WOOD EXTRACTIVES

(A NEW DITERPENE DIOL FROM ARAUCARIA IMBRICATA)

A THESIS

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THE UNIVERSITY OF GLASGOW

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by

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SUMMARY

Wood Extractives.

The thesis has been divided into two sections. Section I deals with the elucidation of the structure of a new diterpene diol, $C_{20}H_{36}O_2$, isolated from the neutral part of the light petroleum extract of the bark of Chile pine (*Araucaria imbricata*). The diol belongs to the labdane series of diterpenoids and has been shown to have a primary hydroxyl group at C_{18} and a secondary hydroxyl group at C_3.

Section II(a) describes the examination of the extractives of the woods of *Dipterocarpus crinitus* and *Araucaria brasiliensis*. The nonsaponifiable portion of the benzene extract of the wood of *D. crinitus* yielded betulin as the major constituent while both woods gave β-sitosterol and ceryl alcohol.

Section II(b) describes the separation of triterpenes by thin-layer chromatography using silica gel. It has been found that triterpenes having different functional groups for example alcohols, ketones and esters can be separated but individual members of the same class can not be separated. It has also been shown that triterpene alcohols may be separated from steroid alcohols by this procedure.

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

The author wishes to take this opportunity to express his sincere gratitude to Professor P. L. Pauson and to Dr. J. McLean for their helpful guidance throughout the course of these investigations. The author gratefully acknowledges the receipt of a maintenance grant from the authorities of the Colombo Plan.

C O N T E N T S

INTRODUCTION

The extraction of the bark of Chile pine (Araucaria imbricata) with light petroleum has led to the isolation of a hitherto unknown diterpene diol and it is pertinent to review the chemistry of diterpenes before discussing our attempts to elucidate the structure of the new diol.

The extraction of the wood of Dipterocarpus crinitus furnished betulin, a pentacyclic triterpene diol as the major constituent and therefore a brief account of the classification and biogenesis of triterpenes is also given in the following pages.

The higher terpenoids are a group of natural products derived mainly from the vegetable kingdom although they are also found in woolfat, fungi and shark liver oil. The triterpenes and diterpenes occur chiefly as oxygenated derivatives having thirty and twenty carbon atoms respectively in their molecular structure and usually obey the isoprene rule. With the discovery of compounds containing thirty one carbon atoms which bear a close relationship to the triterpenes, the term 'triterpenoids' has been introduced to cover the whole group while the term 'diterpenoids' includes the compounds containing twenty carbon atoms present as hydrocarbons, acids, alcohols, ketones, phenols,

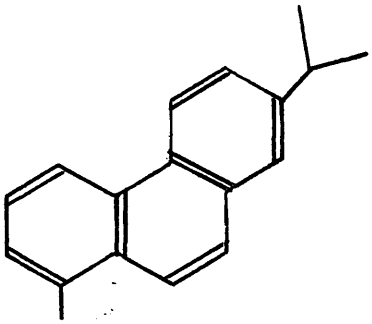
oxides, lactones and glycosides in different parts of the plants.

Classification of Diterpenoids.

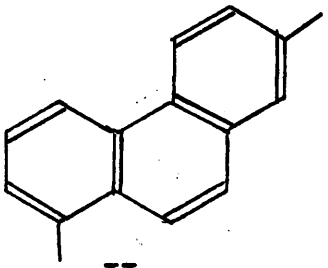
The diterpenoids can be classified into three main groups on the basis of their dehydrogenation products. The first group consists of compounds which on dehydrogenation yield 1-methyl-7-isopropylphenanthrene or retene (I) and includes the tricyclic resin acids, for example, abietic acid and levopimaric acid. Phenolic diterpenoids like ferruginol and hinokiol yield 6-hydroxyretene and sugiol yields 6-methoxyretene on dehydrogenation.

The second group on dehydrogenation gives 1,7-dimethylphenanthrene or pimanthrene (II) and consists of pimaric acid, miropinic acid, phyllocladene, rimuene,[1] rosenonolactone,[2] and darutigenol[3] etc.

