.0094 97	0.0146 105
O(4)	0.0887 78	0.0704 90	0.1040 85	0.0623 148	-0.0191 133	-0.0269 139
C(1)	0.0423 63	0.0472 91	0.0617 69	-0.0285 148	0.0103 105	-0.0060 132
C(2)	0.0681 83	0.0504 93	0.0653 77	-0.0069 157	0.0200 128	-0.0242 155
C(3)	0.0995 116	0.0344 94	0.0572 78	0.0031 130	0.0292 157	-0.0053 161
C(4)	0.0745 81	0.0136 73	0.0611 71	0.0020 122	-0.0224 120	-0.0078 128
C(5)	0.0567 68	0.0040 66	0.0549 62	-0.0072 98	0.0029 105	0.0026 105
C(6)	0.0428 71	0.0559 103	0.0641 82	-0.0030 142	-0.0177 122	-0.0035 129
C(7)	0.0532 80	0.0628 106	0.0613 81	0.0040 146	0.0210 133	-0.0251 146
C(8)	0.0593 78	0.0122 75	0.0694 80	-0.0002 117	0.0287 127	0.0036 117

C(9)	0.0592 75	0.0190 76	0.0550 69	0.0053 109	-0.0142 114	0.0072 113
C(10)	0.0468 66	0.0159 68	0.0507 64	-0.0013 104	-0.0097 104	0.0031 101
C(11)	0.0633 84	0.0332 81	0.0576 73	0.0007 123	-0.0062 129	0.0114 128
C(12)	0.0728 87	0.0291 84	0.0542 70	0.0020 116	-0.0024 127	-0.0060 133
C(13)	0.0591 78	0.0453 97	0.0551 70	-0.0118 129	-0.0155 119	-0.0158 134
C(14)	0.0563 76	0.0485 95	0.0585 72	-0.0024 133	0.0035 117	-0.0076 141
C(15)	0.0656 85	0.0614 106	0.0551 73	0.0046 143	0.0142 129	-0.0053 159
C(16)	0.0981 117	0.0403 103	0.0710 90	-0.0022 151	-0.0091 166	-0.0749 183
C(17)	0.0887 100	0.0119 73	0.0561 72	-0.0140 110	-0.0006 137	0.0149 135
C(18)	0.1027 124	0.0238 85	0.0653 84	-0.0251 132	-0.0099 161	-0.0170 161
C(19)	0.1071 128	0.0318 100	0.0703 93	0.0308 145	-0.0119 173	0.0300 171
C(20)	0.0891 110	0.0573 117	0.0724 92	-0.0119 170	0.0478 165	-0.0407 189
C(21)	0.0677 92	0.0568 103	0.0587 78	-0.0071 149	-0.0088 134	-0.0041 160
C(22)	0.0496 70	0.0479 95	0.0646 78	0.0179 137	0.0002 120	0.0125 130
C(23)	0.0503 71	0.0510 99	0.0597 73	0.0059 134	0.0223 115	0.0251 137
C(24)	0.0727 104	0.0729 122	0.0674 92	-0.0015 175	0.0246 159	-0.0069 179

C(25)	0.0691 95	0.0586 104	0.0705 90	-0.0261 163	0.0287 148	-0.0093 164
C(26)	0.0455 68	0.1169 156	0.0431 60	-0.0244 177	-0.0519 106	-0.0033 181
C(27)	0.0495 81	0.0869 138	0.0730 92	0.0317 184	-0.0254 136	-0.0258 166
C(28)	0.0609 89	0.0579 105	0.0727 92	0.0178 158	0.0127 147	-0.0073 164

TABLE 5.

BOND LENGTHS AND E.S.D.

BOND	ANGSTROMS	SIGMA
Br-C(26)	1.912	±0.013
O(1)-C(8)	1.488	±0.017
O(2)-C(15)	1.196	±0.017
O(3)-C(15)	1.398	±0.019
O(3)-C(21)	1.420	±0.019
O(4)-C(22)	1.246	±0.020
C(1)-C(2)	1.569	±0.019
C(1)-C(10)	1.545	±0.018
C(2)-C(3)	1.548	±0.023
C(3)-C(4)	1.514	±0.022
C(4)-C(5)	1.586	±0.018
C(4)-C(18)	1.535	±0.021
C(4)-C(19)	1.574	±0.023
C(5)-C(6)	1.520	±0.018
C(5)-C(10)	1.563	±0.017
C(6)-C(7)	1.606	±0.021
C(7)-C(8)	1.498	±0.020
C(8)-C(20)	1.507	±0.024
C(8)-C(9)	1.562	±0.019
C(9)-C(10)	1.593	±0.018

BOND	ANGSTROMS	SIGMA
C(9)-C(11)	1.593	±0.020
C(10)-C(17)	1.536	±0.018
C(11)-C(12)	1.511	±0.021
C(12)-C(13)	1.569	±0.020
C(13)-C(14)	1.600	±0.020
C(13)-C(16)	1.510	±0.023
C(14)-C(15)	1.487	±0.020
C(21)-C(22)	1.481	±0.020
C(22)-C(23)	1.504	±0.019
C(23)-C(24)	1.430	±0.025
C(24)-C(25)	1.337	±0.024
C(25)-C(26)	1.458	±0.028
C(26)-C(27)	1.319	±0.028
C(27)-C(28)	1.373	±0.023

TABLE 5.

BOND ANGLES AND E.S.D.S

ANGLE			DEGREES	SIGMA
C(15)	O(3)	C(21)	115.63	±1.13
C(2)	C(1)	C(10)	113.61	±1.11
C(1)	C(2)	C(3)	109.52	±1.14
C(2)	C(3)	C(4)	117.20	±1.25
C(3)	C(4)	C(5)	108.97	±1.08
C(3)	C(4)	C(19)	109.31	±1.14
C(5)	C(4)	C(18)	114.86	±1.10
C(5)	C(4)	C(19)	109.10	±1.04
C(18)	C(4)	C(19)	104.48	±1.15
C(4)	C(5)	C(6)	113.21	±1.04
C(4)	C(5)	C(10)	116.15	±1.00
C(6)	C(5)	C(10)	111.98	±1.01
C(5)	C(6)	C(7)	108.44	±1.13
C(6)	C(7)	C(8)	112.65	±1.17
O(1)	C(8)	C(7)	105.81	±1.12
O(1)	C(8)	C(9)	107.46	±1.06
O(1)	C(8)	C(20)	106.85	±1.14
C(7)	C(8)	C(9)	108.61	±1.12
C(7)	C(8)	C(20)	111.42	±1.26
C(9)	C(8)	C(20)	116.11	±1.18

C(8)	C(9)	C(10)	114.54	±1.05
C(8)	C(9)	C(11)	111.85	±1.06
C(10)	C(9)	C(11)	115.27	±1.06
C(1)	C(10)	C(5)	108.39	±0.98
C(1)	C(10)	C(9)	108.07	±0.99
C(1)	C(10)	C(17)	109.46	±1.12
C(5)	C(10)	C(9)	105.85	±0.96
C(5)	C(10)	C(17)	114.70	±1.07
C(9)	C(10)	C(17)	110.13	±1.04
C(9)	C(11)	C(12)	111.21	±1.17
C(11)	C(12)	C(13)	115.22	±1.20
C(12)	C(13)	C(14)	109.70	±1.09
C(12)	C(13)	C(16)	111.07	±1.26
C(14)	C(13)	C(16)	110.22	±1.18
C(13)	C(14)	C(15)	112.08	±1.15
O(2)	C(15)	O(3)	119.41	±1.32
O(2)	C(15)	C(14)	131.09	±1.50
O(3)	C(15)	C(14)	109.49	±1.22
O(3)	C(21)	C(22)	111.94	±1.32
O(4)	C(22)	C(21)	118.92	±1.32
O(4)	C(22)	C(23)	121.74	±1.38
C(21)	C(22)	C(23)	119.26	±1.29

