

A T H E S I S

=====

submitted to

THE UNIVERSITY OF GLASGOW

in fulfilment of the
requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

James Clark

=====

**Chemistry Department,
The Royal College of Science and Technology,
Glasgow.**

October 1962.

ProQuest Number: 13849332

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 13849332

Published by ProQuest LLC (2019). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

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

Diterpenes from Araucaria Imbricata.

A C K N O W L E D G E M E N T S

The author would like to express his sincere gratitude to Dr. J. McLean for his continued guidance and encouragement, to Professor P. L. Pauson for his active interest in the problem, and to Professor W. Klyne, Imperial College of Science and Technology, for recording the optical rotatory dispersion curves. Finally the author acknowledges receipt of a Research Studentship from the Department of Scientific and Industrial Research.

C O N T E N T S

	<u>Page</u>
<u>Summary</u>	i
 <u>Introduction</u>	
The structural elucidation of the known bicyclic diterpenes	1
The Biogenesis of Diterpenes	24
 <u>Theoretical</u>	
Compounds isolated from the bark of Araucaria Imbricata	31
The Structure determination of labd-8(20)-en-3 β ,15-diol and related diterpenes	32
<u>Experimental</u>	54
<u>Bibliography</u>	87

Summary

The introductory section of the thesis contains a brief account of some of the important steps in the structural elucidation of the known bicyclic diterpenes, followed by some present ideas on diterpene biogenesis.

The theoretical and experimental sections are concerned with the isolation and structural determination of natural products from the bark of Araucaria Imbricata, the Chilean Pine tree, known in Britain as the 'Monkey Puzzle'.

Extraction of the powdered bark with light petroleum gave a gummy extract which was separated into neutral and acidic fractions.

The neutral fraction contains ceryl alcohol, β -sitosterol, and a new diterpene diol, labd-8(20)-en-3 β ,15-diol. The elucidation of the structure of this compound takes up the major part of the thesis.

Dehydrogenation of the diol with selenium gave 1,2,5-trimethylnaphthalene, showing it to be bicyclic and of the labdane type. The position of the exocyclic double bond and the stereochemistry of the side chain were established by comparison of the molecular rotational differences of several derivatives with similar compounds in

the labdanolic acid series. The stereochemistry of the ring junction was shown to be normal by optical rotatory dispersion measurements.

The positions of the hydroxyl groups were established by mass spectral, optical rotatory dispersion, and chemical evidence, but an attempt to convert the diol to a known compound, dihydrocativic acid, did not lead to the isolation of the product in a pure state.

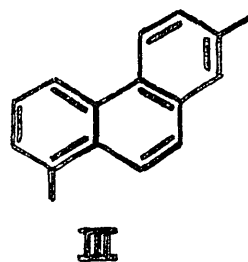
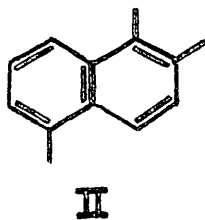
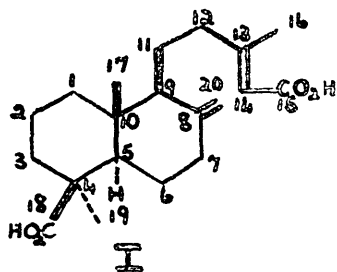
The neutral fraction was also shown to contain 3β -hydroxylabd-8(20)-en-15-al since lithium aluminium hydride reduction gave the diol, but this aldehyde could not be purified for characterisation.

The acidic fraction contains the new diterpene acid 3β -hydroxylabd-8(20)-en-15-oic acid which is also reduced to the diol with lithium aluminium hydride.

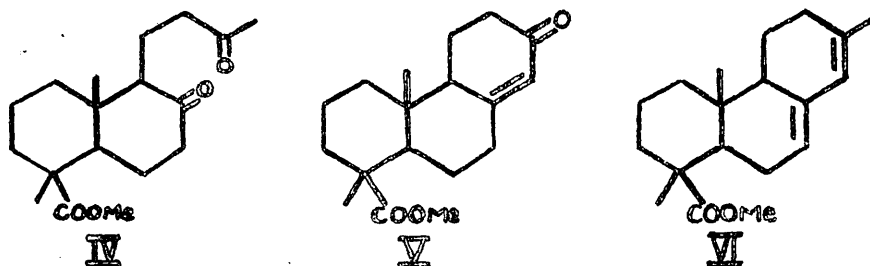
I N T R O D U C T I O N
=====

In the introductory section, an attempt is made to summarise the more important work which led to the structural elucidation of the bicyclic diterpenes and of other closely related diterpenes. The numbering system follows that used by de Mayo¹ (I).

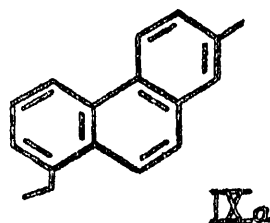
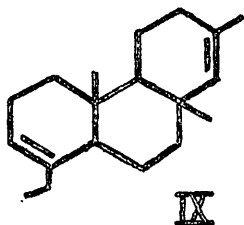
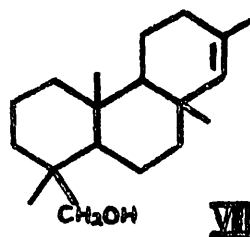
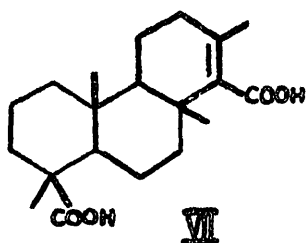
Agathenedicarboxylic acid (I), now known as agathic acid was first isolated in a state of purity by Ruzicka and Hosking.² It is obtained from Kauri copal and also from soft and hard grades of Manila copal. Initial dehydrogenation experiments gave mixtures of naphthalene derivatives containing mainly 1,2,5-trimethylnaphthalene (II), and also the phenanthrene derivative, pimanthrene (III), which was believed to be formed by cyclisation of an unsaturated side chain. This view was supported by similar reactions on saturated derivatives when only the naphthalene compounds were produced.



Spectral data and the ready loss of carbon dioxide indicated an α - β unsaturated acid grouping and, much later, Ruzicka et al⁵ showed, by elegant ozonolysis experiments, the position of the double bonds. The most important product in this work was the diketone (IV), which was cyclised with alkali to compound (V) and converted to the unsaturated ester (VI), dehydrogenation of which gave pimanthrene. This showed that the position of the easily eliminated carboxyl group was at C₁₅.

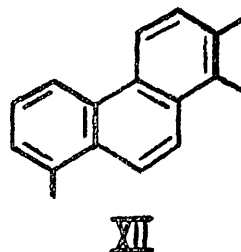
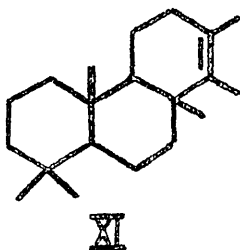
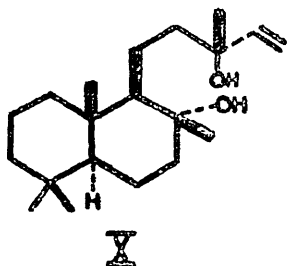


The position of the other carboxyl group was established by isomerising agathic acid with formic acid to the tricyclic acid (VII), which, on decarboxylation and subsequent reduction, gave the alcohol (VIII). This alcohol was dehydrated to the hydrocarbon (IX), which was dehydrogenated to 1-ethyl-7-methyl-phenanthrene (IXa).



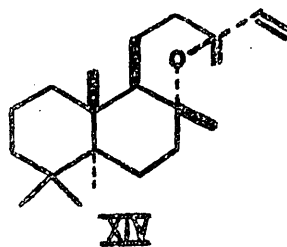
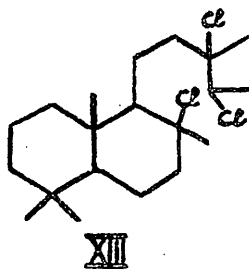
Ruzicka and Bernold⁴ showed by oxidation that the C₄ carboxyl group was in the β-configuration and, later, Ruzicka and co-workers⁵ related agathic acid to manoöl (XV), proving the ring junction to be trans and the side chain equatorial (β).

The structure of sclareol (X), first isolated from the leaves of Salvia Sclarea L.⁶ was assigned, without stereochemical implications, on degradative evidence and also on its relationship to other diterpenes isolated around the same time.



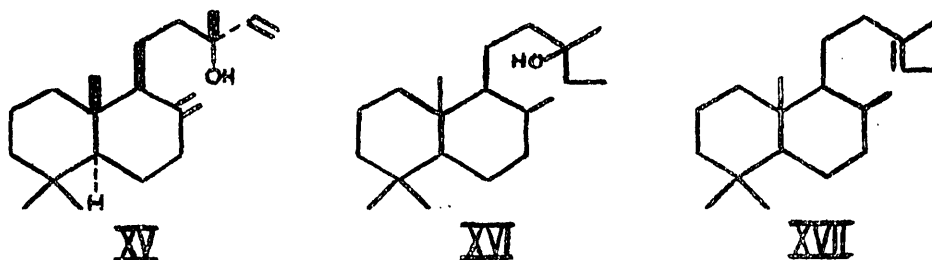
Janot⁷ showed that it was a bicyclic unsaturated diterpene diol and, later, Ruzicka and Janot⁸ partially characterised the skeleton by dehydrogenation to give 1,2,5-trimethylnaphthalene (II). Moreover, they converted sclareol to dihydrocyclosclarene (XI) which dehydrogenated to pimanthrene (III) and 1,7,8-trimethylphenanthrene (XII). These products suggested a carbon skeleton like that of agathic acid and, since ozonolysis yielded a C₁₉ acid and formaldehyde showing the presence of a terminal methylene group, the structure (X) was suggested, the hydroxyl groups being placed at C₉ and C₁₃ to account for the dehydration product (XI).

When treated with hydrogen chloride, sclareol gave the same chloride (XIII) as manoyl oxide (XIV), another diterpene isolated from the wood of the Silver Pine.⁹ Manoyl oxide was shown, in a series of papers by Hosking and Brandt,^{9,10,11} to have the basic structure (XIV) and this received confirmation from the postulated structure of sclareol.



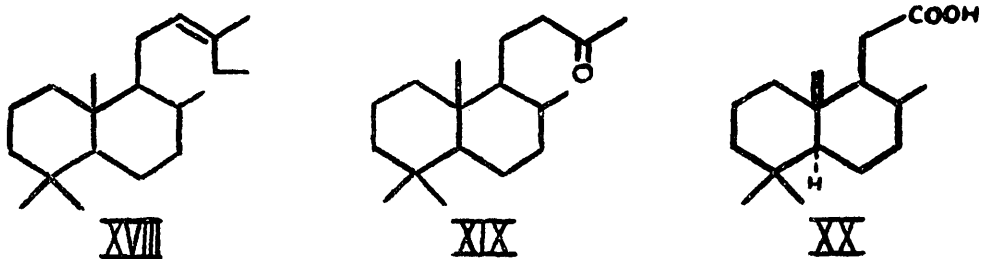
Dehydrogenation of manoyl oxide and sclareol gave the same products and ozonolysis showed the presence of the terminal double bond in each.

Another diterpene alcohol, manoöl (XV), was isolated from the wood of the Yellow Pine,¹² and Hosking and Brandt¹³ showed it to be closely related to manoyl oxide and sclareol since it also gave the same chloride (XIII) on treatment with hydrogen chloride. Manoöl was shown to contain two



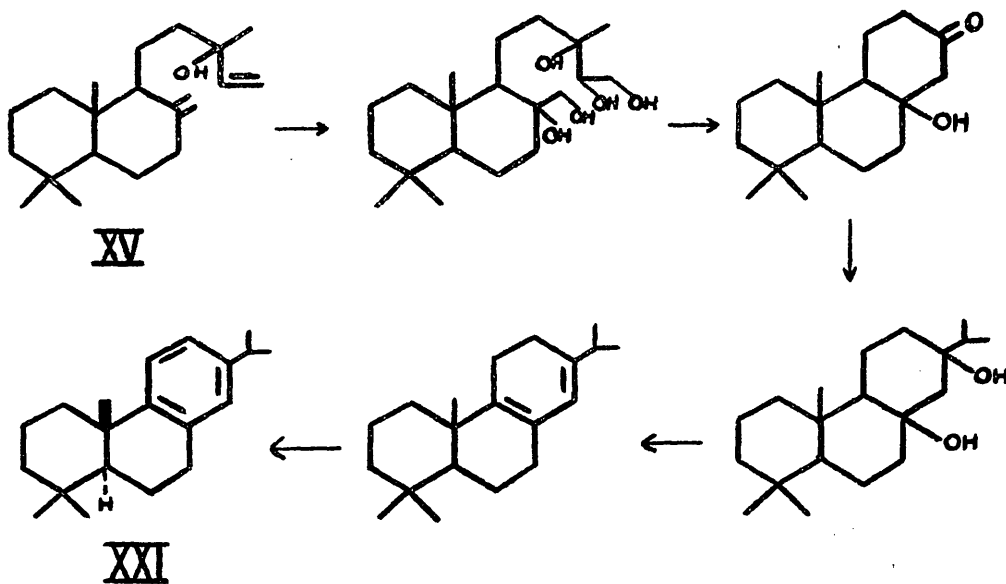
terminal methylene groups, and, although their positions were deduced from the relationship to manoyl oxide, this was also done by oxidation experiments.

The chloride of tetrahydromanoöl (XVI) was converted by base to a mixture of hydrocarbons (XVII and XVIII), which furnished respectively, on ozonolysis, a C_{18} ketone (XIX) and a C_{18} acid (XX). The latter acid has been obtained from ambrein¹³ and furnished evidence of a trans ring junction in

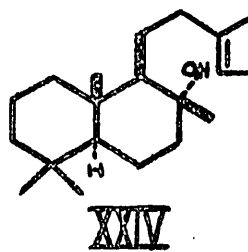
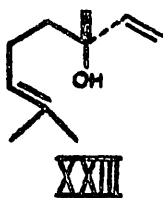
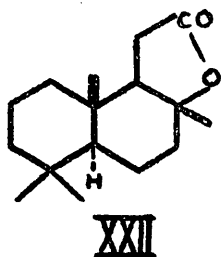


manoöl, which is now known to have the side chain in the equatorial conformation.¹⁴ This series of reactions established the position of the hydroxyl group at C₁₃.

Manoöl was also shown, later, to be related to abietic acid by Jeger and co-workers,¹⁵ who converted *manoöl* (XV) to dehydroabietane (XXI), thus confirming the nature of the ring junction as *trans*.



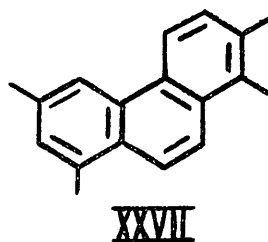
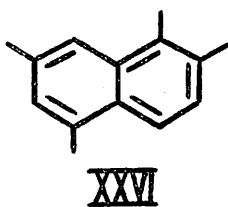
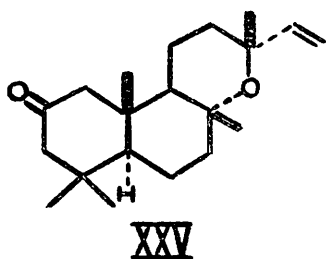
Sclareol itself was directly related to ambrein¹⁹ since both on oxidation furnished the lactone (XXII), and further relations to this compound were established by Stoll and co-workers.^{17,18,19}



Dehydration of sclareol to manoöl was achieved by Büchi and Biemann,²⁰ and also by Ohloff,²¹ thus proving that the C₈ hydroxyl group was in the equatorial configuration (α) since dehydration resulted in an exocyclic double bond. The stereochemistry at C₁₃ had still to be elucidated and this was accomplished by Barltrop, Bigley, and Rogers,²² who synthesised sclareol and its C₁₃ epimer. By studying models of intermediate compounds and giving consideration to hydrogen bonding, they concluded that sclareol had the 13(R)-configuration.²³ This conclusion was supported by careful analysis of molecular rotation data for several derivatives of manoöl and R(+)-linalool²⁴ (XXIII).