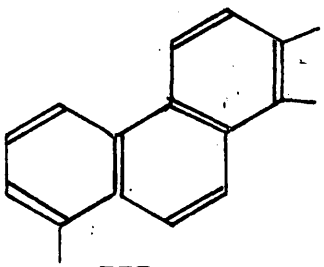
The third group of compounds on dehydrogenation yield 1,7,8-trimethylphenanthrene (III) and includes manoyl oxide, dihydrocyclosclarene - a derivative of sclareol and cassaic acid. The structures of all the dehydrogenation products



I

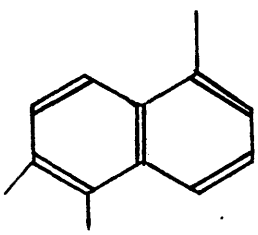


II

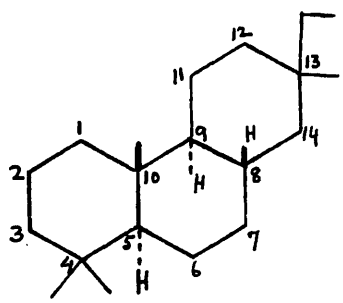


III

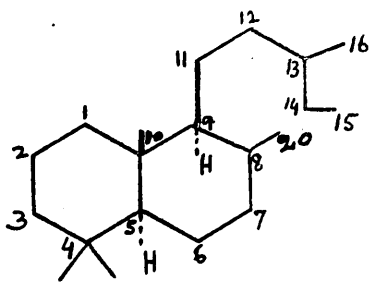
have been confirmed by synthesis. Many bicyclic diterpenoids on dehydrogenation give 1,2,5-trimethylnaphthalene (IV) along with other substituted naphthalenes and these include manool, sclareol, marrubiin, agathenedicarboxylic acid, cativic acid[4,5] and labdanolic acid.[6,7] The recently discovered diol torulosol,[8] the hydroxy-aldehyde torulosal and communic acid[9] are related to manool. The basic skeleton of the bicyclic diterpenoids is the 13,14-secopimarane (VI) or labdane structure which can be theoretically derived by the fission of the hydrocarbon pimarane (V). Vinhaticoic acid



IV



V



VI

and vouacapenic acid containing a furan ring in their structure on dehydrogenation give 1,8-dimethylphenanthrene, 1,6,8-trimethylphenanthrene and 2-ethyl-1,8-dimethyl-phenanthrene.

The bicyclic diterpenoids on appropriate chemical transformations give the same dehydrogenation products as are obtained from the tricyclic compounds for example sclareol gives 1,2,5-trimethylnaphthalene while dihydro-cyclosclarene gives 1,7,8-trimethylphenanthrene and therefore the diterpenoids have not been classified into groups based on the number of rings present.

Classification of Triterpenoids.

Apart from squalene (VII), the triterpenoids are all polyclic compounds and can be classified into three main groups based on the number of rings present: (a) squalene and ambrein, (b) the tetracyclic triterpenoids and (c) the pentacyclic triterpenoids.

(a) The first group consists of only three members squalene (VII), ambrein and onocerin.

(b) The tetracyclic triterpenoids can be further subdivided into two groups, one based on lanosterol (VIII) and the

Biogenesis of Di- and Triterpenoids.

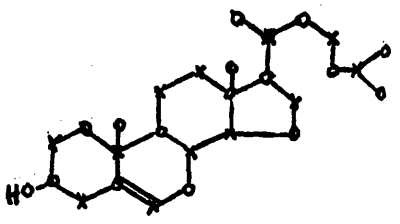
The biogenesis of triterpenes and steroids takes place with acetic acid as the precursor to squalene which cyclises to lanosterol. Lanosterol then loses three methyl groups to give cholesterol. Schemes for biogenesis have been proposed by Robinson,[10] Woodward and Bloch,[11] and Ruzicka.[12]

Experimental confirmation of these postulations has been provided by many workers[13-16] and their combined results have established that acetic acid is the chief source of carbon atoms in the biological synthesis of all the terpenes and steroids investigated so far, for example, cholesterol, squalene, rosenonolactone,[17,18] gibberellic acid,[19] and soyasapogenol-A.[20]

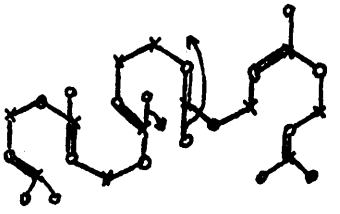
The degradation of cholesterol (XIV) biosynthesised from acetic acid labelled with ^{14}C has proved that both the carbon atoms of acetic acid are present in it and that fifteen carbon atoms originate from the methyl carbon and twelve from the carboxyl carbon of acetic acid (~~XIV~~).