C(22) C(23) C(24)	121.13	±1.37
C(22) C(23) C(28)	122.13	±1.37
C(24) C(23) C(28)	116.63	±1.32
C(23) C(24) C(25)	121.68	±1.65
C(24) C(25) C(26)	119.18	±1.66
Br C(26) C(25)	117.28	±1.37
Br C(26) C(27)	122.92	±1.39
C(25) C(26) C(27)	119.72	±1.37
C(26) C(27) C(28)	122.01	±1.77
C(23) C(28) C(27)	120.72	±1.66

SECTION VII

ATTEMPTS

TO PREPARE AN X -RAY DERIVATIVE

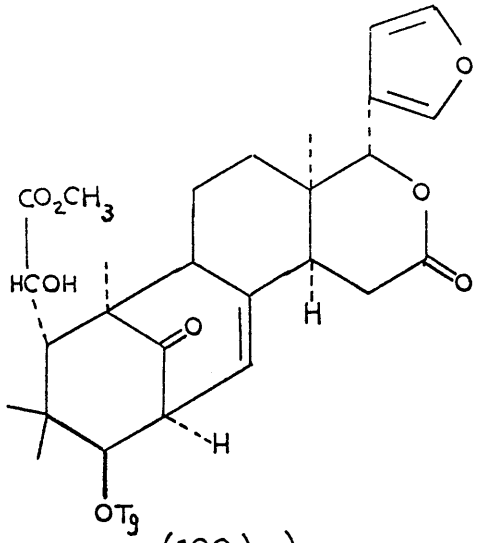
of

SOME SWIETENINE DEGRADATION PRODUCTS

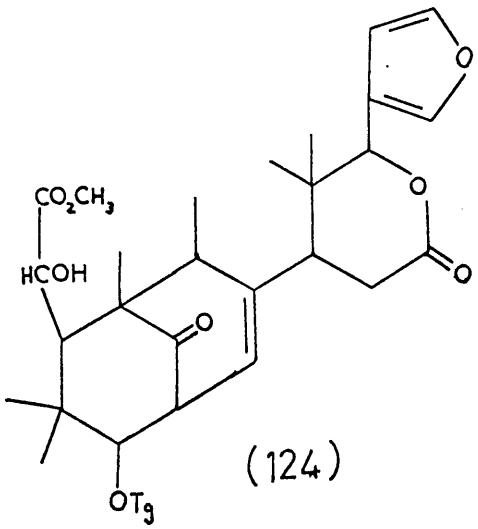
SWIETENINE DEGRADATION PRODUCTS.

Swietenine (123 a) non-bitter principle of Swietenia macrophylla King (Fam. Meliaceae) was first isolated by Sircar and Chakrabarthy[98] who postulated its formula as $C_{18}H_{24}O_5$. From further investigations[99] by Chakrabarthy and Chatterjee it was alleged that Swietenine contained an isolated double bond and was bicarboxylic since substituted naphthalenes were obtained from dehydrogenation experiments. From an X-ray molecular weight determination[100] the formula of Swietenine was revised to $C_{32}H_{42}O_9$ and from n.m.r. evidence it was suggested that the molecule contained a β-furan system, and five C-Methyl groups. From hydrolysis experiments the presence of a tiglate and a methyl ester was suggested. Overton et al[101] re-investigated the chemistry of Swietenine comfirming the molecular formula as $C_{32}H_{40}O_9$ by mass spectroscopic molecular weight of 568.

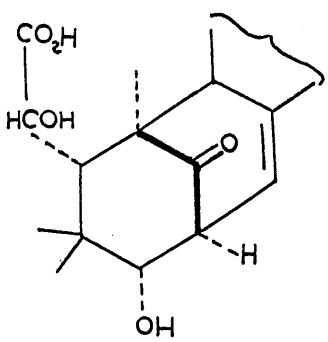
From n.m.r. evidence Overton et al[101] confirmed that Swietenine contained a β-furan ring, a tiglate and a methyl ester and showed that the compound has 4 C-Methyl groups, a secondary hydroxyl group and an isolated double bond. The partial structure[124] was postulated by these workers on the basis of chemical and spectroscopic studies of Swietenine. The X-ray structural analysis of the p-iodo benzoate of destigloyl swietenine showed the structure and stereochemistry to be as shown in (123a).



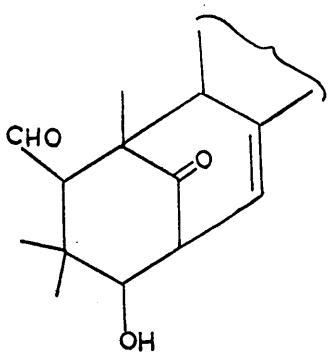
(123) a)



(124)



(123 b)



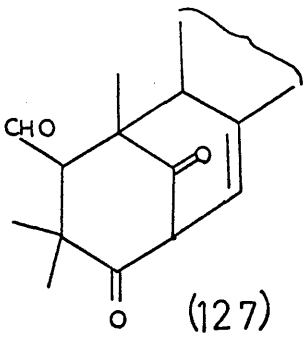
(126)

The bicyclo [3,3,1] nonene systeme gives rise to two different types of rearrangement product since the rearrangements of the three tri-carbonyl compounds (124a), and (125) seem to follow different courses.

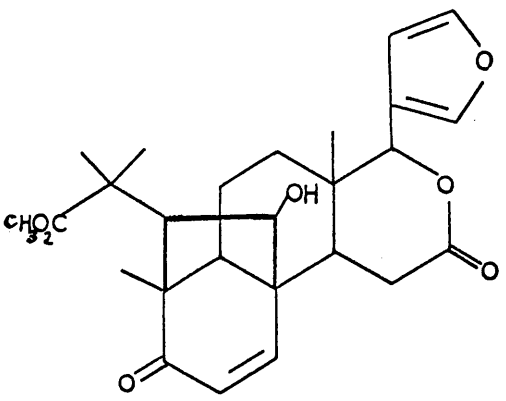
When swietenine is hydrolysed with 5% alcoholic potassium hydroxide a complex mixture of products is formed, from which, owing to its insolubility in chloroform des-methyl des-tigloyl iso-swietenine (123b) can be obtained in 30% yield. Treatment of this compound with lead tetra-acetate in acetic acid decarboxylates the α-hydroxy acid forming the hydroxy keto aldehyde (126). On oxidation with chromic acid (126) is transformed into the β-diketo aldehyde (127). Mild alkali treatment of (127) with subsequent acidification produces an enone acid the methyl ester of which has the formula $C_{26}H_{32}O_7$. The structure of this compound was rationalised as (128) from consideration of its spectroscopic properties and the various mechanistic pathways rearrangement could have followed[103].