The structure given to manoyl oxide (XIV) was therefore shown to be correct and again the only problem remaining concerned the configuration at C₁₃ and at C₈. This was solved by Hodges and Reed²⁵ who prepared 8 α -hydroxy-13 β -ene (XXIV) by hydrogenolysis of manoyl oxide, thus showing the C₈-methyl group to be axial (β). They also studied the electron impact induced fission of manoyl oxide and 13-epimanoyl oxide and suggested a 13(β)-methyl structure. Hence the stereochemistry of manoyl oxide may be represented as in (XIV) assuming that the six-membered oxide ring is in the normal chair form.

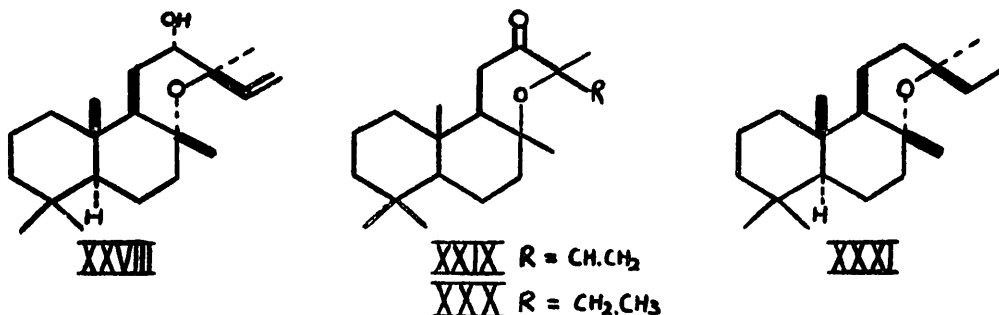
An oxygenated derivative of manoyl oxide, ketomanoyl oxide (XXV), occurs in the wood oil of the silver pine.



Wolff-Kishner reduction of this compound gave manoyl oxide, so the structural problem concerns the position of the keto-group. Hosking and Brandt^{9,11} solved this very neatly by marking the position of the keto-function with a methyl

group using a methyl Grignard reagent; subsequent dehydrogenation gave 1,2,5,7-tetramethylnaphthalene (XXVI) and 1,3,7,8-tetramethylphenanthrene (XXVII) hence the carbonyl function is situated at C₂.

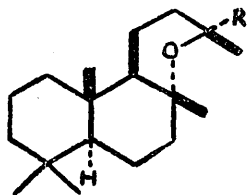
Giles and co-workers²⁶ recently isolated a new diterpene from Turkish tobacco and showed that its structure and stereochemistry is represented by structure (XXVIII), i.e. 12 α -hydroxy-13-epimanoyl oxide.



Spectral comparisons indicated that the diterpene was related to sclareol and this was confirmed as follows. Oxidation of the diterpene and its dihydro-derivative gave the ketones (XXIX) and (XXX) respectively, and stereospecific reduction of (XXX) gave the equatorial alcohol which was different from the dihydro derivative of the natural compound. The natural material therefore must have an axial hydroxyl group a conclusion which is supported by infrared evidence. Wolff-Kishner reduction of the ketone (XXX) gave dihydro-13-epimanoyl oxide (XXXI),

a known compound.

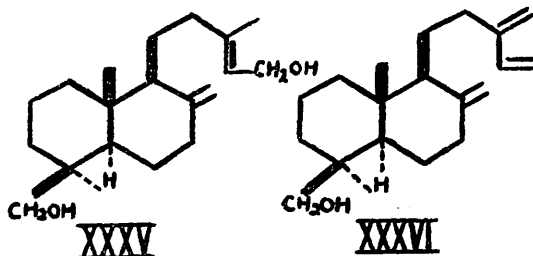
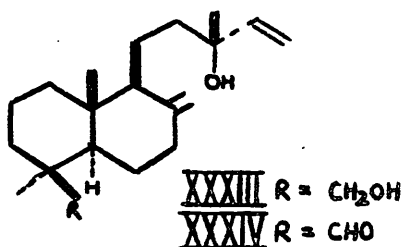
Ozonolysis experiments showed that the hydroxyl group is attached to one of the last five carbon atoms in the side chain which positions it at C₁₂. The compound is therefore represented by formula (XXVIII). Preparation of the epimeric methyl esters (XXXII) and



XXXVII, R = CO₂Me and 13-epimer

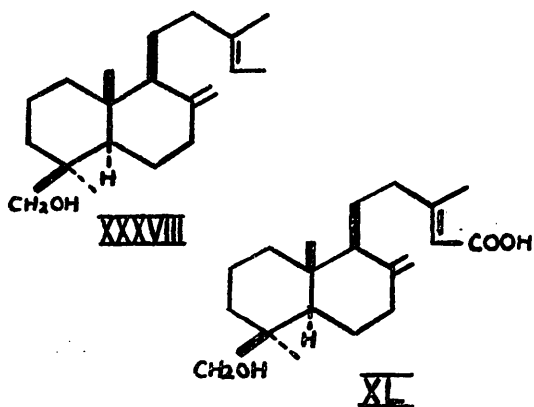
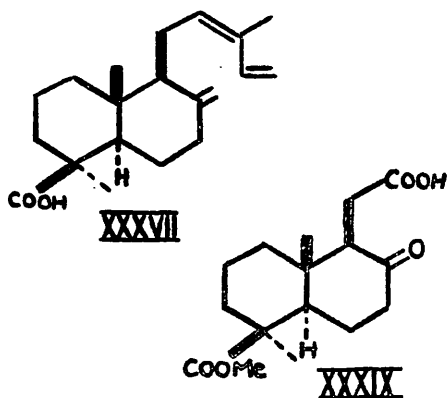
a study of their rates of hydrolysis showed that the 13 β -methyl structure in manoyl oxide proposed by Hodges and Reed²⁵ was indeed correct.

Torulosal (XXXIII) and Torulosal (XXXIV), isolated from Cupressus torulosa were shown to be, respectively hydroxy-manoöl and the related aldehyde. Comparison of the infrared and mass spectral data suggested a relationship to manoöl, and this was confirmed by reduction of torulosal to manoöl which established the stereochemistry at C₁₀, C₅, C₉ and C₁₃. The position of the

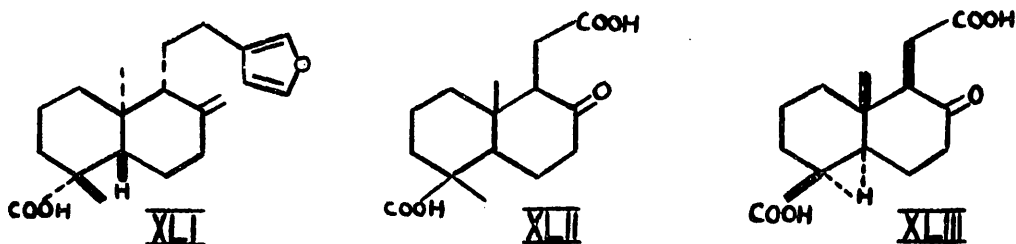


other oxygen function was shown by allylic rearrangement of torulosol to give (XXXV) and (XXXVI), the former being identical with the lithium aluminium hydride reduction product of agathic acid. Hence the second oxy-function is present as a β -oriented hydroxymethylene group attached to C₄.

Compound (XXXVI) and its dihydroproduct were used to establish the structure of a diterpene acid, communic acid (XXXVII), isolated from Juniperus communis L.²⁸ This compound, containing three double bonds, of which two are terminal, formed a maleic anhydride adduct; moreover, lithium aluminium hydride reduction and subsequent hydrogenation gave isodihydrocommunol (XXXVIII), identical with the reduction product of (XXXVI) above. This evidence, taken in conjunction with the formation of an ozonolysis product (XXXIX), proved communic acid to be (XXXVII).



Enzell isolated agatholic acid (XL) from Manila copal,²⁹ and infrared and mass spectral data suggested a close relationship to torulosol, the difference being in the side chain. Reduction of agatholic acid with lithium aluminium hydride gave the compound (XXXV), which had previously been obtained from torulosol, and thus established the structure and stereochemistry of agatholic acid.

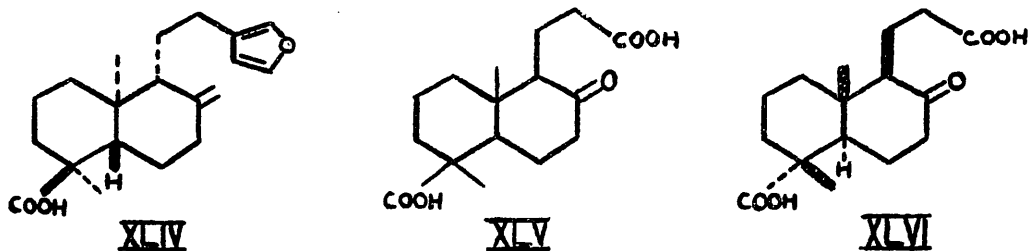


Daniellic acid (XLI), isolated from the African copal tree, was shown³⁰ to have one exocyclic double bond, and spectra showed the presence of a β -substituted furan ring. Dehydrogenation gave 1,2,5-trimethylnaphthalene (II), showing it to be a furan derivative of a bicyclic molecule, and consideration of the isoprene rule suggested the structure (XLI), without stereochemical implications. Comparison of the physical constants of some derivatives, e.g. (XLII), from daniellic acid with those, e.g. (XLIII), from agatholic acid, showed that these

were in fact antipodes. Daniellic acid (XLI) therefore has the inverted stereochemistry at the ring junction, and has an equatorial (α) side chain and an axial (α) carboxyl group at C₄.

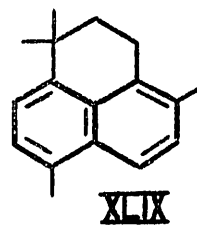
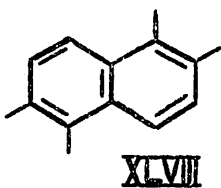
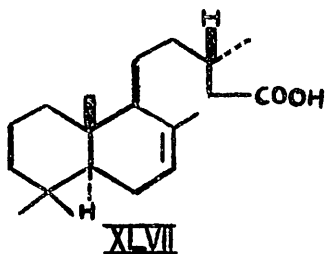
The C₄ epimer of daniellic acid, polyalthic acid, (XLIV), was isolated from Polyalthia fragrans and shown³¹ to have this structure by a series of reactions similar to those employed in the daniellic acid investigations. Dehydrogenation gave 1,2,5-trimethylnaphthalene (II) and a phenanthrene derivative, while ozonolysis gave a six-ring ketone and formaldehyde. Structure (XLIV) was formulated from this and spectral evidence.

The stereochemistry was elucidated by comparing the constants of several derivatives of polyalthic acid, e.g. (XLV), with isomeric compounds obtained from neoabietic acid e.g. (XLVI). Antipodal relationship was evident

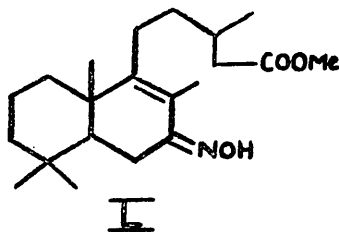
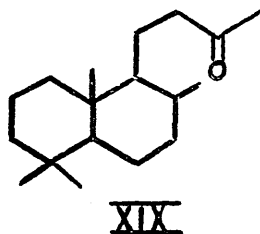


proving the stereochemistry of polyalthic acid to be as (XLIV) i.e. the C₄ epimer of daniellic acid.

Cativic Acid, first isolated from Prioria copaifera Griseb,³² was shown by Grant and Zeiss^{33,34} to have structure (XLVII). Dehydrogenation gave characteristic bicyclic derivatives, 1,2,5-trimethylnaphthalene (II), 1,2,5,6-tetramethylnaphthalene (XLVIII), and also 1,1,4,7-tetramethylphenalan (XLIX) which has been found to be

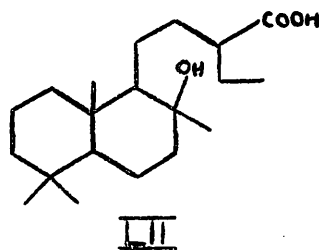
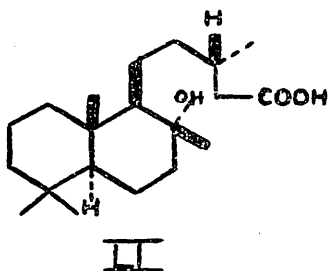


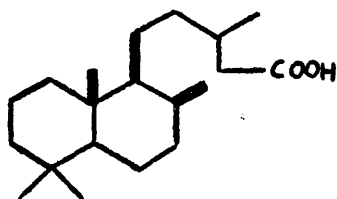
particularly characteristic of this type of structure. Degradation of dihydrocaticvic acid by a two-stage Barbier-Wieland technique gave a C_{18} ketone (XIX), identical with that obtained from manoöl, thus establishing the basic skeleton as in (XLVII) with the normal trans ring junction and β -orientated side chain. The position of the double bond was established by ozonolysis experiments which yielded



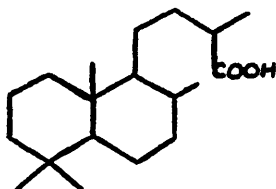
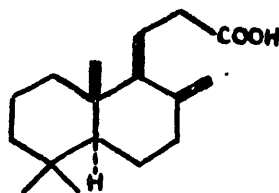
a methyl ketone (iodoform test) without loss of carbon and also by formation of the oxime (L) by reaction with nitrosyl chloride, this being consistent with a trisubstituted double bond, which therefore must be $\Delta^7(e)$. The remaining feature of the stereochemistry, i.e. at C₁₃ has been shown recently to be 13(S) (see labdanolic acid p. 17).

Cocker and Halsall³⁶ showed that labdanolic acid (II), isolated from gum labdanum,³⁶ was closely related to cativic acid and established its structure and stereochemistry except for the configuration at C₁₃. Dehydrogenation of labdanolic acid and cativic acid gave the same naphthalene derivatives, while dehydration gave a homogeneous product which, on ozonolysis, yielded a six-ring ketone and formaldehyde. Consideration of this and other preliminary evidence prompted the suggestion of formulae (II) or (III) or a stereoisomer as a basic structure.



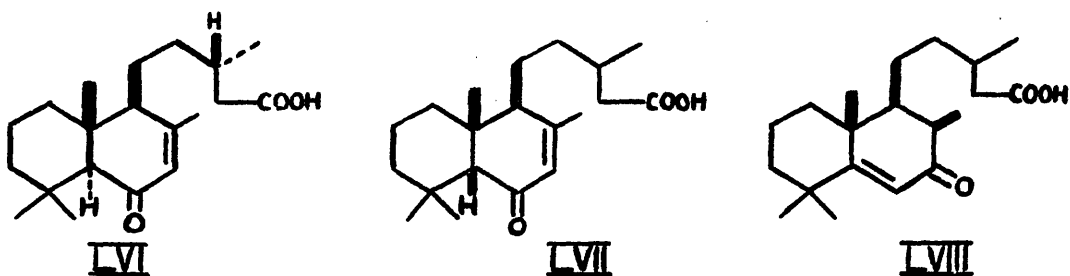
LIII

The side chain structure, and indeed the whole skeleton was established by Barbier-Wieland degradation of compound (LIII), obtained by dehydration and reduction of labdanolic acid, to give, in turn, the C_{19} acid (LIV), the methyl ketone (XIX), obtained also from cativic acid, and the C_{17} acid (LV), the latter being identical with a compound of known constitution and stereochemistry obtained from marrubiin.⁵⁷ This showed the presence of a normal trans ring junction and an equatorial side chain¹⁴ (β), and it followed that the C_8 -hydroxyl group has the α -configuration to account for the dehydration to an exocyclic double bond.

LIVLV

Compound (LIII) was shown to be dihydrocaticvic acid, and the only remaining feature of the stereochemistry of labdanolic and caticvic acids to be determined involved that at C₁₃. This was elucidated conclusively by two independent syntheses^{38,22} of labdanolic ester, and its C₁₃ epimer, when, by physical studies, the 13(S) configuration was assigned to both acids.