The examination of isotopically labelled carbon atoms in the side chain and ring A of cholesterol and of biosynthesised squalene has shown that the C_5 or the isoprene

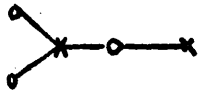
units present therein have the distribution of carboxyl and methyl carbons as shown in (XV).



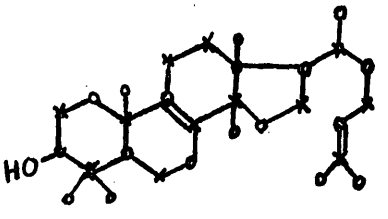
XIV



XVI



XV



XVII

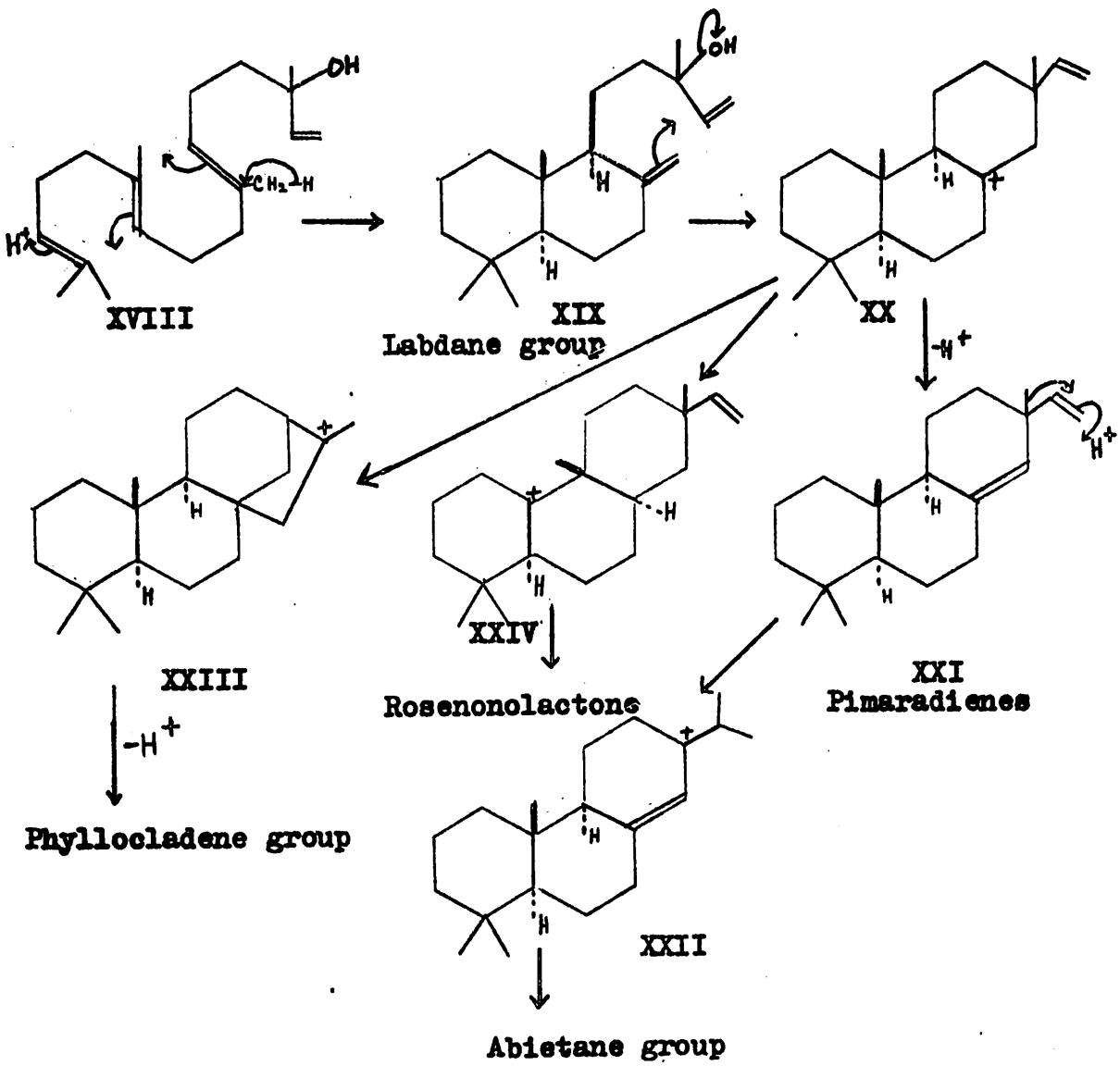
Squalene has the pattern of distribution of the carboxyl and methyl carbons as shown in (XVI) and on cyclisation with subsequent migration of methyl groups gives lanosterol (XVII). The above results are in