The infra-red frequencies of this rearrangement product are $\nu_{max}^{CHCl_3}$ 1737 cm^{-1} (methyl ester and δ-lactone) 1687 cm^{-1} (conjugated enone) 3620 cm^{-1} (free hydroxyl) 3583 cm^{-1} (bonded hydroxyl).

Cleavage of the β-diketone system by hydroxyl ion attack at C_3 produces a carbanion at C_2, whose isolated double bond then isomerises to the conjugated enone system (129). The C_8 carbanion then undergoes condensation with the aldehyde group which on acidification yields the hydroxy enone-acid (130). See flowsheet 1.

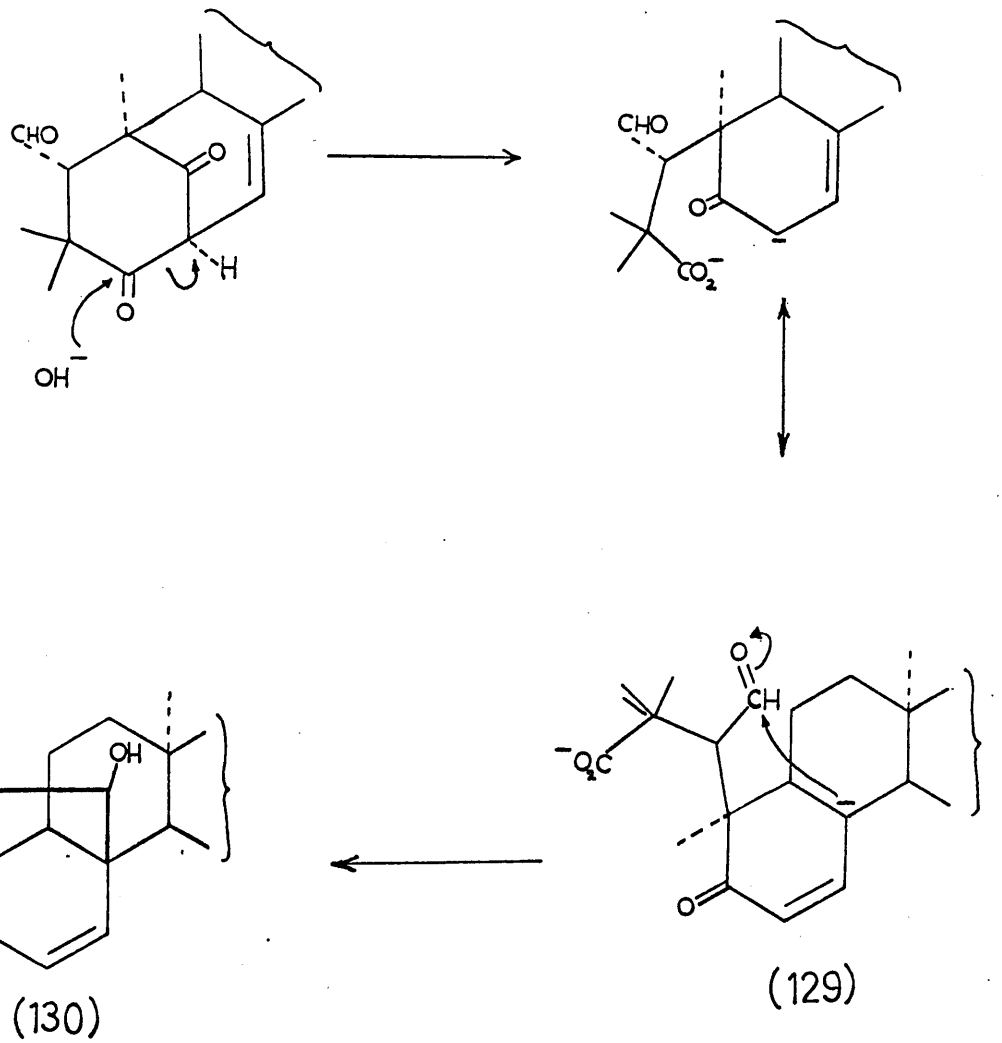


(127)



(128)

FLOWSHEET 1.



(129)

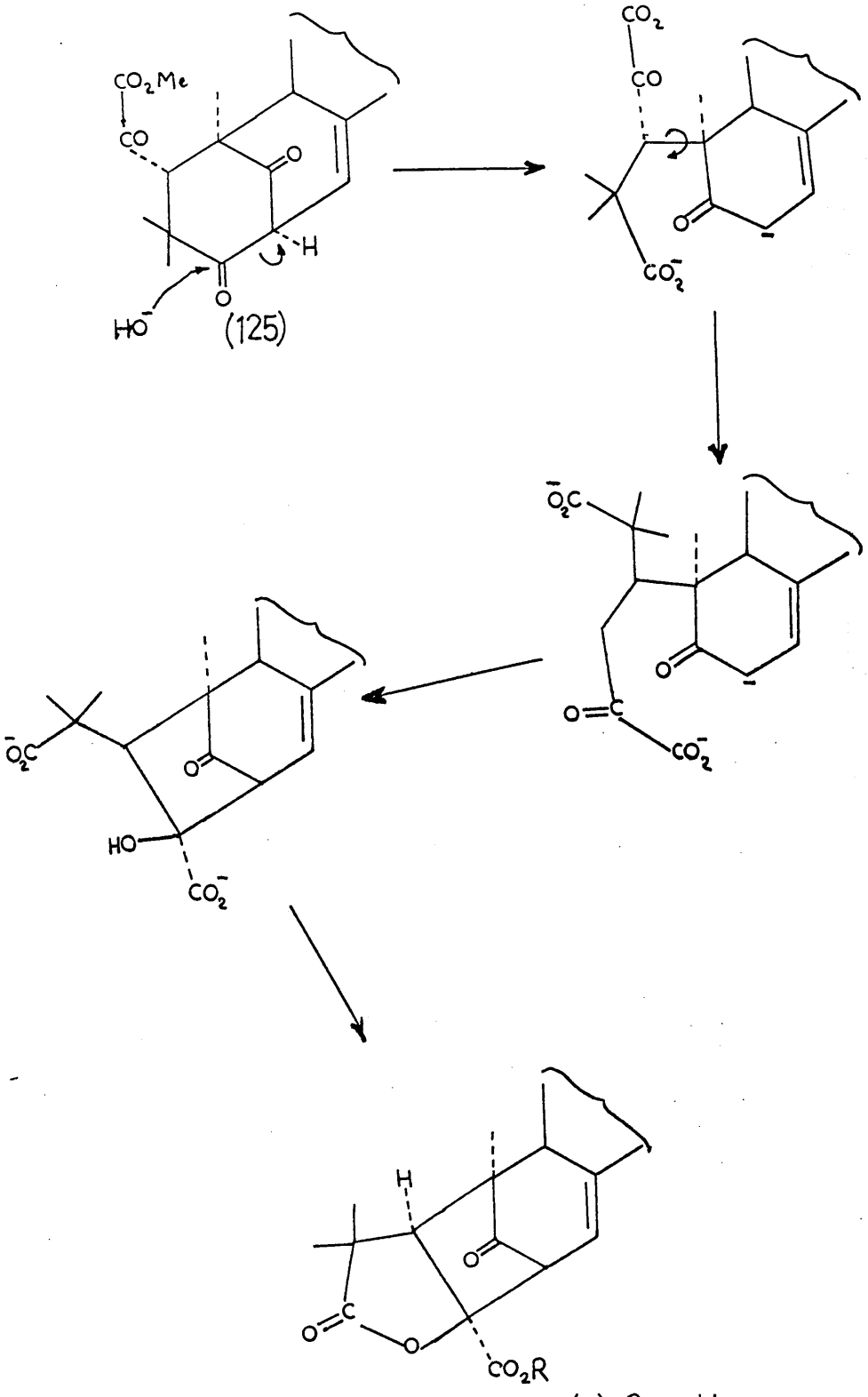
(130)

Oxidation of swietenine with chromic acid yields dehydro swietenine which, on hydrolysis with 5% potassium hydroxide, produces des-methyl des-tigloyl dehydro iso-swietenine which can be isolated from the reaction mixture by crystallisation from chloroform (123b). Methylation with diazomethane and oxidation of this product with chromic acid yields a tri-keto compound (131). This trione on mild alkali treatment rearranges to a keto- γ -lactone acid which has been assigned the structure (133a) methylation with diazomethane forms the corresponding methyl ester, (133b).