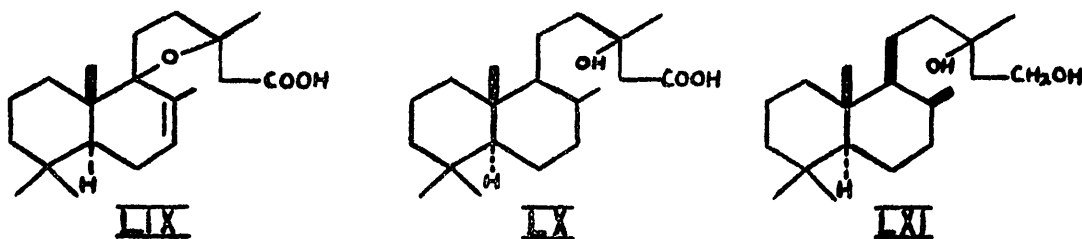
6-Oxocaticvic acid (LVI) was isolated along with labdanolic acid,³⁶ and Halsall and Moyle³⁹ later established its structure. Vigorous Wolff-Kishner reduction of its dihydroproduct yielded dihydrocaticvic acid leaving only the location of the α - β unsaturated carbonyl system to be fixed. Infrared and ultraviolet spectral data suggested a trisubstituted double bond in conjugation with the keto group and this led to consideration of structures (LVI), (LVII) and (LVIII).



Structure (LVII) was ruled out by optical rotatory dispersion studies, and (LVIII) was inconsistent with

the ultraviolet absorption data and also with the extremely inert carbonyl group, hence structure (LVI) was adopted. The stereochemistry follows from its relationship to cativic and labdanolic acids.

Another diterpene acid shown recently to be related to labdanolic acid is grindelic acid (LIX), isolated from the resin of Grindelia robusta.⁴⁰ Dehydrogenation gave 1,2,5-trimethylnaphthalene (II), while vigorous

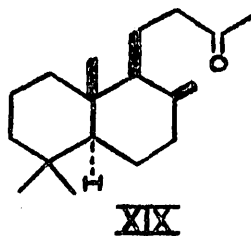
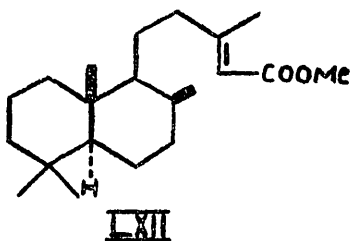


hydrogenation gave the acid (LX).

The opening of the oxide ring was also achieved by refluxing grindelic acid with acetic anhydride but similar reflux with dihydro-grindelic acid had no effect, implying the allylic nature of the ether.

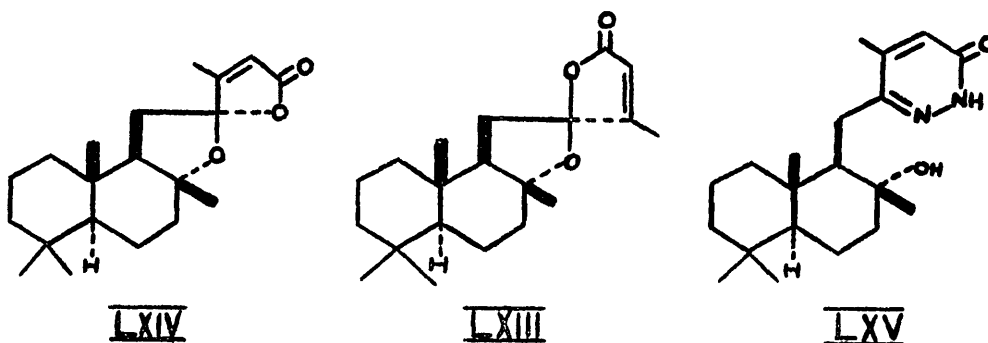
Successive reduction of grindelic acid with lithium aluminium hydride and lithium/ethanolamine, followed by hydrogenation, gave the diol (LXI) which was not a 1:2 diol. Oxidation of the diol gave a hydroxy-

acid thus proving that the newly introduced hydroxyl group is tertiary. Dehydration of the methyl ester of this hydroxy acid gave the conjugated ester (LXII), thereby establishing the position of the hydroxyl group at C₁₃. Confirmation of the location of the hydroxyl group came from ozonolysis of (LXII) when the known 15,16-bis-norlabd-13-one (XIX) was obtained.



Ozonolysis and oxidation experiments established the location of the double bond at C₇(₈), but the stereochemistry at C₉ and C₁₃ is as yet unresolved.

Two isomeric diterpenes, α -levantenolide (LXIII) and β -levantenolide (LXIV), were isolated⁴¹ together from Turkish tobacco. These were shown⁴¹ by physical techniques to be very similar α - β unsaturated γ -lactones, which gave the same triol on reduction and formed the same pyridazone (LXV) with hydrazine. Saponification of

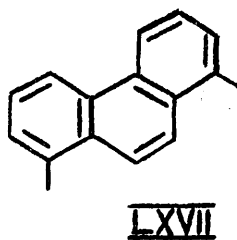
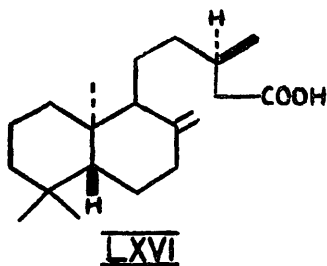


either isomer, followed by lactonisation gave predominantly the α -isomer. From this evidence it was deduced that the isomers differed only in the configurations of the carbon atoms to which the lactone rings were attached and also that the other oxygen was attached to the carbon atom undergoing the change in configuration. In short the compounds must be lactones of a hemi-ketal or hemi-acetal, a conclusion supported by the pyridazone formation.

Ozonolysis and oxidation experiments helped to show the positions of the various functions, and treatment of the pyridazone of the dihydro- β -lactone with potassium hydroxide, under Wolff-Kishner conditions, gave methyl labdanolate^{35, 38} and methyl-13-epilabdanolate³⁸ thus establishing the stereochemistry at C₁₀, C₅, C₈, C₉. Models showed that the formation of the lactones is sterically influenced by the methyl groups at C₈ and C₁₃ and this influence deters the C₁₃ methyl group from assuming

the position required for ring closure to give the β -isomer. Structures (LXIII) and (LXIV) were therefore proposed for the α - and β - isomers respectively.

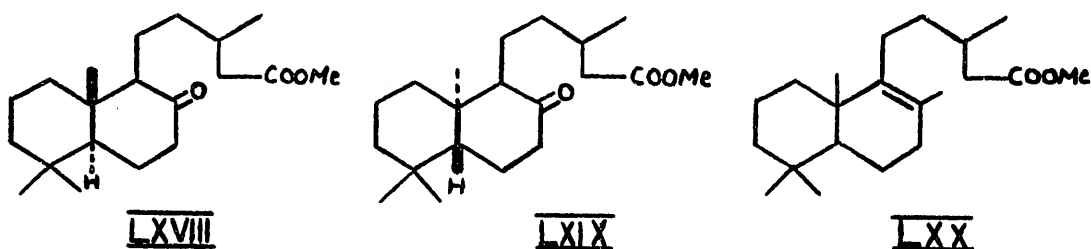
King and Jones⁴² isolated a diterpene acid, eperuic acid, from Eperua falcata and other Eperua species for which they postulated structure (LXVI). Dehydrogenation yielded 1,2,5-trimethylnaphthalene (II), 1,1,7,11-tetramethylphenalan (XLIX) and, from the norketone obtained by ozonolysis, 1,8-dimethylphenanthrene (LXVII) was obtained. Structure (LXVI) readily explained this cyclisation. Stepwise degradation of the side chain



of dihydroeperuic acid yielded a C_{17} acid which differed from the tris-nor acid obtained similarly from labdanolic acid, and it was shown to have the same structure but have different stereochemistry. When the structure and stereochemistry of labdanolic acid were determined, it was suggested⁵⁵ that the two acids were in fact antipodes

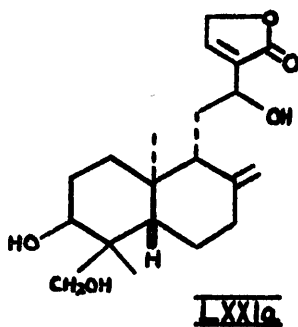
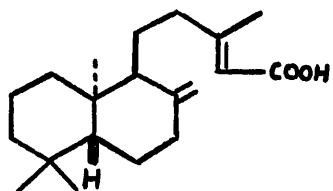
at least as far as the two rings were concerned.

This was shown to be the case by Djerassi and Marshall⁴³ who studied the optical rotatory dispersion curves obtained from the keto-esters (LXVIII) and (LXIX), derived from labdanolic and eperuic acids respectively. The curves were opposite in sign, although not exact mirror



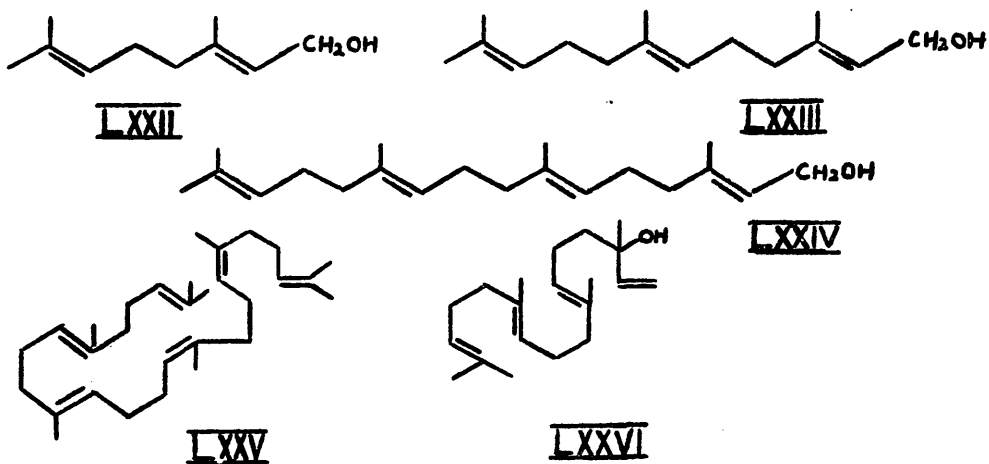
images, which was explained by the suggestion that the side chains were not opposite in configuration. This was supported by the observation that derivatives of the Δ^8 esters (LXX) from the two acids showed physical constants suggestive of complete antipodes. Eperuic acid will therefore have the stereochemistry as in (LXVI).

Copalic acid, isolated from Brazil Copal,⁴⁴ was shown by Djerassi and Nakano⁴⁴ to be mainly Δ^{15} -eperuic acid (LXXI) associated with other double bond isomers, and andrographolide, isolated from Andrographis paniculata Nees, has been shown⁴⁵ to have structure (LXXIa).



Biogenesis of Diterpenes

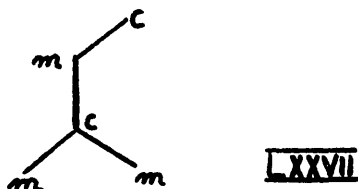
In 1953,⁴⁶ Ruzicka summarised his views on terpene biogenesis by proposing the 'Biogenetic Isoprene Rule'. This rule postulates that terpenoids are formed by preliminary telomerisation of isopentane units into a few aliphatic substances such as geraniol (LXXII), farnesol (LXXIII), geranyl-geraniol (LXXIV), and squalene (LXXV), which then cyclise and rearrange by known mechanisms to give mono-, sesqui-, di-, and tri-terpenes (and steroids) respectively.



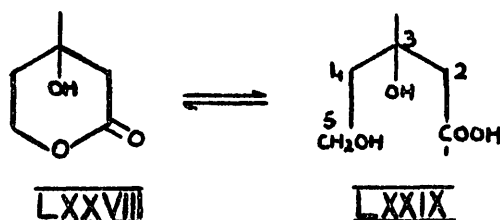
This hypothesis has been of great value in the structure determination of complex terpenes, and has been supported by many recent investigations.

In such a biogenesis, the intermediate required for diterpene production would obviously be geranylgeraniol or a related system e.g. geranylgeranylgeraniol (LXXVI), and it is necessary to consider the mechanism of the production and polymerisation of the active C₅ units, and their subsequent cyclisation and rearrangement to yield the different groups of diterpenes.

The role of acetate as precursor in terpenoid biosynthesis, as indicated by the early work^{47,48} on sterols, has become firmly established by much recent work using labelled carbon, in that acetic acid is a carbon source for the biosynthesis of all sterols and terpenoids so far investigated. This work has shown that both carbon atoms i.e. the methyl C(m) and the carboxyl C(c), are incorporated in the skeleton, appearing at specific points in the molecule, and the position of these labelled atoms has shown the presence of isopentane units labelled as (LXXVII).^{49,50,51}



The nature of the isopentane unit and its mode of synthesis from acetate posed very difficult problems which were not solved until the isolation⁵² of β -hydroxy- β -methyl- γ -valerolactone (LXXVIII), the lactone of mevalonic acid (LXXIX) designed as MVA.



This was shown to be a much better source of cholesterol⁵³ in biosynthesis than any other postulated intermediate and is considered to be a direct precursor. It is known⁵⁴ that MVA decarboxylates on its way to cholesterol, and such decarboxylation would clearly lead to a C₅ unit with the required distribution of carbon atoms, if we consider the derivation of MVA from acetate as in Fig.1

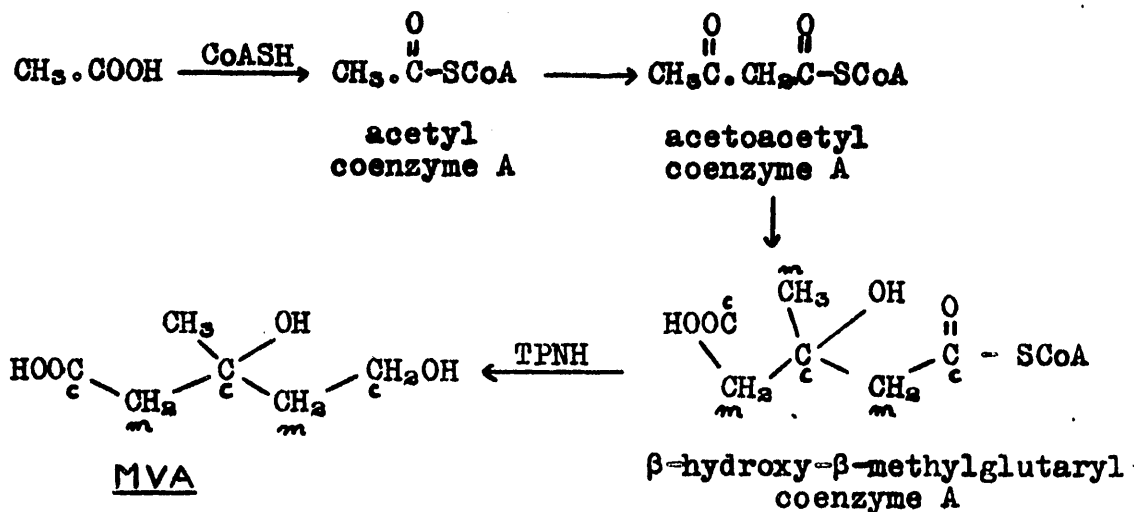


Fig.1 MVA from acetate

Condensation of C_2 of one molecule of MVA with C_5 of another will lead to polyisoprenoids.

The proposed conversion of mevalonic acid into geranyl-geraniol is outlined in Figure 2.

The subsequent cyclisation of geranyl geraniol or a related compound would be effected by initial cation attack after which skeletal rearrangement, oxidation etc. would lead to the different groups of diterpenes. Some of the proposals which have been made are shown in Figure 3.