agreement with the scheme of Woodward and Bloch.[11] Ruzicka and co-workers[12] formulated a scheme called the biogenetic isoprene rule which states that terpenes are compounds formed by the combination of isoprene units to aliphatic substances like squalene or geranylgeraniol and can be derived from these aliphatic precursors by cyclisation and rearrangements. Triterpenes like lanosterol and euphol and diterpenes like rosenonolactone which do not obey the classical isoprene rule comply with the biogenetic rule.

It has been shown[21] that only natural squalene which is an all-trans isomer can be biologically converted into cholesterol and that the conversion of squalene to lanosterol requires the presence of oxygen. It has been suggested[22] that this transformation is initiated by the attack of molecular oxygen or the cation OH^+ at the terminal double bond and the cyclisation thereafter proceeds synchronously.

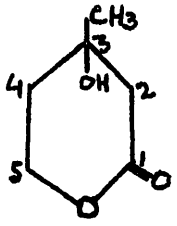
The aliphatic precursor for the biogenesis of diterpenes is geranylgeraniol or geranyllinalool.(XVIII). The cyclisation here is initiated by cationic attack usually of a proton whereby rings A and B are formed first. Thereafter skeletal rearrangements and oxidation take place

to give different groups of diterpenes as shown (XVIII-XXIV).

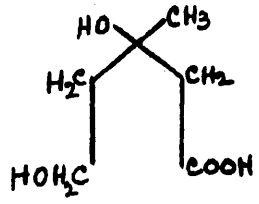


Mevalonic acid and mevalonolactone as precursor.

The isolation of mevalonolactone[23] (XXV) from soluble distillers residues and its subsequent conversion into cholesterol suggests that the lactone is an intermediate in the biosynthetic pathway from acetate to squalene. Tavormina and Gibbs[24] showed that mevalonic acid (XXVI) loses one carbon atom as carbon dioxide during the biosynthesis of cholesterol forming a C_6 unit which by losing two molecules of water could give rise to a C_5 unit having the required distribution of carbon atoms. The C_2 of one