The mechanism involved in the formation of this rearrangement product differs from that involved in the formation of (128). If the β -diketo system is opened by hydroxyl ion attack at C_3 as before and the carbanion localised on C_2, then rotation about the C_5 - C_{10} bond and condensation of the α -keto acid side chain with the carbanion yields the hydroxy acid (132). Lactonisation produces the γ -lactone (133). See flowsheet 2.

There is a third rearrangement product obtainable from the aldehyde (126). On base treatment retro-aldolisation breaks the C_2 -C_3 bond to give the dialdehyde C_2 carbanion. Rotation about the C_5 -C_{10} bond brings C_6 and C_2 within reacting distance. Epimerisation of the C_5 aldehyde before aldolisation reduces the serious interactions between the gem-dimethyl and the aldehyde and the carbon atoms C_8 and C_9. Aldolisation followed by an internal Cannizaro reaction furnishes

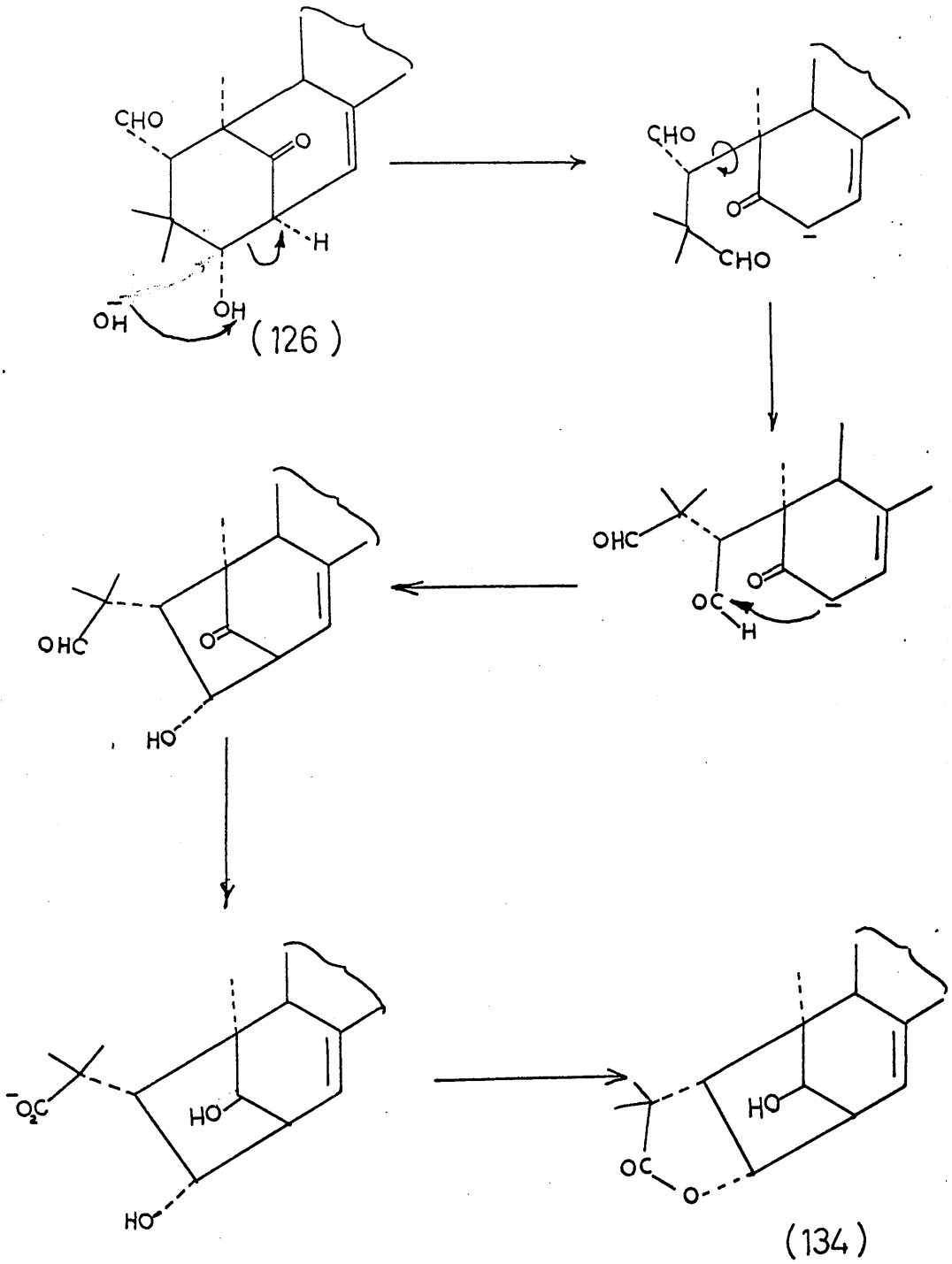
SCHEME 2.



(133) (a) R = H
(b) R = CH_3

the diol acid which on lactonisation yields the γ-lactone (134). See flowsheet 3.

From these three postulated mechanisms it can be seen that three different rearrangement products from the bicyclo [3,3,1] system are possible. It is a matter of considerable interest why the bicyclo system will undergo these different rearrangements but the answer to these questions must await determination of the structure of the rearrangement products.


CHO
O
H
OH
OH
(126)
CHO
O
CHO
OHC
O
OC
H
OHC
O
HO
HO
HO
O_2C
HO
OC
O
(134)

Attempts to prepare Derivatives of the Swietenine Transformation Products (130), (133) and(134),suitable for X-ray Analysis.

The mode of rearrangement of the [3,3,1] bicyclo system in swietenine raises interesting mechanistic problems since different types of product can be formed from essentially the same intermediate carbanion. The product obtained appears to depend on the nature of the side chains produced by cleavage of the β-diketone system. In order to study these rearrangements it is first necessary to confirm the structure and stereochemistry of some key transformation products. It was intended to use X-ray analysis of suitable "heavy" atom derivatives to determine the structures. For a "heavy" atom to dominate the scattering of X-rays by a molecule of molecular formula $C_{25}H_{30}O_7$ the heavy atom must not be lighter than bromine.

Derivatives of the enone-acid(128).