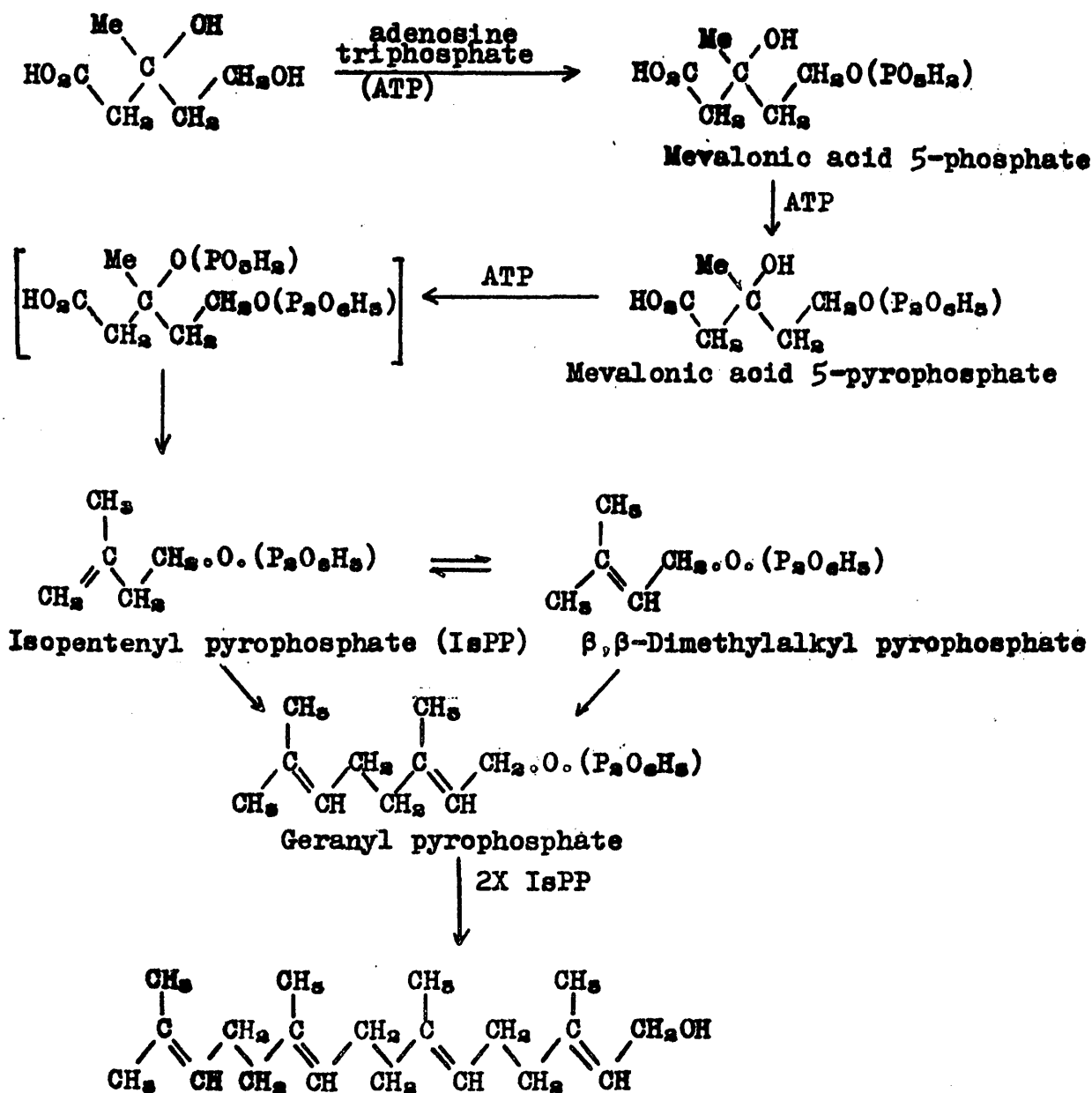


Figure 2. Geranyl geraniol from mevalonic acid.

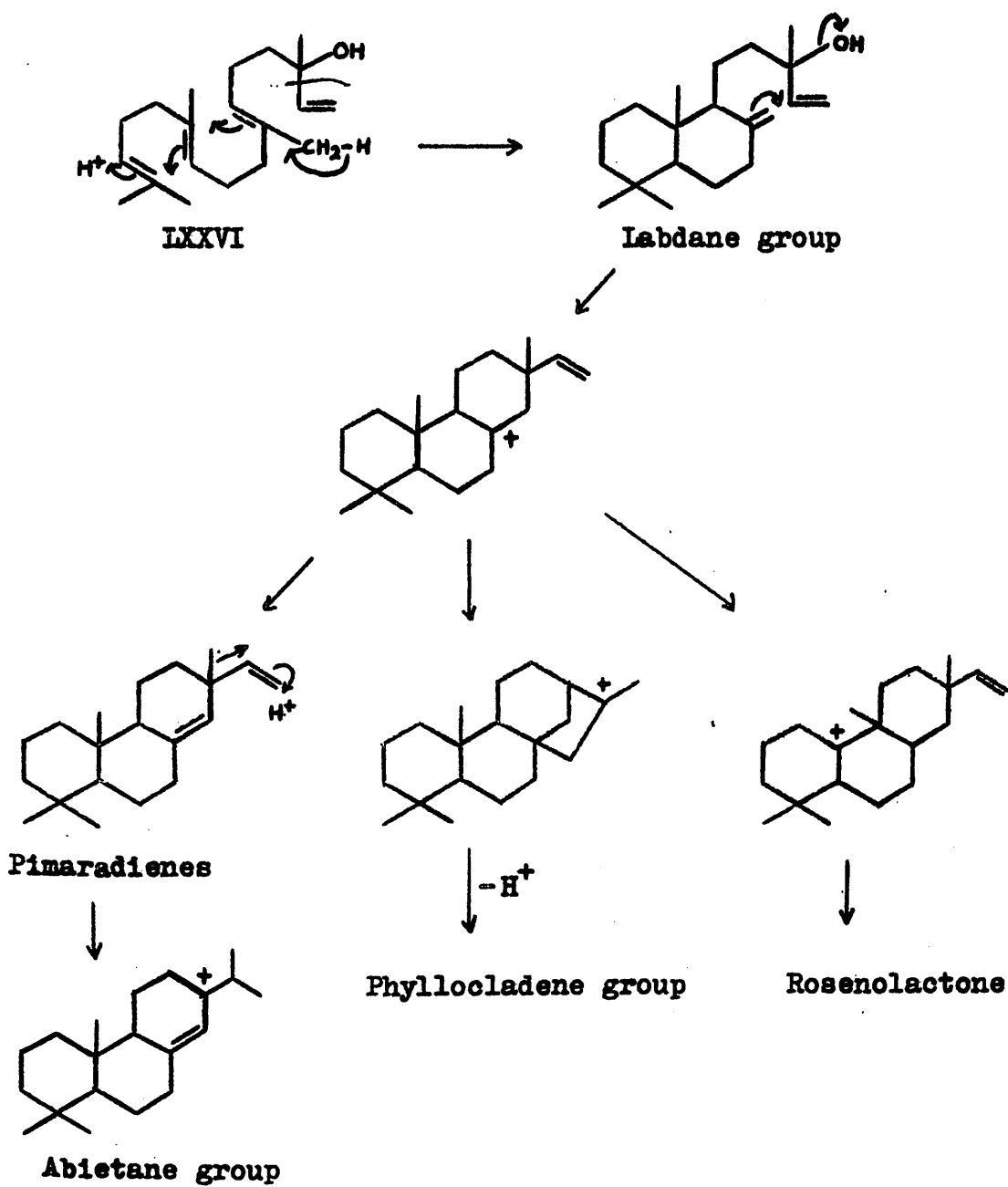
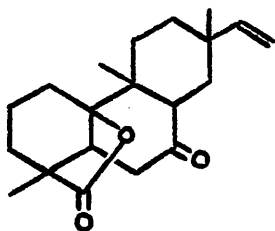


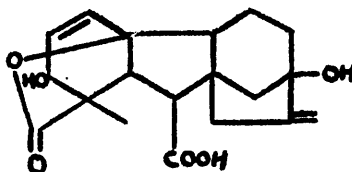
Figure 3.

Although little experimental evidence exists at present to confirm these predictions, it is encouraging that work carried out on rosenolactone (LXXX)^{55,56,57} and gibberellic acid (LXXXI)^{55,58} has shown that these compounds have the same distribution of carbon atoms as predicted by the biogenetic isoprene rule.

In the biosynthesis of diterpenes, the initiating cationoid entity is usually a proton, but exceptions have been found e.g. diterpenes hydroxylated in ring A. A number of these are now known and it is noteworthy that nearly all of them have the oxygen function at C₃, which is to be expected on biogenetic grounds.



LXXX



LXXXI

T H E O R E T I C A L
=====

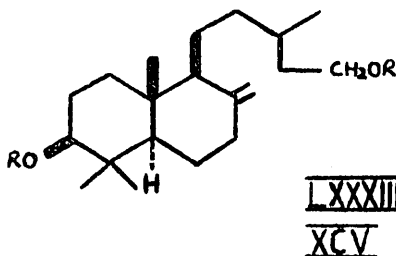
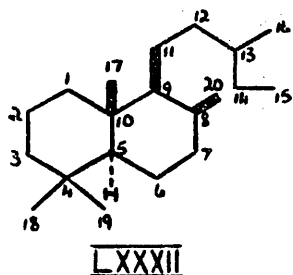
Compounds Isolated from the Bark of Araucaria Imbricata.

The Chilean pine tree, Araucaria imbricata or Araucaria araucana, known in Britain as the 'Monkey Puzzle' tree, occurs in a comparatively restricted area in Chile and south-west Argentina. Introduced into Britain about 150 years ago, it is grown mainly for ornamental purposes although many have been planted in the open countryside.

Extraction of the powdered bark gave a gummy extract which was separated into neutral and acidic fractions. The neutral one afforded ceryl alcohol, β -sitosterol, and a new diterpene diol (LXXXIII). A diterpene aldehyde (CXIV), related to the diol, has also been shown to be present. The acidic fraction contains the new hydroxy-acid (CXVI), another diterpene related to the diol.

The Structure Determination of the New Diol.

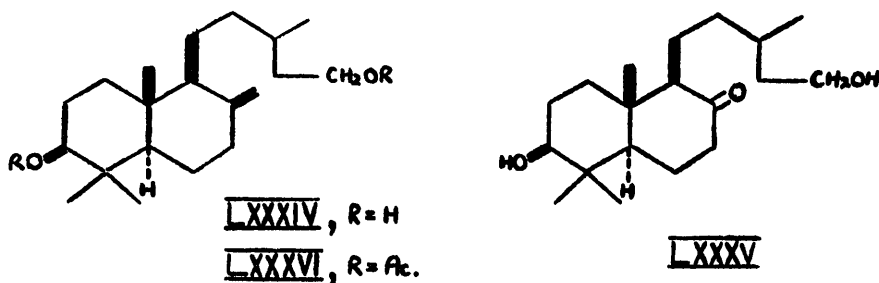
The diol has been shown to be a derivative of labdane (LXXXII), following the nomenclature used by Cocker and Halsall,³⁸ and has the structure (LXXXIII) i.e. labd-8(20)-en-3 β ,15-diol.



Infrared absorption indicated the presence of hydroxyl (bands at 3635 cm.^{-1} and 3440 cm.^{-1}), vinylidene double bond (bands at 1640 cm.^{-1} , 890 cm.^{-1}), and gem-dimethyl (doublet at 1365 cm.^{-1} , 1385 cm.^{-1}), although the latter absorption was not always resolved.

Ultraviolet absorption showed at 2060 $\text{m}\mu$ ($\epsilon_{\text{max.}} = 5000$), confirming the presence of the double bond.

Hydrogenation of the diol gave a saturated dihydrodiol (LXXXIV) with uptake of one mole of hydrogen, while ozonolysis afforded a six-ring ketone (LXXXV) (infrared absorption at 1695 cm.^{-1}) and formaldehyde which proved the double bond to be exocyclic.

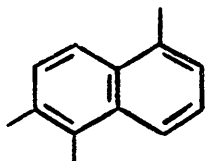


From a consideration of the analytical data for the natural diol and several of its crystalline derivatives, a molecular formula of $C_{20}H_{36}O_2$ was proposed which required the molecule to be bicyclic allowing for the presence of one double bond. Active hydrogen determination showed the presence of two such atoms which, considering the infrared evidence, accounted for both of the oxygen atoms as hydroxyl groups. Molecular weight estimation by the Rast method indicated a mass of approximately 320 which is well within the range of experimental error for the proposed formula and, later, mass spectroscopy showed the parent molecular ion to have mass 308 which agrees entirely with $C_{20}H_{36}O_2$.

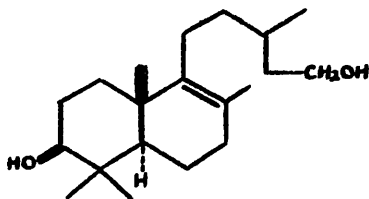
Formation of the acetate, benzoate, and other esters of the diol and its reduction product was

accomplished under very mild conditions, but these diesters were all, with one exception, oily products, the exception being the diacetate (LXXXVI) of the dihydrodiol. The ease of formation and subsequent hydrolysis of these esters, however, pointed to the absence of a tertiary hydroxyl group.

Dehydrogenation of the diol with palladium/charcoal catalyst gave an oily product which showed absorption in the ultraviolet consistent with a naphthalene compound (absorption at 2290Å), and the same degradation carried out using selenium afforded a trimethylnaphthalene, identified as the 1,2,5-trimethyl isomer (II) by formation of the picrate and 1,3,5-trinitrobenzene adduct. The melting point of these derivatives showed no depression when mixed with authentic samples kindly supplied by Dr. K. Overton. The infrared spectra were also identical.



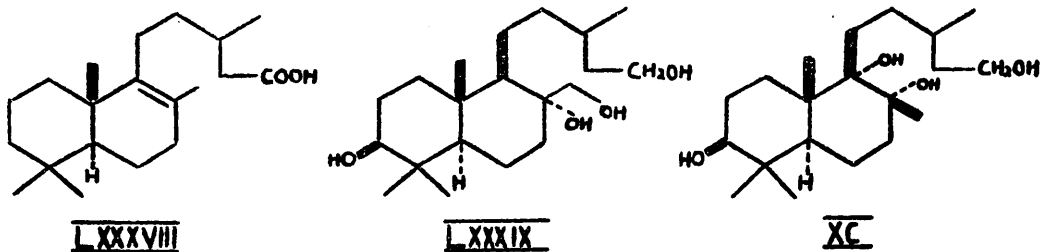
II



LXXXVII

The isolation of 1,2,5-trimethylnaphthalene suggested that the diol was related to the group of bicyclic diterpenes typified by sclareol (X) and manoöl (XV) and that its carbon skeleton was like that of labdane (LXXXII).

When the diol was treated with acid in methanol, it furnished an isomeric diol (LXXXVII) which did not show any absorption in the infrared indicative of a trisubstituted double bond or a vinylidene group. Ultraviolet absorption showed at 2100Å ($\epsilon_{\text{max.}} = 5,500$). The isomer had accordingly a tetrasubstituted double bond. Hydrogenation of the isomer supported this conclusion since only one product was isolated and the change in molecular rotation (-119°) was very similar to that found (-112°) when labd-8-en-15-oic acid (LXXXVIII) was hydrogenated.³⁵



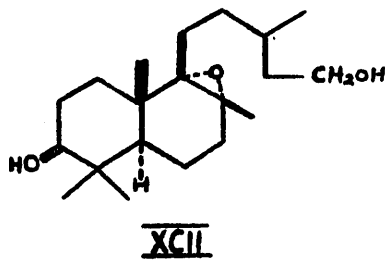
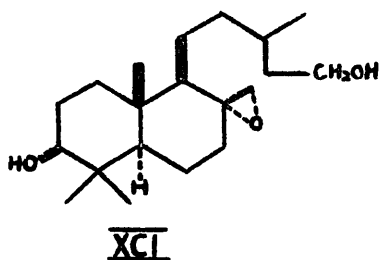
The product obtained from hydrogenation of the isomer was the same as that from the natural diol,

proving that no skeletal rearrangement had occurred during the isomerisation. On the basis of the labdane structure, the double bond would therefore require to be exocyclic at C₈; also the configuration of the side chain at C₉ must be β , and the methyl group at C₈ in the saturated diol must also be β -orientated if hydrogenation occurred at the less hindered (α) face of the molecule. Although only one product was isolated from the hydrogenation of the natural diol, evidence for the formation of the other isomer (C₈, β H) was obtained. Occasionally hydrogenation in ethyl acetate afforded a saturated product which melted over a wide range of temperature. Attempts at separating the saturated isomers from this mixture proved fruitless.