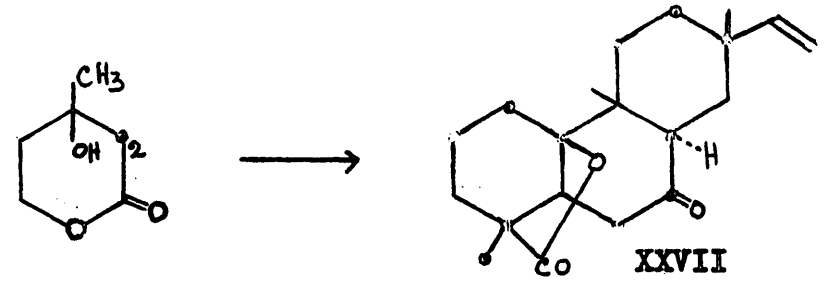
XXV



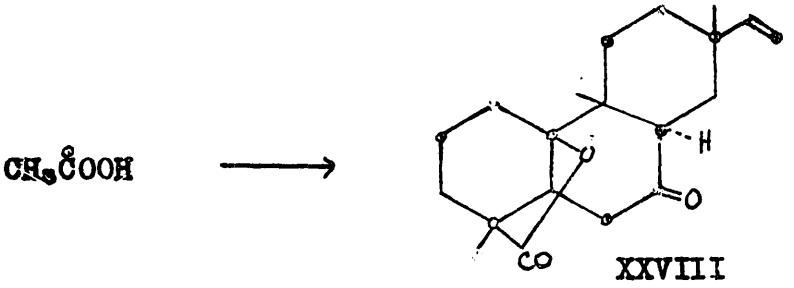
XXVI

unit condenses with the C_5 of another unit and so on to yield the aliphatic precursors like squalene or geranylgeraniol which under suitable conditions cyclise and rearrange to give individual members of triterpenes and diterpenes as postulated by the biogenetic isoprene rule.

Rosenonolactone, the diterpene metabolite of Trichothecium roseum has been biosynthesised from mevalonic acid labelled at C_2 and acetic acid labelled at the carboxyl carbon. An examination of the degradation products confirmed that the labelled isotopes were present at positions predicted by the biogenetic isoprene rule as in (XXVII) and (XXVIII).



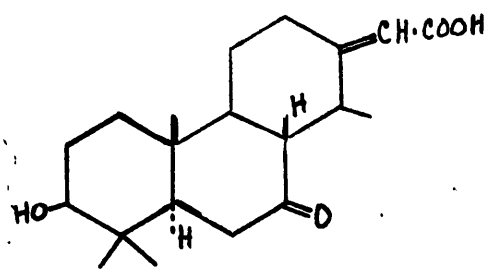
XXVII



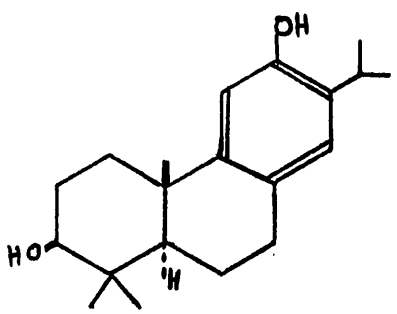
XXVIII

The results show that the methyl group at C_{10} has migrated to C_9 and the methyl group at C_4 is derived from the C_2 of mevalonic acid since a labelled carbon atom has been found at that position. The methyl group of the precursor which has been oxidised to COOH is that which in the end product occupies the axial position. The biogenesis of this lactone has been experimentally verified by Britt and Arigoni.[19]

Diterpenes having α hydroxyl group in ring A are also known, for example, cassaic acid (XXIX), darutigenol and hinokiol (XXX) and in agreement with the biogenetic isoprene rule bear the oxygen function at C_3.



XXIX



XXX

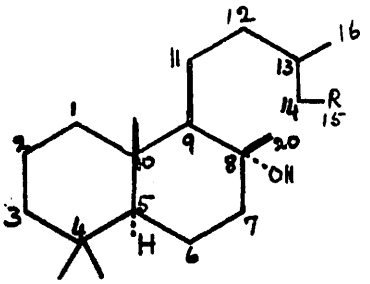
Chemistry of labdanolic acid and related compounds.

A survey of the chemistry of labdanolic acid and related compounds is described in the following pages since we have isolated a new diterpene diol from Araucaria imbricata which belongs to the labdane series.

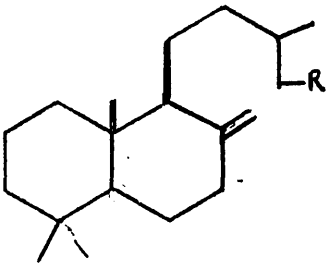
Cocker et al[6], while investigating the Spanish gum labdanum, isolated from the acidic fraction, two new diterpene acids. One of these is labdanolic acid (XXXI) and the other acid, which contains an αβ-unsaturated ketone grouping, has been shown to be 6-oxo-cativic acid (XXXVII).[25] Cocker and Halsall[7] showed that labdanolic acid (XXXI) on methylation gave the methyl ester (XXXII) which could be easily hydrolysed back to the acid and concluded that the carboxyl group is not situated in the usually hindered position 4 of the diterpene acids. The hydroxyl group could not be acetylated on mild acetylation and was assumed to be unreactive. The methyl ester (XXXII) on hydrogenolysis with lithium aluminium hydride gave the diol (XXXIII), which furnished a monoacetate on acetylation and hence the original hydroxyl group present in labdanolic acid must be tertiary. The methyl ester of labdanolic acid on dehydration with phosphoryl chloride gave a compound which