The first derivatives attempted were the rubidium and cesium salts of the enone-acid. A solution of the acid in aqueous ethanol was treated with 0.5mole of the metal carbonate. On evaporation of the solvent the residue was taken up in ethanol, however only the rubidium salt was obtained as a solid, the cesium salt remained as a gum. No solid material was obtained from the cesium salt from any solvent system tried and the rubidium salt could not be obtained in a crystalline form.

It was intended to prepare a heavy atom ester or amide of the enone acid via the acid chloride but on treatment of the enone acid sodium salt with oxalyl chloride a γ-lactone was formed. ν_{max}^{nujol} 1780 cm^{-1} mp 295-298°C., which did not exhibit any absorbtion in the infra-red corresponding to a hydroxyl group, or an acid carbonyl. This indicates that the acid group and the hydroxyl group in the molecule have internally esterified which implies that the carboxyl group and the hydroxyl group are 1,3 cis to each other. Owing to this lactonisation of the acid chloride, this method of heavy atom ester formation was abandoned.

Attempts were made to prepare the p-bromo phenacyl ester of the enone acid by refluxing the sodium salt of(128) with p-bromo phenacyl bromide in ethanol. The product obtained from this reaction rapidly decomposed on attempted crystallisation, one of the decomposition products having the same TLC behaviour as the γ-lactone prepared above. Possibly some internal lactonisation occurred during the work up of the reaction product.

Refluxing the enone acid with p-iodo phenyl hydrazine[108] formed a substance mp. 235-238°C, which showed a complex carbonyl absorbtion region in the infra-red (1730-1690 cm^{-1}), aromatic peaks at 1590 and 840 cm^{-1}, and a furan peak at 890 cm^{-1}. On attempted crystallisation this material rapidly decomposed to a variety of products .

Reduction of the enone methyl ester with sodium borohydride at room temperature for two hours yielded two alcohols α and β of which only the minor component (α) was crystalline, mp. 215-218°C, the major component (β) was a clear gum. Treatment of alcohol α with p-iodo benzoyl chloride in pyridine afforded two p-iodo benzoates both crystalline with virtually identical infra-red spectra. ν_{max}^{nujol} 1708 (methyl ester+lactone) 1690 (benzylic carbonyl) 1655 (double bond) 1600 (Aromatic ring) 890 cm^{-1} (furan). The major product mp. 122-123.5°C on slow evaporation from an ether-chloroform solution crystallised as stout plates which, although crystallising in the favourable orthorhombic crystal system ($P_{2_12_12_1}$), was unsuitable owing to an extremely high temperature factor along the short axis. Along the long axis there were alternate dark and light spots on the photographic film which indicate that the heavy atom is in a special position which would give rise to troublesome pseudo symmetry problems. The minor product from p-iodo benzoylation mp. 138-141°C of the alcohol was found to be unsuitable since the long thin needles formed on crystallisation from ether-chloroform were twinned.

Derivatives of the γ-lactone keto acid (133a).

A cesium salt of the acid was prepared as for the enone-acid (128) which crystallised as white plates from methanol mp. 330°C. Although this derivative crystallised in the

orthorhombic system, it was found to be unsuitable owing to the irregular stacking pattern in the crystals.

Unsuccessful attempts were made to prepare the p-bromo phenacyl ester of the γ-lactone keto acid by refluxing the sodium salt with p-bromo phenacyl bromide in aqueous ethanol. Attempts to form the acid chloride by treatment of the sodium salt with oxalyl chloride also failed.

When the γ-lactone keto methyl ester (135b) is reduced with sodium borohydride two epimeric alcohols (A and B) are formed, alcohol A only, being crystalline mp. 316-321°C. Treatment of alcohols A and B with p-iodo benzoyl chloride yields the corresponding p-iodo benzoates. Crystallisation of these derivatives from chloroform-petrol yields white rectangular plates. p-iodo benzoate A mp. 178-180°C

p-iodo benzoate B mp. 161-163°C.

Both of these derivatives unfortunately, crystallise in the monoclinic system (P_{2_1}) which involves a troublesome pseudo symmetry. This leads to a pseudo mirror plane lying through the heavy atom which makes it extremely difficult from an electron density map to locate atoms belonging only to one molecule, hence these derivatives were considered unsuitable.

The m-iodo benzoate of B was prepared which crystallised from ethanol as white plates mp. 235-237°C. Although this derivative crystallised in the orthorhombic system, irradiation of the crystal by X-rays for two days produced decomposition which made this derivative unsuitable for an X-ray analysis.

The 3,5 dibromo benzoate of A was non-crystalline.

EXPERIMENTAL.

Hydrolysis of Swietenine (123a).

Swietenine (123a) was heated for 10 min. with 5% potassium hydroxide in ethanol (100 ml.) in an atmosphere of nitrogen. On cooling, the reaction mixture was acidified with 6N hydrochloric acid and extracted with ethyl acetate (3 x 30 ml.). The extract was washed with sodium bicarbonate solution (3 x 20 ml.), water (2 x 20 ml.) and the combined washings on acidification with 6N hydrochloric acid and extraction with ethyl acetate (3 x 30 ml.) yielded a yellow gum of acidic material (760 mg.). Crystallisation of the acidic material from chloroform yielded des tigloyl des methyl iso-swietenine (123b) (250 mg.). mp. 249-251°C

The neutral ethyl acetate solution furnished 156 mg. of neutral products.

Lead Tetra-acetate oxidation of des tigloyl des methyl iso-swietenine (123b).

Des tigloyl des methyl swietenine (200 mg.) in analaR acetic acid (20 ml.) was treated with lead tetra-acetate (264 mg.) and left in the dark for three days. The reaction mixture was diluted with water, extracted with chloroform (3 x 20 ml.) and the chloroform extract washed with sodium bicarbonate (2 x 20 ml.) and water (2 x 10 ml.) to yield on evaporation a gum (184 mg.). Chromatography of this gum over grade IV acid alumina (2g.) eluted the hydroxy-keto aldehyde (126) (110mg.) with chloroform-benzene (3:7). mp. 230-237°C

Oxidation of the hydroxy-keto aldehyde (126).

The hydroxy-keto-aldehyde (126) (165 mg.) in dry pyridine (5 ml.) was treated with chromium trioxide (183mg.) at room temperature for 16 hours. Methanol was added to the reaction mixture and, after 15 min., the solvents were removed in vacuo. The residue was stirred with water (10 ml.) and ethyl acetate (8 ml.), filtered, and the layers separated. The aqueous layer was extracted (3 x 5 ml.) with ethyl acetate, the combined ethyl acetate extracts yielding (130mg.) of gum. Filtration through grade IV acid alumina (o.5g.) and crystallisation from chloroform-ether furnished the β-diketo-aldehyde (127) (118mg.) mp. 217-221°C.

Alkaline hydrolysis of the β-diketo aldehyde (127).

The β-diketo-aldehyde (33mg.) was dissolved in ethanol (1.5 ml.) and a 5N sodium hydroxide (1.5 ml.) and refluxed under nitrogen for 1 hour. The reaction mixture was cooled, diluted with water and acidified with 6N HCl. The mixture was extracted with ethyl acetate (3 x 8ml.) and yielded the enone-acid (128) (30mg.) as a colourless gum.

DERIVATIVES OF THE ENONE ACID.

Attempted preparation of the p-iodo anilide of enone-acid (128).