On treatment with osmium tetroxide in dioxan, the diol afforded the tetrol (LXXXIX) in medium yield and the glycol, when reacted with periodic acid, gave the norketone (LXXXV) (which had been obtained by ozonolysis of the diol) and formaldehyde. Similar treatment of the isomeric diol (LXXXVII) with osmium tetroxide yielded the dihydroxy derivative (XC). The stereochemistry of the addition of the hydroxyl groups

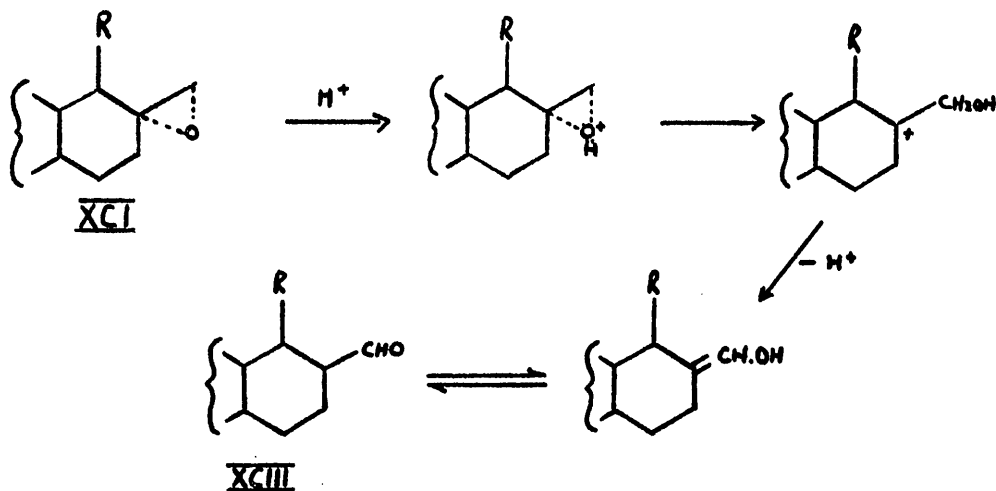
in these reactions is envisaged as taking place from the under (α) side of the molecule, but there is evidence in the literature of a similar addition leading to the opposite stereochemistry.⁵⁹ Splitting of the glycol system in the isomeric tetrol was not attempted due to lack of material.

The diol (LXXXIII) and the isomeric diol (LXXXVII) both furnished crystalline oxides on treatment with excess of monopero-phthalic acid in ether at 0° and the stereochemistry of the oxides is considered to be as shown in structures (XCI) and (XCII) respectively on the basis of the argument developed for the osmylation products. The oxide (XCI) of the natural diol was



formed in good yield, and it was hoped to convert the oxide into the norketone (LXXXV) by hydration to the glycol (LXXXIX) and subsequent cleavage with periodic acid. The norketone (LXXXV) was sought in quantity as

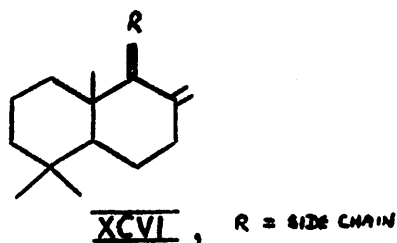
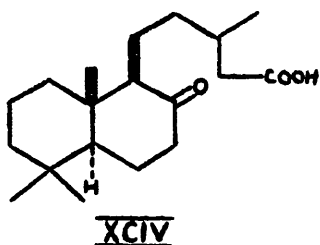
dehydrogenation with selenium should give a naphthol in which the hydroxyl group would mark the position of the oxo-function in the norketone and thereby reveal the location of the exocyclic double bond. However, attempts to hydrate the oxide led to rearrangement with formation of an intractable aldehyde (ν_{max} 1724 cm.^{-1}) presumably via the sequence (XCI) \rightarrow (XCIII).



A close similarity between the chemistry of the new diol and labdanolic acid had now become apparent and this prompted us to compare the molecular rotational differences of several compounds in the diol series with

the corresponding compounds in the labdanolic acid series. The compounds compared are those resulting from ozonolysis, isomerisation, reduction and osmylation and are shown in Table I.

The very close agreement in sign and magnitude between the pairs of values is sufficient proof for the positioning of the exocyclic group at C₈, as suspected, and it follows from this that the configuration of the side chain, and the methyl group in the reduction product, is the same as in labdanolic acid i.e. β -oriented. Cocker and Halsall¹⁴ showed that the side chain in labdanolic acid had the stable equatorial configuration by treating the keto-acid (XCIV) with alkali, when no isomerisation occurred, and they concluded that the compound could not have the axial configuration. Ozonolysis of the oily diacetate (XCV)



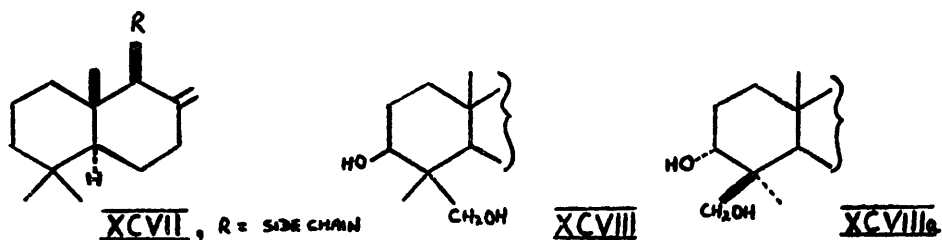
of the natural diol followed by alkaline hydrolysis, gave

Table I

			<u>ΔM_D</u>
Labd-8(20)-en-15-oic acid	$\xrightarrow{O_3}$	20-nor-8-oxo-labdan-15-oic acid	-200°
Diol (LXXXIII)	$\xrightarrow{O_3}$	norketone (LXXXV)	-209°
Labd-8(20)-en-15-oic acid	$\xrightarrow{H^+}$	Labd-8-en-15-oic acid	+136°
Diol (LXXXIII)	$\xrightarrow{H^+}$	isomer (LXXXVII)	+154°
Labd-8-en-15-oic acid	$\xrightarrow{H_2}$	Labdan-15-oic acid	-112°
Isomeric diol (LXXXVII)	$\xrightarrow{H_2}$	dihydrodiol (LXXXIV)	-119°
Labd-8(20)-ene-15-ol	$\xrightarrow{OsO_4}$	Labd-8 α ,15,20-triol	-172°
Diol (LXXXIII)	$\xrightarrow{OsO_4}$	tetrol (LXXXIX)	-150°
Labd-8-en-15-oic acid	$\xrightarrow{OsO_4}$	8,9-Dihydroxylabdan-15-oic acid	-218°
Isomeric diol (LXXXVII)	$\xrightarrow{OsO_4}$	tetrol (XC)	-229°

the same norketone (LXXXV) as had been obtained by ozonolysis of the diol itself. This constitutes a similar proof of the equatorial side chain in the new diol. A partial structure (XCVI) was therefore postulated.

The stereochemistry of the ring junctions in labdanolic and eperuic acids was proved conclusively by Djerassi and Marshall⁴⁵ to be as in structures (LI) and (LXVI) respectively by optical rotatory dispersion studies. The norketone (LXXXV) displayed a very strong negative Cotton effect curve (amplitude -132), and comparison with the curves obtained from the corresponding ketones in the labdanolic and eperuic acid series showed that the new diol has the normal trans ring junction. The partial structure now becomes (XCVII).



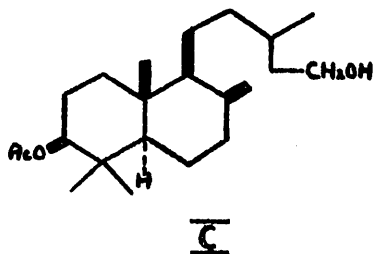
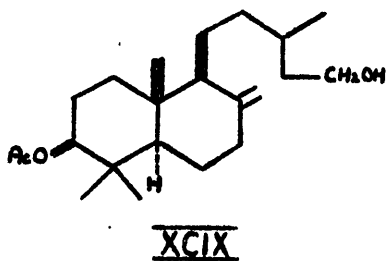
At this stage little was known about the

nature and position of the two hydroxyl groups, and, assuming the presence of the normal side chain common to all members of this group of diterpenes, there remained only this problem of the hydroxyl groups to be elucidated. Absence of a tertiary hydroxyl group had already been demonstrated, and the failure to react with periodic acid eliminated the possibility of a vicinal glycol system. The diol did not condense with acetone, acetaldehyde or benzaldehyde in acid media, which precluded the 1,3 diol structure as in (XCVIII) except the trans-diaxial system (XCVIIIa) since this conformation would not form such condensation products.

Oxidative degradations, using a variety of oxidising agents, were carried out on the diol, the isomer, and the saturated diol in an attempt to obtain some evidence, which would have settled the location of either or both of the hydroxyl functions. This approach proved to be abortive, since none of the oxidation products could be crystallised and derivatives of the carbonyl and acidic products remained as intractable gums. However, the infrared spectra of

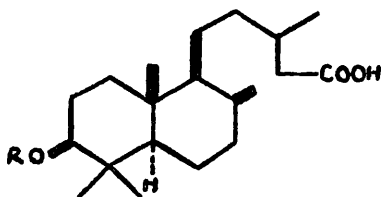
these products showed that both hydroxyl groups had been oxidised and thereby supplied evidence for postulating the existence of one secondary and one primary function. Moreover, the ultraviolet spectra of the products resulting from the oxidation of the unsaturated diols showed no absorption indicative of α - β unsaturated carbonyl conjugation which suggested that C₇ and C₉ were not hydroxylated.

Partial hydrolysis of the diol diacetate (XCV) using sodium carbonate in methanol afforded the monoacetate (XCIX) in rather poor yield, and this allowed the preferential oxidation of one of the hydroxyl groups to be carried out. Similar treatment of the dihydro diol diacetate (LXXXVI) gave the monoacetate (C) in 50% yield. It was observed during this hydrolysis that dilute aqueous-methanolic sodium carbonate solution at room temperature hydrolysed both acetate groups, although only one equivalent of carbonate was added. This demonstrated the freedom of the ester groups from steric hindrance, a fact also shown by the ease of esterification.

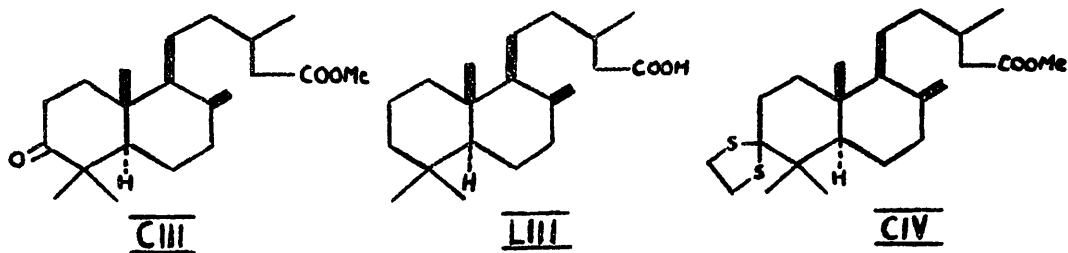


We assumed the presence of one primary and one secondary hydroxyl group and considered the possible sites for these functions. A primary group could be situated on carbons, 15, 16, 17, 18, 19 but three of these, C₁₇, C₁₈, and C₁₉, would be expected to show some degree of steric hindrance. C₁₅ seemed the most likely of the remaining pair since this position is very often oxygenated in this type of diterpene whereas C₁₆ is not. We further assumed that the hydroxyl group liberated during the above partial hydrolysis would be the primary group.

Oxidation of the dihydrodiolmonoacetate (C) with Kiliani mixture afforded an acetoxy-acid (CI), which, on hydrolysis with sodium hydroxide gave the hydroxy-acid (CII). The formation of this hydroxy-acid provided proof of the presence of a primary hydroxyl group, since no



loss of carbon had occurred during the oxidation reaction. Further oxidation of the methyl ester of the hydroxy-acid with chromium trioxide in pyridine gave the non-crystalline keto-ester (CIII) (infrared absorption at 1740 cm.^{-1} , 1720 cm.^{-1}), which was then reduced under Wolff-Kishner conditions to dihydro-cativic acid (LIII). The latter acid was also prepared by desulphurisation of the thioketal (CIV), prepared by



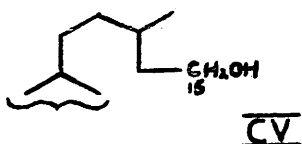
the reaction of ethanedithiol on the ketone (CIII). The acid could not be crystallised nor could its methyl ester, although both are known crystalline compounds, m.p. $80-82^\circ$ and $43-44^\circ$ respectively. The failure to

crystallise may be explained by the fact that only a very small amount of product (30 mgs.) was available and this may not have been sufficiently pure. The infrared spectrum of the acid (LIII) was identical with that obtained from a sample of dihydrocavitic acid, kindly supplied by Dr. T. C. Halsall, The production of the keto ester (CIII) confirmed the presence of a secondary hydroxyl group.

Although this conversion to a known compound had not led to the isolation of the expected product in a pure state, it did furnish a considerable amount of information and in an effort to confirm the positioning of the primary hydroxyl group at C₁₅, several compounds in the series were analysed in a mass spectrometer. We are very grateful to Dr. R. I. Reed and (Miss) F. M. Tabrizi for carrying out these analyses and for their very helpful interpretation and discussion of the results.

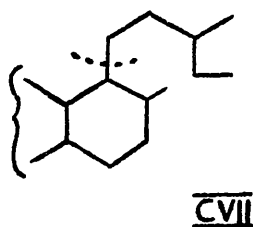
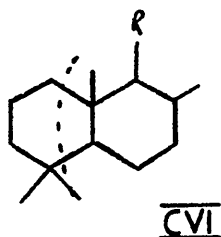
The diol (LXXXIII), the isomer (LXXXVII), the dihydrodiol (LXXXIV) and the norketone (LXXXV) all exhibit spectra characteristic of those diterpenes which

possess two rings and a side-chain.^{60,61} The molecular weights of the ions correspond to $C_{20}H_{36}O_2$ which agrees with the chemical analysis. The parent molecular ion of all of these compounds degrades readily with the elimination of thirty-one mass units, which, since it is a single entity, must correspond to a methoxyl or a hydroxymethylene group. Since both oxygen atoms have been accounted for as hydroxyl groups, then this elimination is due to the loss of CH_2OH i.e. the primary hydroxyl group. The next important elimination is one of forty-five units which must contain oxygen and have the formula C_2H_5O , and, since a hydroxymethylene group is necessarily terminal, this fragmentation favours a partial structure of $CH_2 \cdot CH_2OH$. Moreover, this elimination is so facile that it suggests that the $CH_2 \cdot CH_2OH$ group is joined at a branch in the carbon chain. By consideration of the normal type of side chain, it can easily be seen that only a structure with a C_{15} hydroxyl group (CV) could give this fragmentation pattern.



With the primary hydroxyl group fixed at position 15, the number of sites possible for the placing of the secondary group can be reduced to three, from considerations of chemical evidence. A C_{14} hydroxyl would constitute a vicinal glycol system and this has been shown to be absent; the carboxyl group at C_{16} in the hydroxy-acid (CII) would be expected to form a lactone with a hydroxyl group at C_{11} or C_{12} , but no sign of lactone formation was evident. It has already been suggested (p. 43) that C_7 and C_{11} are not hydroxylated. Also treatment of the tetrol (LXXXIX) with periodic acid did not cause glycol splitting between C_7 and C_8 thus eliminating C_7 . A hydroxyl group at C_8 would be expected to dehydrate readily in the norketone (LXXXV) (β -hydroxy-ketone) but this compound proved quite stable. This leaves positions 1, 2 and 3.

Previous studies^{62, 63, 64} in mass spectrometry of this class of compound have indicated that a common form of fission is as shown in (CVI) with a possible single



hydrogen migration in either direction. A very abundant ion $M/e = 100$ corresponds with this fragmentation which must have the formula $C_6H_{12}O$. Therefore a hydroxyl group is present in ring A, agreeing with chemical evidence.

A further common form of fission in these bicyclic compounds results in the elimination of the entire side-chain as in (CVII). This elimination shows abundantly since, unfortunately, it has the same molecular weight as the fragment obtained from ring A. Therefore, in order that the position of the oxygen atom in ring A might be examined without this complication, the spectrum of the keto-ester (CIII) was taken. In all of the other spectra, a very ready elimination of methyl is apparent from the gem-dimethyl group and the methyl group at C_{10} . In the keto-ester, however, this loss is much reduced, as would be expected if there was an adjacent keto-group. This would exclude position 2. Position 1 is unlikely on biogenetic grounds, and also from the fact that these compounds all show an abundant parent molecular ion; a compound with a C_1 -hydroxyl group would be a crowded molecule and would be unlikely to show this ion. Mass spectral examination therefore favours a 3-hydroxyl group.