showed peaks at 890 cm.$^{-1}$ and 1645 cm.$^{-1}$ in the infrared spectrum, characteristic of a vinylidene group. This was confirmed by ozonolysis of the dehydrated product (XXXIV) when formaldehyde and the ketoester (XXXVIII) were obtained.

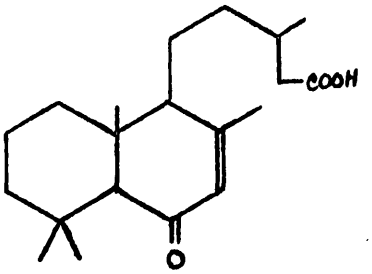
Hydrogenolysis of the dehydration product (XXXIV) with lithium aluminium hydride gave the alcohol (XXXV) which furnished a triol (XXXIX) on treatment with osmium tetroxide, the addition being assumed to take place at the less hindered α-face.



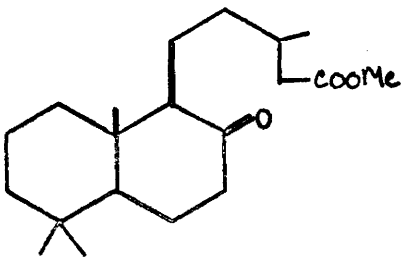
XXXI, R = COOH
XXXII, R = COOMe
XXXIII, R = CH_2OH



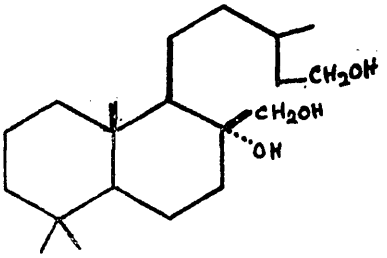
XXXIV, R = COOMe
XXXV, R = CH_2OH
XXXVI, R = COOH



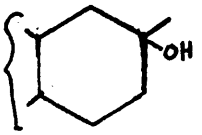
XXXVII



XXXVIII

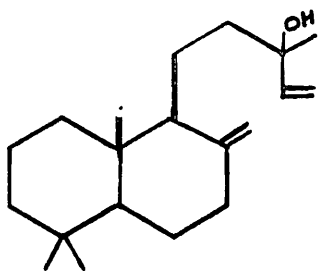


XXXIX

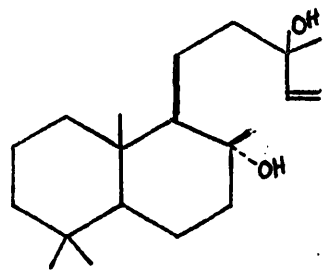


XL

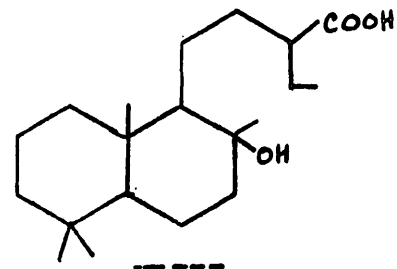
The keto ester (XXXVIII) showed peaks at 1735 cm.$^{-1}$ and 1712 cm.$^{-1}$ in the infrared spectrum indicating that the keto group is in a six membered ring and is slightly hindered as it does not form a 2,4-dinitrophenylhydrazone but gives an oxime. Labdanolic acid therefore contains the grouping (XL), the hydroxyl group being equatorial since dehydration gives an exocyclic and not an endocyclic double bond.