Enone acid (20mg.) in aqueous ethanol (3 ml.) was neutralised with sodium bicarbonate (5mg.). The solvents were evaporated and the sodium salt was dried under reduced pressure (0.01 m m of mercury) for 16 hours at 40°C, then suspended in oxalyl chloride (2 ml.) for 10 hours. Filtration and evaporation of excess oxalyl chloride yielded the acid chloride which was dissolved in dry benzene (2 ml.) and treated with p-iodo aniline (30 mg.) for 6 hours. After filtration and evaporation, the residue was taken up in chloroform and chromatographed on a silica chromatoplate (0.25 mm) with methanol-chloroform (3:97) as eluant. The major band on the chromatoplate (other than the band on the base line) yielded a γ-lactone enone ν_{max}^{nujol} 1780cm^{-1}(γ-lactone), 1687cm^{-1}(enone). as white rods from chloroform-ether mp. 295-298°. No anilide was formed.

Attempted bromination of the enone methyl ester (128).[111][112]

a) Enone methyl ester (13 mg.) was dissolved in AnalaR chloroform (2 ml.) and treated with 1 mole equivalent of bromine in AnalaR chloroform at room temperature for 1 hour. The solvent was removed and the starting material was recovered unchanged.

b) Enone methyl ester (13 mg.) was dissolved in AnalaR acetic acid (1 ml.) and treated with 1 mole equivalent of bromine in acetic acid at room temperature for 2 hours. The mixture was diluted with water and extracted with ethyl acetate (3 x 5 ml.), washed with sodium bicarbonate solution (2 x 5 ml.), water (2 x 5 ml.) and the extract dried over anhydrous sodium sulphate. The product was filtered through grave IV acid alumina. All attempts to crystallise this material failed. Solvents used: chloroform-ether, ethylacetate-petroleum ether, ethanol, methanol.

Attempted preparation of the p-bromo phenacyl ester of the enone acid (130).

The enone acid (14mg.) dissolved in aqueous ethanol (2ml.) was neutralised with sodium carbonate (3.5mg.). After ensuring that the solution was acid to phenolphthalein (to prevent hydrolysis of p-bromo phenacyl bromide), p-bromo phenacyl bromide (9mg. : 1 molar equivalent) in ethanol (1 ml.) was added. The mixture was refluxed 1 hour on the steam bath, diluted with water and extracted with ethyl acetate (3 x 5 ml.); the extract was washed with water (2 x 3 ml.) and dried over anhydrous sodium sulphate. The product from the reaction decomposed to a number of products on attempted crystallisation from ethanol.

$NaBH_4$ reduction of the enone acid ( ).

The enone acid (30mg.) in AnalaR methanol (5 ml.) was treated at room temperature with excess sodium borohydride for 2 hours. The excess sodium borohydride was destroyed with 2N HCl and the solution extracted with ethyl acetate (3 x 8ml.). The extract was washed with water (2 x 5 ml.) and dried over anhydrous sodium sulphate. The residue from the extract (28mg.) was methylated with diazomethane and chromatographed on a 20 x 20 cm silica plate (0.1 mm) eluant methanol-chloroform (1:19). Two products were obtained; non-crystalline product (alcohol) (16mg.) ν_{max}^{nujol} 1710cm^{-1}(methyl ester), 1715cm^{-1} (δ-lactone), 3490cm^{-1} (alcohol). Crystalline product (alcohol) (5mg.) mp. 215-218°C ν_{max}^{nujol} 1702cm^{-1}(methyl ester) 1708cm^{-1} (δ -lactone) 3400cm^{-1} (hydroxyl).

Iodo benzoylation of alcohol α .

Alcoholα (5mg.) was treated with p-iodo benzoyl chloride (30mg.) in dry, redistilled pyridine (1 ml.) at room temperature 16 hours. Crushed ice was added and, after warming to room temperature, the mixture was extracted with ethyl acetate (3 x 5 ml.) and the extract washed with water (2 x 5 ml.) and dried over anhydrous sodium sulphate. The total product was chromatographed on a silica plate (0.1 mm) eluant methanol-chloroform (1:19). Two cyrstalline iodo benzoates were extracted; major product (10mg.) mp. 122-123°C square plates

from chloroform-ether. : minor product (2mg.)
mp. 138-141°C thin needles from chloroform-ether.

The major product was unsuitable for an X-ray analysis since the crystal had a high temperature factor. The minor product was also unsuitable since the crystals were twinned.

m-iodo benzoylation of alcohol β .

Alcohol β (16mg.) was treated with m-iodo benzoyl chloride (95mg.) in dry, redistilled pyridine (1 ml.) at room temperature 16 hours. Crushed ice was added and the mixture extracted with ethyl acetate (3 x 8 ml.). The extract was washed with water (2 x 5 ml.) and dried over anhydrous sodium sulphate. The product separated by TLC was non-crystalline.

DERIVATIVES OF THE ISO-TRIONE.(33)

Preparation of dehydro-swietenine.

Swietenine (200mg.) in AnalaR acetone (15 ml.) at 0°C was treated with excess Jones reagent. On warming to room temperature the solution was diluted with water and extracted with ethyl acetate (3 x 8 ml.). The extract was washed with water (2 x 5 ml.) and dried over anhydrous sodium sulphate. The product crystallised from chloroform - ether yielded dehydro-swietenine (164mg.) mp. 258-263°C.

Preparation of des tigloyl dehydro-iso-swietenine.

Dehydro-swietenine (164mg.) was refluxed under nitrogen 30 min. with 5% ethanolic potassium hydroxide (15 ml.). After cooling under nitrogen, the solution was acidified with 2N HCl and allowed to crystallise. A white crystalline solid (85mg.) was filtered, dissolved in methanol and treated with excess ethereal diazomethane. The product crystallised from chloroform-ether yielded destigloyl dehydro-iso-swietenine (80mg.) mp. 258-265°C

Extraction of the acidic solution with ethyl acetate (3 x 8 ml.) and methylation and crystallisation of the product as above yielded a further 35 mg. of destigloyl dehydro-iso-swietenine.

Oxidation of destigloyl dehydro-iso-swietenine.

Des tigloyl dehydro-iso-swietenine (115mg.) in AnalaR acetone (5 ml.) at 0°C was oxidised with excess Jones' reagent. On warming to room temperature, the solution was diluted with methanol and water, and extracted with ethyl acetate (3 x 10 ml.). The extract was washed with water (3 x 5 ml.) and dried over anhydrous sodium sulphate. Crystallisation of the product from chloroform-ether yielded the trione (125) (98mg.) mp. 230-234°C.

Alkaline hydrolysis of the trione.

Trione (**125**) (95mg.) in dioxan (1.5 ml.) was treated with 5% ethanolic potassium hydroxide (1.5 ml.) at room temperature for 90 min. The solution was acidified with 2N HCl and extracted with ethyl acetate (3 x 8 ml.) and the extract washed with water (2 x 5 ml.) and dried over anhydrous sodium sulphate. The product was dissolved in methanol and treated with excess ethereal diazomethane to yield the iso-trione (**133**) (61mg.). mp. 294-299°C.

$NaBH_4$ reduction of iso-trione (**133**).