An optical rotatory dispersion curve of the keto-ester (CIII) has completely ruled out the possibility of the secondary hydroxyl group being situated at C_2 , since such a 2-ketone would show a large positive amplitude ^{ee} (about +70). The curve obtained from the keto-ester shows a weakly negative amplitude (-15) and is reproduced in Fig. 4 (curve A).

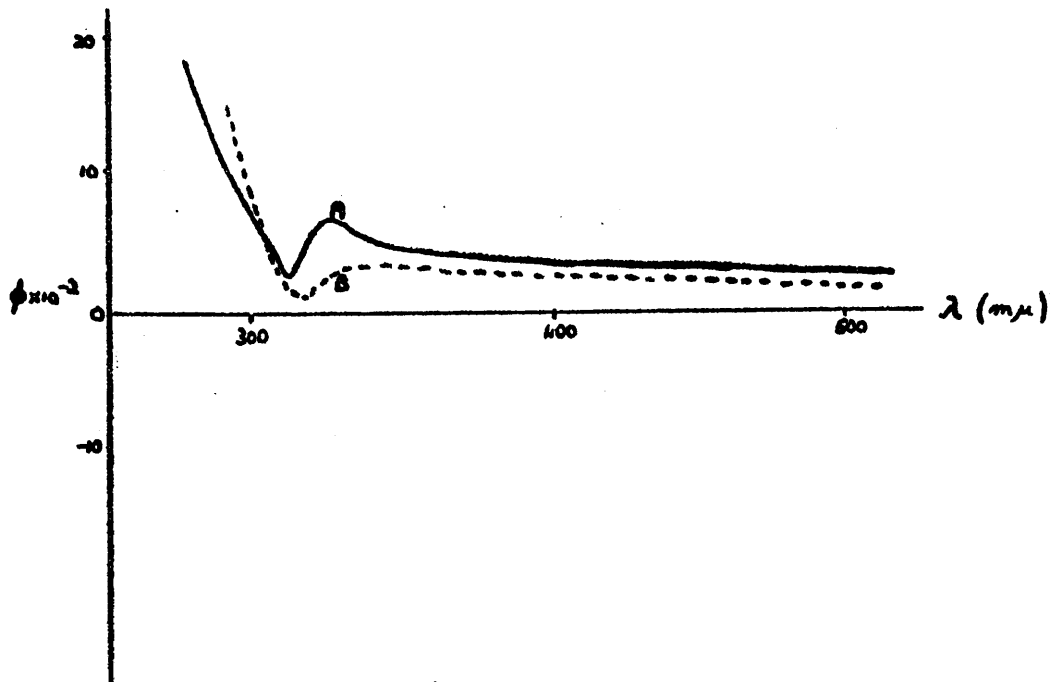
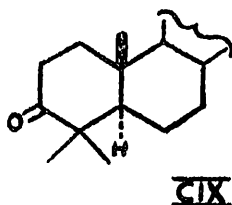
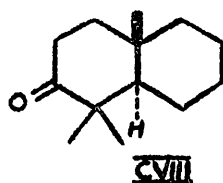
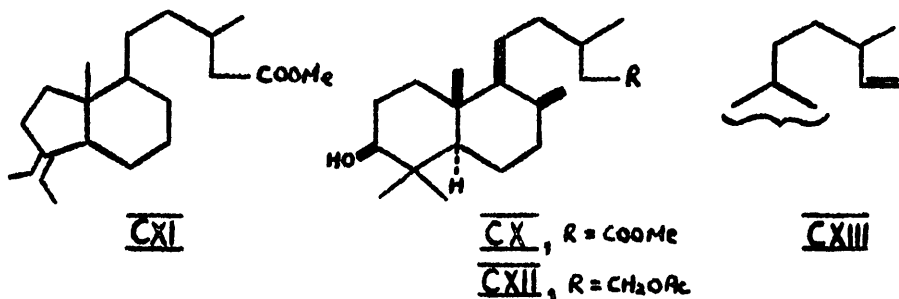


Fig. 4

This may be compared with the curve obtained by Djerassi and Marshall⁶⁶ for 4,4,9-trimethyldecalone-3 (CVIII) (on enantiomer) which shows a similar weakly negative curve, amplitude -11. The curve from the keto-ester can also be compared with that of lanostan-3-one (CIX) (curve B in Fig. 4), to which it shows a strong resemblance.



Although the optical rotatory dispersion measurements have ruled out position 2 as being oxygenated, C₁, though unlikely for the reasons already stated, cannot be ignored since 1-ketones give dispersion curves similar to the 3-ketones. For this reason the hydroxy-methyl ester (CX) was treated with phosphorous pentachloride in benzene in an attempt to bring about the typical ring contraction undergone by 3 β -hydroxy-triterpenes. Such a rearrangement should lead to the ester (CXI) which on ozonolysis would yield acetone.

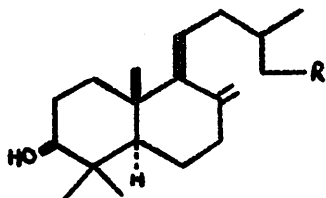


The product from the dehydration reaction showed strong absorption in the infrared at 1025 cm.⁻¹ (broad band), attributed to the presence of phosphorus. This was verified by elementary analysis. The phosphorus was presumed to be present as a phosphate ester but attempted hydrolysis with sodium hydroxide was unsuccessful. (Phosphate esters are known to be resistant to hydrolysis⁶⁷). The dehydration reaction was repeated using the monoacetate (CXII) which was prepared by treating the dihydrodiol with one equivalent of acetic anhydride in pyridine. Phosphorus oxychloride was substituted for phosphorus pentachloride and the reaction was conducted in pyridine at room temperature. Ozonolysis of the dehydration product afforded acetone which was identified by thin layer chromatography of the 2,4-dinitrophenylhydrazone. A second volatile product was shown to be formaldehyde which presumably originated from the

olefin (CXIII) formed by deacetylation of the acetate. The hydroxyl group must therefore be in the 3-position and have the equatorial configuration.

The structure and stereochemistry of the diol are therefore established as in structure (LXXXIII); the only feature of the stereochemistry not elucidated is that at C₁₃.

The structure of the aldehyde shown to be present in the non-saponifiable fraction must be as in (CXIV), since lithium aluminium hydride reduction afforded the new diol. Similar reduction of the ester (CXV), obtained from the methylation product of the acidic fraction, also gave the diol. The natural acid, characterised as its methyl ester, must therefore have structure (CXVI).



CXIV, R = CHO

CXV, R = COOMe

CXVI, R = COOH

EXPERIMENTAL
=====

All melting points are uncorrected. The specific rotations were determined in a one decimetre tube at room temperature using the solvents stated. The ultra-violet spectra were determined in ethanol solution and the infrared spectra were recorded as nujol mulls unless otherwise stated. Spence's grade H alumina was used for chromatography, and neutral alumina was prepared by shaking this alumina in ethyl acetate for 4 hr. and reactivating it at 100° for 24 hr. Light petroleum refers to the fraction of b.p. 60-80°.

The mass spectra were determined using a Metropolitan-Vickers M.S.2 Mass Spectrometer.

Extraction of Bark with Light Petroleum.

The bark (7 lbs.) was powdered and extracted continuously for 20 hours with light petroleum (25 litres). The bulk of the solvent was evaporated and the solution filtered. Complete evaporation of solvent then afforded a dark green gum (132 gms.).

Separation of the Non-Saponifiable Fraction.

The extract was hydrolysed with 10% methanolic potassium hydroxide; as much methanol as possible was distilled off and the mixture poured into a large excess of water, when a small amount of solid matter was precipitated. This material was not filtered. Ether extraction of the mixture, followed by drying (anhydrous sodium sulphate) and solvent removal afforded the non-saponifiable fraction as a waxy solid (35 gms.). Emulsions which formed during the ether extraction were broken by adding small amounts of ethanol.

The non-saponifiable material was dissolved in light petroleum/benzene (1:1) and adsorbed on alumina (1000 gms.) Elution of the column proceeded as follows, the solvents used being light petroleum, benzene, ether and methanol,

in that order:-

- (1) All fractions up to solvent 100% ether yielded small amounts of sweet smelling gum. The first fractions also probably contained hydrocarbon material (6 gms.).
- (2) 1% methanol in ether; traces of gum.
- (3) 3% methanol in ether; 27 gms. of waxy material.

The fractions from (3) yielded a crystalline solid (9.6 gms.) from chloroform/methanol, identified as ceryl alcohol, m.p. and mixed m.p. 79° (Found: C, 81.45; H, 14.2. Calc. for $C_{26}H_{54}O$: C, 81.6; H, 14.2%). The mother liquors contained gummy material which yielded β -sitosterol (1.2 g.) as plates from ethanol, m.p. and mixed m.p. 139° , $[\alpha]_D + 36.4^{\circ}$ in chloroform (c, 1.0), infrared spectrum identical with that of an authentic specimen.

After removal of ceryl alcohol and β -sitosterol, there remained 16 gms. of gummy material which was again chromatographed on alumina (500 g.) and eluted as follows:-

- (1) Solvents up to 0.5% methanol in ether gave traces of the same sweet-smelling gum; total yield 4 gms.
- (2) 1% methanol in ether; 10 gms. gum.

(3) 2% methanol in ether; .1 gms. gum.

(4) 5% methanol in ether; 1.1 gms. gum.

The infrared spectrum of fraction (2) showed absorption corresponding to hydroxyl, vinylidene double bond and carbonyl functions. Girard separation of the carbonyl material was effected as follows.

Girard Separation of Carbonyl Material.

The carbonyl-containing material (10 gms.) was refluxed for 1 hr. in absolute ethanol (80 ml.) and acetic acid (10 ml.) containing Girard T reagent (7 gms.) i.e. trimethylaminoacetohydrazide chloride. Glycol (80 ml.) was then added and the mixture was extracted with dry ether. The ethereal solution was washed and dried (anhydrous sodium sulphate) and, on evaporation of the solvent, gave the non-carbonyl material (7 gms.). The glycol solution was then poured into an excess of water which was made 1N with hydrochloric acid and left for 1 hr. Isolation through ether gave the carbonyl material (3 gms.).

The non-carbonyl fraction (7 gms.) gave 1ab_d-8(20)-en-3 β ,15-diol (LXXXIII) (1.2 g.) as blades (from light petroleum) m.p. 114°, $[\alpha]_D + 29^\circ$ in chloroform (c, 0.5).

(Found: C, 78.0; H, 11.6. $C_{20}H_{36}O_2$ requires : C, 77.9; H, 11.8%). Light absorption max. (in EtOH) at 2060\AA ($\epsilon = 5000$). Infrared absorption bands at 3635 cm.^{-1} , 3440 cm.^{-1} , 1643 cm.^{-1} , 890 cm.^{-1}

The following procedure was adopted after subsequent extractions to minimise the time required to obtain the crystalline diol:-

The non-saponifiable material was dissolved in hot ethyl acetate, and allowed to stand overnight. The ceryl alcohol, which precipitated, was filtered off and the residue, after evaporation of ethyl acetate, was adsorbed on alumina from benzene. The column was immediately eluted with benzene/ether (1:9, 3 litres), after which followed careful elution with ether and ether/methanol mixtures. In most cases β -sitosterol was eluted with ether and the diol with ether-methanol (99:1), although in some cases the compounds overlapped. The yield of diol varied, as did the total extract, with the locality of the bark on the tree. The initial extractions, carried out on bark from the base of the tree, yielded approximately 1.2 gms.

pure diol but this decreased to 0.35 g. from bark taken from the middle and upper trunk.

The carbonyl material obtained from many extractions was isolated as above and chromatographed several times on alumina to give a fraction (3.5 gms.) which showed absorption in the infrared at 3400 cm.^{-1} (hydroxyl); 2730 cm.^{-1} and 1715 cm.^{-1} (aldehyde), 1645 cm.^{-1} and 890 cm.^{-1} (double bond). Attempts to form carbonyl derivatives gave no crystalline products. Further chromatography gave a fraction (1 gm.) which, on lithium aluminium hydride reduction in ether, afforded labd-8(20)-en-3 β ,15-diol (LXXXIII) in 40% yield. The aldehyde could not be isolated pure enough to establish its physical constants.

Separation of the Acidic Fraction.

The basic solutions, after extraction of the non-saponifiable fraction, were acidified with hydrochloric acid. The precipitated acid material was extracted with ether to give the acidic fraction (500 gms. from many extractions). ν_{max} broad peak at 3300 cm.^{-1} , 1710 cm.^{-1} (carboxyl) 1645 cm.^{-1} and 890 cm.^{-1} (vinylidene double bond).

Methylation of a sample of this acid material (21 gms.) with diazomethane in ether gave the methyl ester which was chromatographed on neutral alumina (500 g.) to give methyl 3 β -hydroxyabd-8(20)-en-15-oate (CXVI) b.p. 220° (oil bath)/0.5 mm. n_D 1.5073, $[\alpha]_D$ + 52° (c, 0.5).
 (Found: C, H, C₂₁H₃₆O₅ requires :
 C, 74.95; H, 10.78%). ν_{\max} . 3500 cm.⁻¹ (hydroxyl), 1735 cm.⁻¹ (ester), 1650 cm.⁻¹ and 890 cm.⁻¹ (vinylidene double bond).

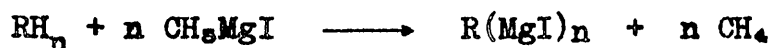
Reduction of Methyl 3 β -Hydroxyabd-8(20)-en-15-oate.

The methyl ester (228 mgs.) was refluxed with lithium aluminium hydride (100 mgs.) in ether for 1 hr. The excess hydride was destroyed with crushed ice and the ether layer was washed and dried (anhydrous sodium sulphate). Evaporation of the solvent gave abd-8(20)-en-3 β ,15-diol (LXXXIII) m.p. 114° (95% yield).

Active Hydrogen Determination.

The method used for this determination was that of Tschugaeff and Zerevitinoff, Ber., 1907, 40, 2027, as

described in "Laboratory Methods of Organic Chemistry", 24th Edition, Gattermann, p.84. Labd-8(20)-en-3 β ,15-diol was reacted with methylmagnesium iodide and the volume of methane gas produced was related to the number of active hydrogens in the molecule by the equation:-



where n is the number of active hydrogens per mol.

According to this equation, one mole of substance liberates n x 22.4 litres of methane. Therefore a grams of substance ($\frac{a}{M}$ mol) liberate $\frac{n \cdot 22400 \cdot a}{M}$ ml. of methane

$$\therefore n = \frac{M \cdot V}{22400a} \quad \text{where V is volume produced at N.T.P.}$$

Weight of diol used = .04 g.

Vol. of methane produced (N.T.P.) = 5.6 ml.

$$\therefore n = \frac{308 \times 5.6}{22400 \times .04}$$

$$= 1.9$$

The limits of error are 5-10%. Therefore the number of active hydrogens per mol of diol is two.

Reaction of Labd-8(20)-en-3 β ,15-diol with Periodic acid.

Labd-8(20)-en-3 β ,15-diol (100 mgs.) was dissolved in methanol (5 ml.) and periodic acid (150 mgs.), dissolved in a small amount of water, was added. The solution was left at room temperature for three days, then poured into excess of water and extracted with ether. The ether extract was dried (anhydrous sodium sulphate) and the solvent evaporated. Crystallisation from petrol gave starting material in almost quantitative yield, m.p. and mixed m.p. 114°.

Dehydrogenation of Labd-8(20)-en-3 β ,15-diol (LXXXIII) with Palladium/Charcoal.

Labd-8(20)-en-3 β ,15-diol (1 gm.) was heated with palladium/charcoal catalyst (5%; 1 gm.) in a stream of carbon dioxide at 250° for one hour. The gas evolved amounted to 160 ml. (calculated for complete dehydrogenation, approximately 400 ml.). Extraction of the residue with ether afforded a yellow oil (600 mgs.) which showed absorption in the ultraviolet at 2290A° (very low

intensity). After chromatography on alumina (20 g.), attempts to prepare 1,3,5-trinitrobenzene adducts of the naphthalenic fractions gave only coloured gums, which could not be crystallised.

Dehydrogenation of Labd-8(20)-en-3 β ,15-diol with Selenium.