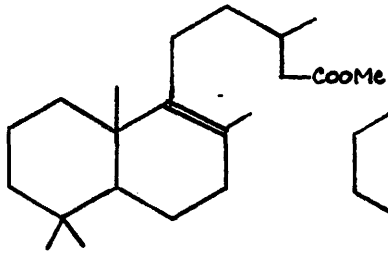
XLI



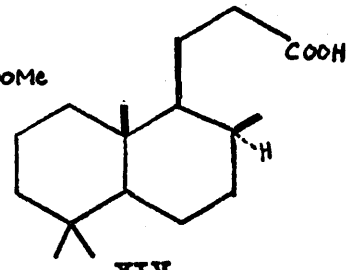
XLII



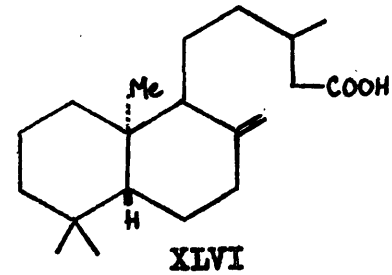
XLIII



XLIV



XLV



XLVI

Dehydrogenation of the unsaturated alcohol (XXXV) with platinised charcoal afforded 1,2,5-trimethylnaphthalene and 1,2,5,6-tetramethylnaphthalene which suggested that the alcohol and its parent compound must be related to manool (XLI) and sclareol (XLII). Labdanolic acid can therefore be represented by (XXXI) or a stereoisomer, or by (XLIII).

King and Jones[26] isolated another new diterpene acid, eperuic acid from the oleo resin of Eperua falcata and showed its methyl ester to be a stereoisomer of (XXXIV). Since methyl-eperuate had an optical rotation which was equal in magnitude but opposite in sign to that of methyl labd-8,20-en-15-oate (XXXIV), the two compounds appeared to be enantiomorphs.

The methyl ester (XXXIV) on treatment with acid gave a product (XLIV) in which the double bond had shifted to a tetra-substituted position, as shown by the absence of a vinylidene peak in the infrared spectrum. The isomerisation product on hydrogenation gave the same solid saturated compound as is obtained by hydrogenation of (XXXIV) implying that no skeletal rearrangement had taken place during acid isomerisation.

The solid hydrogenation product from the methyl ester

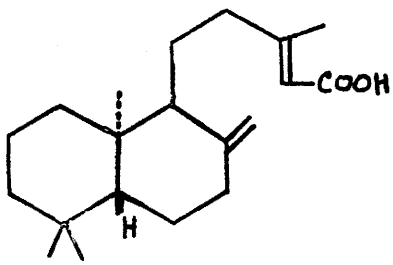
(XLIV) or (XXXIV) was degraded by the Barbier-Wieland method to the nor-acid and thence to a methyl ketone which on oxidation gave the C_{17} acid (XLV). This acid had also been obtained from ambrein and from marrubiin.[27] Thus the degradation of labdanolic acid to the common degradation product (XLV) showed that it should be represented by (XXXI) and not by (XLIII). It also showed that the rings A and B in labdanolic acid are trans fused and the configuration at C_{10} is the same as in ambrein i.e. the methyl group at C_{10} has β-orientation. The configuration of the hydroxyl group at C_8 is accordingly α and that of the methyl group at C_8 is β.

Additional proof for this has been provided by the comparison of the molecular rotation differences of sclareol and manool and of labdanolic acid and its dehydration product.

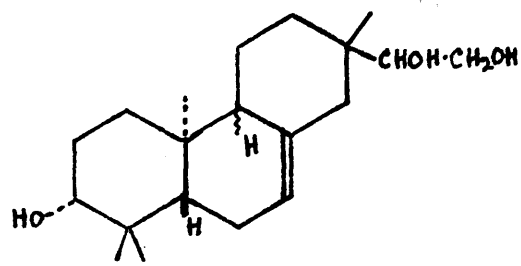
The solid isomer of the hydrogenation product of (XXXIV) was identical with methyl dihydro-cativate, which had been shown[4,5] to possess the β-configuration at C_9 (methyl dihydro-cativate when subjected to a two step Barbier-Wieland degradation gives a C_{18} methyl ketone which had also been obtained from manool). Hence labdanolic acid has the

β-configuration at C_9. Methyl dihydrocativate should accordingly have the methyl group at C_8 in the β position, similar to sclareol and labdanolic acid, hence the latter is represented by (XXXI) except for the stereochemistry at C_{13}.