Iso-trione (175mg.) in methanol (20ml.) was treated with sodium borohydride (520mg.) at room temperature 16 hours. The excess sodium borohydride was destroyed with 2N HCl and the solution extracted with chloroform (3 x 10 ml.). The residue from the extract was chromatographed over alumina grade III acid (12g.). Two alcohols were eluted. Alcohol A (69mg.) mp. 313-317°C was eluted with chloroform-benzene (2:3) and Alcohol B, non-crystalline (68mg.) was eluted with chloroform-benzene (4:5).

p-iodo benzoylation of alcohol B.

Alcohol B (12mg.) was treated with p-iodo benzoyl chloride (70mg.) in dry, redistilled pyridine (3 ml.) at room temperature 16 hours. Crushed ice was added and on warming to room temperature, the precipitated material was filtered and

recrystallised from chloroform-petrol as white plates mp. 161-163°C. ν_{max} 1780cm^{-1} (γ-lactone) 1730cm^{-1}(δ-lactone and methyl ester), 1690cm^{-1} (benzoate) 860cm^{-1} (1,4-aromatic substitution).

m-iodo benzoylation of alcohol B.

Alcohol B (10mg.) was treated with m-iodo benzoyl chloride (70mg.) in dry, redistilled pyridine (3 ml.) at room temperature 16 hours. Crushed ice was added and, on warming to room temperature, the solution was extracted with ethyl acetate (3 x 5 ml.). The extract was washed with sodium bicarbonate (2 x 5 ml.) and water (2 x 5 ml.) and dried over anhydrous sodium sulphate. The m-iodo benzoate (6 mg.) was separated by TLC and crystallised in white rods from chloroform-ethanol mp. 235-237°C.

p-iodo benzoylation of alcohol A.

Alcohol A (12mg.) was treated with p-iodo benzoyl chloride (70mg.) in dry, redistilled pyridine (3 ml.) at room temperature 16 hours. Crushed ice was added and, on warming to room temperature, the solution was extracted with ethyl acetate (3 x 5 ml.) and the extract washed with water (2 x 5ml.). The p-iodo benzoate was separated by TLC and crystallised from chloroform-ether as white plates mp. 178-180°C.

Hydrolysis of the iso-trione.

Iso-trione (20mg.) in dioxan (0.5 ml.) was treated with 5% methanolic potassium hydroxide (0.5 ml.) at room temperature for 30 min. The solution was diluted with water, acidified with 2N HCl and extracted with chloroform (3 x 5 ml.). The extract was washed with water and dried over anhydrous sodium sulphate. This yielded the iso-trione acid (19mg.).

Iso-trione acid cesium salt.

Iso-trione acid (18mg.) was dissolved in aqueous methanol (2 ml.) and neutralised with cesium carbonate (11.8mg. :0.5 molar equivalent). The mixture was shaken until the cesium carbonate had dissolved. ca 30 min. The solvents were removed in vacuo and the residue crystallised from methanol as thin white plates. mp. above 330°C.

Attempted preparation of iso-trione acid p-iodo anilide.

The acid (15mg.) dissolved in aqueous ethanol (1:1 v/v) (3 ml.) was neutralised with sodium bicarbonate (2.7 mg.). The solvents were removed in vacuo and the sodium salt thoroughly dried under vacuum, on the steam bath (1 hour). The dried salt was scraped off the sides of the flask and treated with excess oxalyl chloride at room temperature 6 hours. The excess oxalyl chloride was removed in vacuo and the residue taken up in dry benzene and added to p-iodo aniline (30 mg.) in dry benzene (1 ml.). The mixture was

left at room temperature 16 hours, filtered and the residue examined by T.L.C. but no product had been formed. The residue was dissolved in chloroform and extracted with sodium bicarbonate solution (3 x 5 ml.) and water (2 x 5 ml.). The combined aqueous extracts were acidified and extracted with chloroform (3 x 5 ml.). The iso-trione acid (13mg.) was recovered.

Preparation of iso-trione acid p-bromo phenacyl ester.

The acid (20mg.) in ethanol (1 ml.) was neutralised with sodium carbonate (4.6mg.) in water (0.5 ml.) and the solution tested to ensure that it was acid to phenolphthalein. p-bromo phenacyl bromide (24 mg.) was added and the solution refluxed on the steam bath 1 hour. The reaction mixture was evaporated to dryness under reduced pressure and the residue chromotographed on a silica chromatoplate (20 x 20cm, 0.1 mm thick, eluant methanol-chloroform (1:19)). A non-crystalline p-bromo phenacyl ester (17mg.) was isolated.

ν_{max} 1785cm^{-1} (γ -lactone), 1750cm^{-1} (δ -lactone), 1690cm^{-1} (carbonyl α to aromatic ring), 1670cm^{-1} (double bond) 1600cm^{-1} (aromatic bands).

REFERENCES

1. Williams, Chem. News, (1860), 2, 206.

2. Ruzicka. Experientia, (1953), 9, 357.

3. Bloch and Rittenberg. J. Biol. Chem. (1942), 145, 625.

4. Bloch. Vitamins and Hormones, (1957), 15, 119.

5. Langdon and Bloch. J. Biol. Chem. (1953), 200, 129.

6a. Birch Rickards, Smith, Harris and Whalley. Proc. Chem. Soc. (1958) 223.

6b. Britt and Arigoni. ibid. 224.