Labd-8(20)-en-3 β ,15-diol (1 gm.) and selenium powder (1 gm.) were well mixed and heated for six hours at 350° (Wood's metal bath). The residue was continuously extracted for one hour to give a yellow oil (350 mgs.), which was chromatographed on alumina (10 g.). Fractions showing absorption in the ultraviolet at around 2300Å were treated with 1,3,5-trinitrobenzene, giving an impure adduct which was shown to be a mixture by thin layer chromatography on silica gel. However, one of the components of this mixture showed the same R_f value as authentic 1,2,5-trimethylnaphthalene adduct. The material was bulked and chromatographed several times on alumina yielding eventually a fraction (20 mgs. oil) which absorbed at 2290Å ($\epsilon = 40,000$), and proved to be 1,2,5-trimethylnaphthalene (II) since it yielded a picrate, orange needles (from ethanol),

m.p. and mixed m.p. 133-136° (literature 136-137°), and a trinitrobenzene adduct, yellow needles (from ethanol), m.p. and mixed m.p. 152-155° (literature 153-156°). Infrared comparison with an authentic sample showed complete identity.

Hydrogenation of Labd-8(20)-en-3 β ,15-diol.

Labd-8(20)-en-3 β ,15-diol (112 mgs.) was dissolved in ethyl acetate (redistilled; 20 ml.) and added to freshly reduced platinum oxide catalyst (120 mgs.) in the same solvent. The mixture was shaken for four hours and then filtered. Evaporation of the solvent afforded a clear gum (114 mgs.) which crystallised on standing.

Crystallisation from light petroleum gave labdan-3 β ,15-diol (LXXXIV) as blades, m.p. 122-124°, $[\alpha]_D + 39^\circ$ (c, 1.0, chloroform) (Found: C, 77.6; H, 12.45. C₂₀H₃₂O₂ requires: C, 77.4; H, 12.3%). The compound showed no absorption in the ultraviolet and no absorption in the infrared at 1640 cm.⁻¹ and 980 cm.⁻¹

Hydrogenation in ethyl acetate usually proceeded in good yield to give one isomer (β -Me at C_6) but in some cases a mixture of isomers was obtained which could not be separated by chromatography. It was found that by using ethanol as solvent, this difficulty did not arise.

Labdan-3 β ,15-diol-3,15-diacetate (LXXXVI).

Labdan-3 β ,15-diol (200 mgs.) was dissolved in pyridine (5 ml.) and acetic anhydride (1 ml.) was added. The solution was heated on the steam bath for one hour, then poured into water. Extraction with ether afforded a clear gum which was filtered through alumina in benzene to give labdan-3 β ,15-diol-3,15-diacetate crystallised as needles (from aqueous methanol), m.p. 78°, $[\alpha]_D + 24^\circ$ (c, 1.0 in chloroform) (Found: C, 71.55; H, 10.7 C₂₄H₄₂O₄ · $\frac{1}{2}$ CH₃OH requires C, 71.7; H, 10.7%). The compound showed a broad peak at 1725 cm.⁻¹ (OAc), but hydroxyl absorption was absent.

Acetylation of Labd-8(20)-en-3 β ,15-diol.

Labd-8(20)-en-3 β ,15-diol (100 mgs.) was dissolved in pyridine (5 ml.) and acetic anhydride (1 ml.) was

added. After heating on the steam bath for one hour, the solution was poured into water and extracted with ether to give labd-8(20)-en-3 β ,15-diol-3,15-diacetate (XCV) which could not be crystallised. Hydrolysis of the diacetate with sodium hydroxide gave the diol in quantitative yield.

The preparation of the following esters, using, in most cases, a recipe similar to the above acetylation procedure did not give crystalline derivatives, although in each case infrared evidence showed the diesters to have formed:- (a) benzoate, (b) 3,5-dinitrobenzoate, (c) p-toluene sulphonate, (d) α -naphthylisocyanate.

Ozonolysis of Labd-8(20)-en-3 β ,15-diol.

Labd-8(20)-en-3 β ,15-diol (150 mgs.) was dissolved in chloroform (25 ml.) and ozone was bubbled through the solution for 30 minutes at -70° . When the solution had attained room temperature, acetic acid (4 ml.) was added and the solution was stirred while zinc dust (400 mgs.) was slowly added. After stirring for a further 30 minutes, the solution was filtered and the zinc was thoroughly washed with chloroform. The combined

chloroform solutions were washed with water, after which evaporation of the chloroform afforded a clear gum (80 mgs.). Chromatography on alumina (4 gms.) gave 20-nor-8-oxolabdan-3 β ,15-diol (LXXXV) as plates (from light petroleum), m.p. 107-107.5°, $[\alpha]_D = 39.7^\circ$ (c, 0.5 in chloroform) (Found: C, 73.3; H, 11.2. C₁₉H₃₄O₃ requires, C, 73.5; H, 11.0%). $\nu_{\text{max.}}$ 3320 cm.⁻¹ (hydroxyl), 1695 cm.⁻¹ (ketone).

The aqueous washings were treated with a saturated solution of dimedone and left at room temperature for 24 hr. Filtration of the solid derivative, and crystallisation from ethanol gave formaldehyde dimedone m.p. and mixed m.p. 191°, showing infrared identity with an authentic sample.

Attempts to increase the yield of the ozonolysis product (~ 30%) by conducting the experiment in ethyl acetate as solvent at 0° and by using the oily labd-8(20)-en-3 β ,15-diol-3,15-diacetate (XCV) were unsuccessful.

8 α ,20-epoxylabdan-3 β ,15-diol (XCI).

A solution of labd-8(20)-en-3 β ,15-diol (100 mgs.) in ether (10 ml.) was mixed with monoperphthalic acid in

ether (10 ml; 0.34M) and left at 0° for 72 hr. The mixture was poured on to crushed ice (50 g.), containing ethanol (25 ml.), sodium dithionite (1.5 g.) and N-sodium hydroxide (25 ml.), and the ether was removed at room temperature. The aqueous alkaline phase so obtained was extracted with ether, and the extracts were washed with water, dried (anhydrous sodium sulphate) and evaporated. The oxide crystallised from light petroleum as prisms, m.p. 116-117°, $[\alpha]_D + 8.5^\circ$ (c, 0.5 in chloroform) (Found: C, 74.4; H, 11.5 C₂₀H₃₆O₃ requires, C, 74.0; H, 11.2%). The compound showed no absorption in the infrared at 1645 cm.⁻¹ and 890 cm.⁻¹ (double bond) and was transparent to ultraviolet light.

8 α , 9 α -epoxylabdan-3 β , 15-diol (CXII).

The above procedure was repeated using labd-8-en-3 β , 15-diol (100 mgs.) and afforded a gum which crystallised after several months. Recrystallisation from light petroleum gave 8 α , 9 α -epoxylabdan-3 β , 15-diol as plates m.p. 88°, $[\alpha]_D + 56^\circ$ (c, 0.25 in chloroform). (Found: C, 73.4; H, 10.8. C₂₀H₃₆O₃ requires, C, 74.0; H, 11.2%). The compound showed no selective absorption

in the ultraviolet.

Attempted Acid Hydrolysis of 8 α ,20-Epoxyabdan-3 β ,15-diol (XCI).

8 α ,20-Epoxyabdan-3 β ,15-diol (200 mgs.) in acetone (40 ml.) and water (4 ml.) containing 2N sulphuric acid (0.2 ml.) was left at room temperature for four days. Removal of acetone in vacuo and extraction with ether afforded a gum (190 mgs.) which could not be crystallised.

ν_{max} . 3595 cm.^{-1} , 3460 cm.^{-1} (hydroxyl) and 1720 cm.^{-1} (carbonyl). The product may have been the C₂₀ aldehyde (XCIII) formed by isomerisation of the epoxide.

Isomerisation of Labd-8(20)-en-3 β ,15-diol.

The diol (200 mgs.) in methanol (5 ml.) was heated under reflux with methanolic sulphuric acid (6.7%; 25 ml.) for three hours. The solvent was then reduced to quarter bulk at low temperature in vacuo, after which the mixture was poured into excess of water and extracted with ether. The ethereal solution was dried (anhydrous sodium sulphate) and, on evaporation of the solvent, afforded a clear gum. Crystallisation from

light petroleum gave labd-8-en-3 β ,15-diol (LXXXVII) as blades, m.p. 121°, $[\alpha]_D + 78^\circ$ (c, 0.5 in chloroform) (Found: C, 77.54; H, 11.9. C₂₀H₃₆O₂ requires, C, 77.86; H, 11.76) λ_{max} in ethanol 2100 μ ($\epsilon = 5,500$). There were no bands corresponding to a trisubstituted double bond or a vinylidene group in the infrared.

The diacetate and the dibenzoate of labd-8-en-3 β ,15-diol were prepared in acetic anhydride/pyridine and benzoyl chloride/pyridine respectively but these compounds could not be crystallised.

Hydrogenation of Labd-8-en-3 β ,15-diol (LXXXVII).

Labd-8-en-3 β ,15-diol (50 mgs.) in ethyl acetate (20 ml.) was shaken with freshly reduced platinum oxide catalyst for several hours. No uptake of hydrogen was apparent and, on isolation of the product as before, a quantitative yield of the diol was obtained.

The reaction was repeated using acetic acid as solvent. The solution was shaken for 24 hrs. and then filtered. The solvent was evaporated under high vacuum and water (100 ml.) was added. Ether extraction and isolation of the product in the usual way gave labdan-3 β ,15-

diol, m.p. and mixed m.p. 121-124°, identical in all respects with the hydrogenation product of labd-8(20)-en-3 β ,15-diol.

Reaction of Labd-8(20)-en-3 β ,15-diol (LXXXIII) with Osmium Tetroxide. Method I.

A solution of labd-8(20)-en-3 β ,15-diol (300 mgs.) in pyridine (5 ml.) was added to a solution of osmium tetroxide (300 mgs.; 1.2 equivalents) in ether (4.5 ml.), and the mixture allowed to stand in the dark for six days. Ether (10 ml.) was added and the reaction mixture was refluxed for one hour with excess of lithium aluminium hydride (1 gm.), cooled, and poured on to ice. The mixture was allowed to attain room temperature, and then extracted with ether. The extract was washed with hydrochloric acid (2N), then sodium bicarbonate, and water before drying over anhydrous sodium sulphate. Evaporation of the solvent gave labdan-3 β ,8 α ,15,20-tetrol (LXXXIX) as needles (from light petroleum), m.p. 138-139°, $[\alpha]_D - 17^\circ$ (c, 0.5 in methanol-chloroform) (Found: C, 70.0; H, 11.3. C₂₀H₃₈O₄ requires, C, 70.1; H, 11.2%). The compound was transparent in the

ultraviolet and did not show double bond absorption in the infrared. Overall yield 60 mgs. (< 20%).

Method II.

A solution of labd-8(20)-en-3 β ,15-diol (250 mgs.) in dioxan(15 ml.) was added to a solution of osmium tetroxide (245 mgs.) in dioxan(15 ml.) and left in the dark for five days. Hydrogen sulphide gas was then bubbled through the solution until there was no further precipitate of black osmium sulphide. The solution was washed and dried (anhydrous sodium sulphate) and on evaporation of the solvent gave a clear gum (220 mgs.) which afforded labdan-3 β ,8 α ,15,20-tetrol (LXXXIX) as needles (from light petroleum) m.p. 138-139°. Overall yield 150 mgs. (54%).

Reaction of Labd-8-en-3 β ,15-diol (LXXXVII) with Osmium Tetroxide. Method I.

Labd-8-en-3 β ,15-diol (200 mgs.) in pyridine (5 ml.) was allowed to stand for five days in the dark with a slight excess of osmium tetroxide in ether. After refluxing with lithium aluminium hydride for one hour,

the excess hydride was destroyed by ice, and the mixture was continuously extracted with ether to give a red gum which would not crystallise. The gum was therefore acetylated in the usual way to give an oily product which, after chromatography, was hydrolysed by sodium hydroxide to give the labdan-3 β ,8 α ,9 α ,15-tetrol (XC) as needles (from light petroleum), m.p. 120-121°, $[\alpha]_D + 3^\circ$ (c, 0.5 in chloroform-methanol) (Found: C, 70.4; H, 11.3. C₂₀H₃₈O₄ requires, C, 70.1; H, 11.2%). The compound showed no absorption in the ultraviolet. Overall yield 35 mgs. (< 20%).

Method II.

The preparation of labdan-3 β ,8 α ,9 α ,15-tetrol (XC) was achieved in the same way as above using hydrogen sulphide to decompose the osmium complex giving an overall yield of 40% of pure product, m.p. 120-121°.

Treatment of Labdan-3 β ,8 α ,15,20-tetrol (LXXXIX) with Periodic Acid.

Labdan-3 β ,8 α ,15,20-tetrol (100 mgs., crude) was dissolved in methanol (10 ml.) and periodic acid (150 mgs.),

dissolved in a little water, was added. The solution was left for three days at room temperature. Water (50 ml.) was then added and the methanol evaporated in vacuo. The aqueous solution was extracted with chloroform to give, after washing and drying (anhydrous sodium sulphate), a gum (100 mgs.) which showed absorption in the infrared at 1695 cm.^{-1} (ketone). Chromatography on alumina (3 gms.) gave 20-nor-8-oxolabdan- $3\beta,15$ -diol (LXXXV), m.p. 107° identical with that obtained from ozonolysis of labd-8(20)-en- $3\beta,15$ -diol. Treatment of the aqueous solution with dimedone afforded formaldehyde dimedone m.p. and mixed m.p. 191° , (20 mgs; 40%).

Acetylation of Labdan- $3\beta,8\alpha,15,20$ -tetrol.

Labdan- $3\beta,8\alpha,15,20$ -tetrol (30 mgs.) was acetylated using pyridine (1 ml.) and acetic anhydride (0.5 ml.) at 100° for 1 hr. Isolation in the usual manner gave a gum (35 mgs.) which was chromatographed on neutral alumina to give an oil showing acetate absorption and hydroxyl absorption in the infrared, but crystallisation could not be achieved. Hydrolysis with methanolic

potassium hydroxide (5%) under reflux for 1 hr. gave a quantitative yield of starting material.

Attempted Reaction of Labd-8(20)-en-3 β ,15-diol (LXXXIII) with Acetone.

Concentrated sulphuric acid (0.5 ml.) was added to a solution of labd-8(20)-en-3 β ,15-diol (50 mgs.) in acetone (10 ml.). After 24 hrs. at room temperature, water (20 ml.) was added and the acetone was evaporated under reduced pressure. Extraction of the aqueous solution with ether gave, after washing with water and drying (anhydrous sodium sulphate), a gum (50 mgs.) which showed no acetonide absorption in the infrared. Crystallisation from light petroleum gave starting material (45 mgs.) m.p. 114°.

Condensation of the diol with acetaldehyde and benzaldehyde in an attempt to form the ethylidene and benzylidene derivatives respectively resulted in the recovery of starting material in almost quantitative amounts.

Labd-8(20)-en-3 β ,15-diol-3-monoacetate (XCIX).

Labd-8(20)-en-3 β ,15-diol-3,15-diacetate (135 mgs. oil) was dissolved in methanol (25 ml.), and anhydrous sodium carbonate (16 mgs.; 1 equiv.), dissolved in a minimum of water, was added. After 24 hrs. at room temperature, water (25 ml.) was added and the methanol removed in vacuo at room temperature. Extraction of the aqueous solution gave a gum (116 mgs.) which showed absorption in the infrared corresponding to hydroxyl and acetate. Chromatography on alumina (3 gms.) gave, on elution with benzene ether (3:1), labd-8(20)-en-3 β ,15-diol-3-monoacetate (65 mgs.) needles, (from light petroleum at 0°) m.p. 69°, $[\alpha]_D + 35^\circ$ (c, 0.5 in chloroform). (Found: C, 75.0; H, 10.9. C₂₈H₃₈O₃ requires, C, 75.4; H, 10.9%). λ_{max} (in ethanol 2060Å ($\epsilon = 5000$)). ν_{max} 3520 (hydroxyl); 1721 cm.⁻¹, 1276 cm.⁻¹ (acetate); 1645 cm.⁻¹, 889 cm.⁻¹ (vinylidene double bond).

Labdan-3 β ,15-diol-3-monoacetate (C).

A solution of anhydrous sodium carbonate (0.19 g.; 1 equiv.) in water (3 ml.) was added to a solution of labdan-3 β ,15-diol-3,15-diacetate (1.42 g.) in methanol

(250 ml.) and the mixture allowed to stand at room temperature overnight. Methanol was evaporated off under vacuum and periodically replaced by the same amount of water. Extraction of the aqueous solution with ether gave, after the usual method of isolation, a gum which slowly solidified. Chromatographed on alumina and elution with benzene and benzene-ether (4:1) afforded labdan-3 β ,15-diol-3-monoacetate (0.81 g.), needles (from light petroleum at 0°), m.p. 78-79°, $[\alpha]_D + 26.4^\circ$ (c, 1.0 in chloroform) (Found: C, 74.8; H, 11.5. $C_{22}H_{40}O_3$ requires C, 74.95; H, 11.4%). ν max. 3480 $cm.^{-1}$ (hydroxyl); 1715 $cm.^{-1}$, 1270 $cm.^{-1}$ (acetate).

Oxidation of Labdan-3 β ,15-diol-3-monoacetate (C).

Kiliani[§] oxidation mixture (2.77 ml.) was added dropwise to a solution of labdan-3 β ,15-diol-3-monoacetate (0.78 g.) in acetone (15 ml.) with stirring, and, after all the oxidant had been added, stirring was continued for

[§] Sodium dichromate (17.62 g.) in conc. H_2SO_4 (14 ml.) made up to 100 ml. with water.

a further $1\frac{1}{2}$ hr. Excess water was added, the acetone evaporated, and the product isolated through ether.

The ethereal extract was washed with water until neutral, and then with saturated sodium carbonate. Acidification of the carbonate solution liberated the free acid which was extracted with ether, to give 3 β -acetoxyabdan-15-oic acid (CI) needles (from aqueous acetone), m.p. 93-94°, $[\alpha]_D + 17^\circ$ (c, 1.0 in chloroform). (Found: C, 72.0; H, 10.7. C₂₂H₃₃O₄ requires, C, 72.1; H, 10.45%) γ max. 1735 cm.⁻¹, 1280 cm.⁻¹ (acetate); 1710 cm.⁻¹ (carboxyl).

3 β -Hydroxyabdan-15-oic acid (CII).

3 β -Acetoxyabdan-15-oic (0.62 g.) in 5% methanolic potassium hydroxide (50 ml.) was refluxed on the steam bath for 1 hr. After cooling, the solution was poured into excess water and the methanol removed in vacuo. Acidification of the solution with hydrochloric acid (2N) and isolation through ether gave 3 β -hydroxyabdan-15-oic acid, needles (from light petroleum), m.p. 154-156°, $[\alpha]_D + 20^\circ$ (c, 1.0 in chloroform) (Found: C, 73.7; H, 11.25. C₂₀H₃₃O₃ requires C, 74.0; H, 11.2%) γ max. 3450 cm.⁻¹ (hydroxyl); 1716 cm.⁻¹ (carboxyl).

Methylation of 3 β -Hydroxylabdan-15-oic acid (CII).

Excess of an ethereal solution of diazomethane was added slowly to a solution of 3 β -hydroxylabdan-15-oic acid (45 mgs.) in ether (2 ml.). After 24 hrs. at room temperature, the solution was filtered and the ether evaporated to give a gum which slowly crystallised. Crystallisation from light petroleum gave methyl 3 β -hydroxylabdan-15-oate (CXI), needles, m.p. 74°, $[\alpha]_D + 29^\circ$ (c, 0.5 in CHCl₃) (Found: C, 74.9; H, 11.6. C₂₁H₃₈O₃ requires, C, 74.5; H, 11.3%) ν_{\max} . 3540 cm.⁻¹ (hydroxyl), 1735 cm.⁻¹ (ester).

Oxidation of the Methyl 3 β -Hydroxylabdan-15-oate (CXI).

A solution of methyl 3 β -hydroxylabdan-15-oate (0.28 g.) in pyridine (5 ml.) was added to chromium trioxide-pyridine complex [chromium trioxide (0.3 g.) dissolved in small portions in pyridine (5 ml.) with stirring] and the mixture left at room temperature for 24 hr. Methanol (5 ml.) was added to destroy the excess of oxidant and, after dilution with water, the solution was extracted with ether to give, after the usual work-up, an oily product (0.240 g.). Chromatography

on neutral alumina (1.5 g.) gave the oily methyl 3-oxo-labdan-15-oate (CIII) showing no hydroxyl absorption in the infrared, and a band at 1740 cm.^{-1} with a shoulder at 1720 cm.^{-1} (ketone). The product could not be crystallised nor did it form crystalline carbonyl derivatives.

Wolff-Kishner Reduction of Methyl 3-oxolabdan-15-oate (CIII).

A mixture of keto-ester (0.07 g.), potassium hydroxide (0.2 g.) and anhydrous hydrazine (0.3 ml.) in diethylene glycol (2.5 ml.) was refluxed in an oil bath (external temperature $180\text{--}185^\circ$) for 5 hr. The excess hydrazine was distilled off and the reaction mixture refluxed for 5 hr. (external temperature $225\text{--}230^\circ$). The product, after cooling, was diluted with water, acidified with dilute hydrochloric acid and extracted with ether. The ethereal solution was washed and dried (anhydrous sodium sulphate) to give an acidic gum (0.05 g.) which could not be crystallised. Esterification of the acid with a solution of diazomethane in ether and isolation of the neutral product in the usual way gave an oily methyl ester which was purified by chromatography. Regeneration of the free acid (LIII) again afforded a gum

which could not be crystallised. The infrared spectrum of the acid was identical with that of dihydrocaticvic acid and the acid had $[\alpha]_D + 22.5^\circ$ (c, 0.4 in CHCl_3) [Grant and Zeiss³⁵ give $[\alpha]_D + 25.4^\circ$ for dihydrocaticvic acid].

Preparation of the Thioketal from the Methyl 3-oxolabdan-15-oate.

Methyl-3-oxolabdan-15-oate (0.07 g.) in glacial acetic acid (2.5 ml.) was treated with ethanedithiol (0.2 ml.) and boron trifluoride etherate (0.2 ml.) at room temperature for 1 hr. The product was extracted with ether in the usual way, excess reagent being removed by washing with sodium carbonate solution (5%). Removal of the ether afforded a gummy product which was chromatographed on alumina (2 g.), but could not be obtained crystalline. The product gave a positive test for sulphur.

Desulphurisation of the Thioketal (CIV).

The gummy thioketal (0.05 g.) in absolute ethanol (20 ml.) was refluxed with Raney nickel (0.5 g.) for 10 hr.

Filtration and evaporation of solvent gave a gum (0.04 g.) which was filtered through alumina in light petroleum. The product was free from sulphur and showed infrared identity with that obtained by Wolff-Kishner reduction of the ketoester.

Attempted Dehydration of Methyl 3 β -Hydroxyabdan-15-oate with Phosphorus Pentachloride.

Methyl 3 β -hydroxyabdan-15-oate (210 mgs.) and phosphorus pentachloride (220 mgs.) were shaken in dry benzene (20 ml.) until solution was complete and the shaking continued for a further hour. After the solution had been well washed with water and dried (anhydrous sodium sulphate) the solvent was evaporated to give a gum (200 mgs.) which showed no hydroxyl or double bond absorption in the infrared. Chromatography on neutral alumina (6 gms.) from light petroleum-benzene gave a gummy product, which showed a strong broad peak in the infrared at 1000-1050 cm.^{-1} suggestive of phosphate ester, the presence of which was confirmed chemically. Hydrolysis with 2N potassium hydroxide for 3 hrs. did not effect hydrolysis of the phosphate ester.

Monoacetylation of Labdan-3 β ,15-diol (LXXXIV).

Labdan-3 β ,15-diol (310 mgs.) was dissolved in pyridine (5 ml.) and a solution of acetic anhydride (26.5 mg.) in pyridine (4 ml.) was added dropwise with stirring which was continued for a further 3 hrs. The solution was then allowed to stand overnight. Excess water was added and the solution was extracted with ether. After washing with dilute acid and water, the solvent was evaporated to give a gum (350 mgs.) which was chromatographed on neutral alumina (10 gms.) to give, on elution with benzene, labdan-3 β ,15-diol-15-monoacetate (CXII) needles (from light petroleum) m.p. 81-2°, $[\alpha]_D + 26.4^\circ$ (c, 1.0 in CHCl₃) (Found: C, 74.3; H, 11.1. C₂₂H₄₀O₃ requires, C, 74.95; H, 11.4%) γ max. 3540 cm.⁻¹ (hydroxyl); 1720 cm.⁻¹, 1270 cm.⁻¹ (acetate).

Dehydration of Labdan-3 β ,15-diol-15-monoacetate (CXII).

Phosphorus oxychloride (2.5 ml.) was added dropwise with stirring to a solution of labdan-3 β ,15-diol-15-monoacetate (130 mgs.) in pyridine (7.5 ml.). The solution was heated for a further $\frac{1}{2}$ hr. on the steam bath, then left at room temperature for 24 hrs. after

which it was carefully poured into an excess of water. Ether extraction followed by isolation in the usual manner gave a gum (110 mgs.) which was chromatographed on neutral alumina (3.0 g.). Elution with petrol gave an oil (30 mgs.) which showed no absorption in the hydroxyl region of the infrared.

Ozonolysis of the dehydration Product.

A solution of the dehydration product (30 mgs.) in carbon tetrachloride (5 ml) was treated with ozone (10%) for $\frac{1}{2}$ hr. at -5° . The solvent was evaporated in vacuo at room temperature. Water (10 ml.) and zinc dust (100 mgs.) were added and the solution was boiled. The first few drops of distillate caused a saturated solution of 2,4-dinitrophenylhydrazine in dilute hydrochloric acid to turn cloudy. Extraction with ether gave a product which was shown by thin layer chromatography on silica gel to be a mixture of the 2,4-dinitrophenylhydrazones of acetone and formaldehyde.

	<u>Solvent</u>	<u>Spot</u>	<u>R_f value</u>
plate 1.	Benzene	reaction product	0.28 and 0.36
	"	acetone 2,4-D.N.P.	0.28
plate 2	Benzene	reaction product	0.2 and 0.26
	"	acetone 2,4-D.N.P.	0.2
	"	formaldehyde 2,4-D.N.P.	0.26

The product was chromatographed on alumina and the material in the last fraction tested using several solvent systems.

	<u>Solvent</u>	<u>Spot</u>	<u>R_f value</u>
	Benzene	product	0.32
	"	acetone 2,4-D.N.P.	0.32
	Benzene ether (1:1)	product	0.80
	"	acetone 2,4-D.N.P.	0.80
	Ether	product	0.85
	"	acetone 2,4-D.N.P.	0.85

Tritylation of Labd-8(20)-en-3 β ,15-diol.

Attempts to form the monotrityl ether of labd-8(20)-en-3 β ,15-diol (200 mgs.) with trityl chloride (200 mgs.) in

pyridine at room temperature and at 100° for 4 hr. were unsuccessful.

Oxidation Reactions.

Oxidation of the natural diol, the isomer, and the saturated reduction product in pyridine and acetic acid with chromium trioxide did not lead to the isolation of crystalline products. Kiliani oxidation in acetone likewise gave negative results.

BIBLIOGRAPHY

1. de Mayo, "The Higher Terpenoids", Interscience 1959.
2. Ruzicka and Hosking, Annalen, 1929, 469, 147.
3. Ruzicka, Bernold and Tallichet, Helv. Chim. Acta, 1941, 24, 223.
4. Ruzicka and Bernold, ibid, 1941, 24, 931.
5. Ruzicka, Zwicky and Jeger, ibid, 1948, 31, 2143.
6. Volmar and Jermstad, Compt. rend., 1928, 186, 517, 783
7. Janot, ibid, 1930, 191, 847; 1931, 192, 845.
8. Ruzicka and Janot, Helv. Chim. Acta, 1931, 14, 645.
9. Hosking and Brandt, Ber., 1934, 67, 1173; New Zealand J. Sci. Tech., 1936, 17, 750.
10. Hosking and Brandt, Ber., 1935, 68, 37.
11. Hosking and Brandt, ibid, 1936, 69, 780.
12. Hosking and Brandt, ibid, 1935, 68, 1311; New Zealand J. Sci. Tech., 1936, 17, 755.
13. Ruzicka, Dürst and Jeger, Helv. Chim. Acta, 1947, 30, 353.
14. Cocker and Halsall, J., 1957, 4401.
15. Jeger, Dürst and Büchi, Helv. Chim. Acta, 1947, 30, 1853.
16. Lederer and Mercier, Experientia, 1947, 3, 188.
17. Stoll and Hinder, Helv. Chim. Acta, 1950, 33, 1251.
18. Hinder and Stoll, ibid, 1950, 33, 1308.
19. Lederer and Stoll, ibid, 1950, 33, 1345.

20. Büchi and Biemann, Croat. Chem. Acta, 1957, 29, 163-171.
21. Ohloff, Helv. Chim. Acta, 1958, 41, 845.
22. Baltrop, Bigley and Rogers, J., 1960, 4613.
23. Cahn, Ingold and Prelog, Experientia, 1956, 12, 81.
24. Prelog and Watanabe, Annalen, 1957, 603, 1.
25. Hodges and Reed, Tetrahedron, 1960, 10, 71.
26. Giles, Schumacher, Mims and Bernasek, Tetrahedron, 1962, 18, 169.
27. Enzell, Acta. Chem. Scand., 1961, 15, 1303.
28. Arya, Enzell, Erdtman and Kubota, ibid, 1961, 15, 225.
29. Enzell, ibid, 1961, 15, 1303.
30. Häuser, Lombard, Lederer and Ourisson, Tetrahedron, 1961, 12, 205.
31. Gopinath, Govindachari, Parthasarathy and Viswanathan, Helv. Chim. Acta, 1961, 44, 1040.
32. Kalman, J. Amer. Chem. Soc., 1938, 60, 1423.
33. Grant and Zeiss, ibid, 1954, 76, 5001.
34. Grant and Zeiss, ibid, 1957, 79, 1201.
35. Cocker and Halsall, J., 1956, 4262.
36. Cocker, Halsall and Bowers, J., 1956, 4259.

37. Burn and Rigby, Chem. and Ind., 1955, 386.
38. Bory and Lederer, Croat. Chem. Acta, 1957, 29, 157.
39. Halsall and Moyle, J., 1960, 1324.
40. Panizzi, Mangoni and Belardini, Tetrahedron Letters, 1961, 376.
41. Giles and Schumacher, Tetrahedron, 1961, 14, 246.
42. King and Jones, J., 1955, 658.
43. Djerassi and Marshall, Tetrahedron, 1957, 1, 238.
44. Djerassi and Nakano, J. Org. Chem., 1961, 26, 167.
45. Cava, Chan, Haynes, Johnson and Weinstein, Tetrahedron, 1962, 18, 397 (and references cited therein).
46. Ruzicka, Experientia, 1953, 9, 357.
47. Sonderhoff and Thomas, Annalen, 1937, 530, 195.
48. Bloch and Rittenberg, J. Biol. Chem., 1942, 145, 625.
49. Cornforth, Hunter and Popják, Biochem. J., 1953, 54, 597.
50. Wüersch, Huang and Bloch, J. Biol. Chem., 1952, 195, 439.
51. Cornforth and Popják, Biochem. J., 1954, 58, 403.
52. Wolf, Hoffman, Aldrich, Skeggs, Wright and Folkers, J. Amer. Chem. Soc., 1956, 78, 4499.

53. Tavormina, Gibbs and Huff, J. Amer. Chem. Soc., 1956, 78, 4498.
54. Tavormina and Gibbs, J. Amer. Chem. Soc., 1956, 78, 6210.
55. Birch and Smith, Ciba Foundation Symposium, Biosynthesis of Terpenes and Sterols, p.245, Churchill 1959.
56. Britt and Arigoni, Proc. Chem. Soc., 1958, 224.
57. Arigoni, as ref.55, p.231.
58. Birch, Rickards and Smith, Proc. Chem. Soc., 1958, 192.
59. Dietrich, Lederer and Mercier, Helv. Chim. Acta, 1954, 37, 705.
60. A.P.I. Project No.44.
61. Reed, ASTM E-14 Committee on Mass Spectrometry, Atlantic City, July 1960.
62. Reed, J., 1958, 3432.
63. Friedland, Lane, Longman, Train and O'Neal, Analyt. Chem., 1959, 31, 169.
64. Genge, Anal. Chem., 1959, 31, 1750.
65. Klyne, Personal communication.
66. Djerassi and Marshall, J. Amer. Chem. Soc., 1958, 80, 3986.