7. Birch, Richards and Smith. Proc.Chem.Soc.(1958)192.

8. Wolf, Hofmann, Aldritch, Skeggs, Wright and Folkers. J.Amer.Chem.Soc. (1956), 78, 4499.

9. Tavormina, Gibbs and Huff. J.Amer. Chem. Soc. (1956), 78, 4498, 6210.

10. Chaykin, Law, Phillips, Tchen and Bloch. J.Biol. Chem. (1960), 235, 326.

11. Lynen, Eggerer, Henning and Kessel. Angew.Chem. (1958), 70, 738.

12. Rilling and Bloch. J.Biol. Chem. (1959), 234, 1424.

13. Phillips, Tchen and Bloch. Fed.Proc. (1958), 17, 289.

14. Agranoff, Eggerer, Henning and Lynen. J.Amer. Chem. Soc. (1959), 81, 1254.

15. Robinson. Chem. and Ind. (1934), 53, 1062.

16. Langdon and Bloch. J.Amer. Chem.Soc. (1952), 74, 1869.

17. Cornforth, Gore and Popjak. Biochem. J. (1957), 65, 94.

18. Cornforth, Hunter and Popjak. Biochem. J. (1953), 54, 597.

19. Birch and Smith. Ciba Foundation Synposium, Biosynthesis of Terpenes and Sterols p.245 Churchill 1959.

20. Arigoni. Ciba Foundation Symposium, Biosynthesis of Terpenes and Sterols p.231 Churchill 1959.

21. Demole, Lederer. Bull. Soc. Chim. France, 1958, 1128.

22. Cava, and Weinstein. Chem. and Ind. 1959, 851.

23. Chan, Haynes and Johnston. Chem. and Ind. 1960, 22.

24. Cava, Chan, Haynes, Johnston and Weinstein. Tetrahedron, (1962), 18, 397.

25. Graham and Overton. J.Chem. Soc. 1965, 126.

26. Gopinath, Govindachari, Parthasarathy and Viswanathan. Helv.Chim.Acta. (1961), 44, 1040.

27. Nakano and Djerassi. J. Org.Chem. (1961), 26, 167.

28. Haeuser, Lombard, Lederer and Ourisson. Tetrahedron (1961), 12, 205.

29. Scott, Sim, Ferguson, Young and McCapra. J.Amer.Chem. Soc. (1962), 84, 3197.

30. McCapra, Scott, Sim and Young. Proc.Chem.Soc. 1962, 185.

31. Connolly, McCrindle, Murray and Overton. Tetrahedron Letters (1964), 29, 1983.

32. Bigley, Rogers, Barltrop. J.Chem. Soc. 1960, 4613.

33. Overton and Renfrew. J. Chem. Soc., 1967, 931.

34. Bjåmer, Ferguson, Melville and Overton. unpublished results.

35. Simonsen and de Mayo. The Terpenes, Vol. V. Cambridge University Press, London. 1957,p.596.

36. Overton, Weir and Wylie. Proc. Chem. Soc. 1961, 211.

37. Sim and Halsall. J.Chem. Soc. 1966, 633.

38. Paul, Sim, Hamor and Robertson. J.Chem. Soc. 1962, 4133.

39. Fritel and Fetison. J. Org. Chem. (1958), 23, 481.

40. Wessley, Kinjaski, Isemann and Singer. Monatsch, (1936), 66, 87.

41. Wessley, Schonol and Isemann. Monatsch, (1936), 68, 21.

42. Wessley, Isemann, Singer and Schonol. Annalen, (1936), 522, 41.

43. Wessley, Munster and Schonol. Monatsch, (1936), 70, 30.

44. Wessley and Jentzsch. Monatsch, (1936), 68, 313.

45. Wessley and Schonol. Monatsch, (1938), 71, 10.

46. Feist, Rintelen and Kuntz. Annalen, (1935), 517, 119.

47. Feist, Kuntz and Brachvogel. Annalen, (1935), 519, 124.

48. Feist, Kuntz and Brachvogel. Annalen, (1935), 521, 184.

49. Feist and Brachvogel. Annalen, (1936), 522, 185.

50. Feist, Brachvogel and Volksen. Annalen, (1936), 523, 289.

51. Feist and Volksen. Annalen, (1938), 524, 41.

52. Barton and Elad. J. Chem. Soc. 1956, 2090.

53. Cava and Soboczenski. J. Amer. Chem. Soc. (1956), 78, 5317.

54. Barton and Elad. J. Chem. Soc. 1956, 2085.

55. Cava, Weinstein and Malhotra. Tetrahedron Letters, (1959), 15, 1.

56. Klyne, Chem. and Ind., 1954, 1198.; Barton and Gilman, Proc. Chem. Soc., 1959, 391.

57. Fridricksons and Mathieson. Acta. Cryst., 1963, 206.

58. Weir, N.G., Ph.D. Thesis, Glasgow, 1962.

59. Huisgen. Angew. Chem. (1963), 10, 565.

60. Barbulescu, Grunager, Langella and Quilico. Tetrahedron Letters, 1961, 89.

61. Huisgen, Graschey, Laur and Leitermann. Angew. Chem. (1960), 72, 416.

62. Huisgen, Seidel, Wallbillich and Knupfer. Tetrahedron, (1962), 17, 3.

63. Quilico, Stagno, d'Alcontres and Grünanger.
Gazz. Chim. Ital. (1950), 80, 479.

64. Schmitz. Chem. Ber. (1958), 91, 1495.

65. Schmitz. Chem. Ber. (1958), 91, 1133.

66. Knorr. Annalen, (1896), 293, 1.

67. Nicholls and Whiting. J. Chem. Soc. 1959, 551.

68. Chattaway and Walker. J. Chem. Soc. 127, 981.

69. Werner and Buss. Chem. Ber. (1894), 27, 2193.

70. Piloty and Steinbok. Chem. Ber. (1902), 35, 3112.

71. Janse. Rec. trav. chim. Pays Bas.(1921), 40, 297.

72. Huisgen and Mack. Tetrahedron Letters, 1961, 583.

73. Bullock and Hand. J. Amer. Chem. Soc. (1956), 78, 5854.

74. Brand and Timmins. J. Chem. Soc. 1953, 3136.

75a. Cheung, Overton and Sim. Chem. and Ind. 1966,

75b. Overton, Weir and Wylie. J. Chem. Soc. 1966, 1482.

76. McConnel, Mathieson and Schoenborn.
Acta Cryst., (1964), 17, 472.

77. Schoenborn and McConnel. Acta Cryst., (1962), 15, 779.

78. Cheung, Melville, Overton, Robertson and Sim.
J. Chem. Soc. (B), 1966, 853.

79. Arnott, Davie, Robertson, Sim and Walker.
J. Chem. Soc. 1961, 4183.

80. McPhail and Sim., Tetrahedron Letters, 1964, 2598.

81. Kartha and Hass. J. Amer. Chem. Soc. (1964), 86, 3630.

82. Brown, Sim and Tamura. unpublished results.

83. Dreyer, Tetrahedron, (1965), 21, 83.

84. Barton, Overton and Wylie. J. Chem. Soc. 1962, 4809.

85. Wylie, A. Ph. D. Thesis, Glasgow, 1960.

86. Balasubramanian, Barton and Jackman.
J. Chem. Soc. 1962, 4816.

87. Tori, Tetrahedron Letters, (1964), 559.

88. Tiers' Tables.

89. Tori, Komeno and Nakagawa. J. Org. Chem. (1964), 29, 1136.

90. Jeffries, Tetrahedron Letters, (1963), 1353.

91. Williamson and Johnson, J.Amer.Chem.Soc. (1961), 83, 4623.

92. Cocker and Halsall, J. Chem. Soc. 1956, 4262.

93. Burn and Rigby. Chem. and Ind. 1955, 386.

94. Grant and Zeiss. J.Amer. Chem. Soc. (1954), 76, 5001.

95. Ledered and Bory. Croat. Chem. Acta, (1957), 29, 136.

96. Bigley, Barltrop and Rogers. Chem. and Ind. 1962, 1570.

97. Baker, Eglinton, Gonzalez, Hamilton and Raphael.
J. Chem. Soc. 1962, 4705.

98. Sircar and Chakrabarthy. J. Ind. Chem. Soc.(1951), 28, 207.

99. Chakrabarthy and Chatterjee. J.Ind. Chem.Soc.(1955), 32, 179.
idem, ibid, (1957), 34, 117.

100. Ghosh, Chakrabarthy and Chatterjee.
ibid, (1960), 37, 440.

[redacted].

101. Connolly, Henderson, McCrindle and Overton.
Tetrahedron Letters, 1964, 2593.

102. McPhail and Sim. Tetrahedron Letters, 1964, 2598.

103. Henderson, R., Ph. D. Thesis, Glasgow, 1965.

104. Brockmann and Schodder, Ber., 1941, 74, 73.

105. Stahl, Chem. -Ztg., 1958, 82, 323.

106. Connolly, Gunn, M^{c}Crindle, Murray and Overton, Tetrahedron Letters, 1966, 2109.

107. Corsano, Mellor and Ourisson, Chem. Comm., 1965, 185.

108. Barton, Sklartz, de Koch, Enslin and Norton, Tetrahedron Letters, 1962, 309.

109. Fawcett and Trotter, J.Chem.Soc., 1965, 125.

110. Stempel and Schaffel, J.Amer.Chem.Soc., (1942), 64, 470.

111. Green and Long, J.Chem.Soc., 1961, 2532.

112. Kirk, Patel and Petrow, J.Chem.Soc., 1956, 627; 1184.