Eperuic acid for which structure (XXXVI) was proposed by King and Jones[26] apart from stereochemistry is represented by (XLVI) because its degradation products have similar but opposite optical rotations to the corresponding degradation products of labdanolic acid. Thus in eperuic acid, the carbon skeleton is the mirror image of rings A and B in labdanolic acid. Copalic acid[28] which has been shown to be $\Delta^{13,14}$ eperuic acid (XLVII) and darutigenol[3] (XLVIII), a glycosidic diterpene triol, also possess the same stereochemistry as eperuic acid.

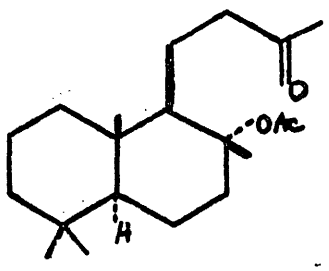


XLVII

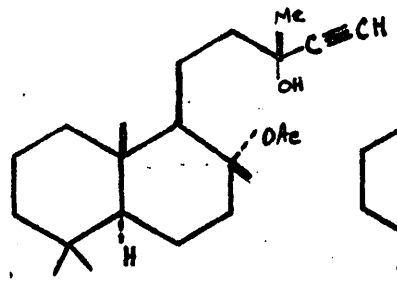


XLVIII

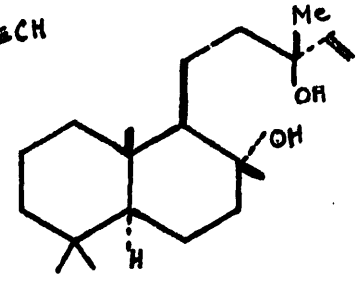
In the synthesis of sclareol and its C_{13} epimer[29] the ketone (XLIX) on ethynylation gave two carbinols(L), one of which was intramolecularly hydrogen-bonded. A study of the models showed that this type of hydrogen-bonding could take place only when the ethynyl group was β-oriented. The non-bonded isomer on reduction with lithium aluminium hydride furnished sclareol and therefore the methyl group at C_{13} is β-oriented (LI). As manool[30,31] has been obtained from sclareol, its configuration (LII) is similar to it at C_{13}. This is supported by the fact that the molecular rotation differences of derivatives of manool closely resemble those of the derivatives of linalool[32] (LIII) which is known to have a similar configuration.



XLIX



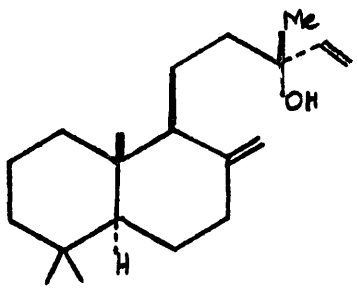
L



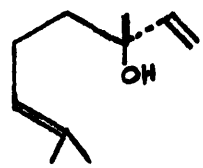
LI

The configuration of labdanolic acid at C_{13} was shown to be as represented by (LIV) by comparison of the molecular rotation differences of methyl labdanolate and its C_{13} epimer, both being synthesised from sclareol.[33] Eperuic acid having the opposite stereochemistry then becomes (LV).

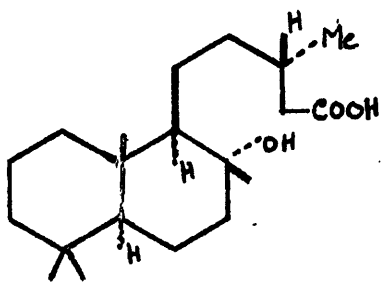
These results have been confirmed by an independent synthesis of both acids.[29]



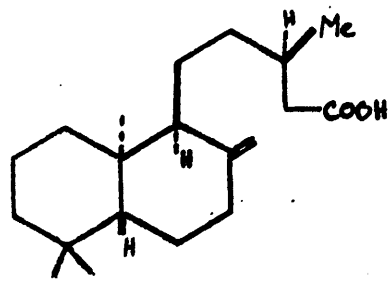
LII



LIII



LIV



LV

SECTION I

Extractives from the Bark of Chile Pine (Araucaria imbricata).

The bark of Araucaria imbricata on extraction with light petroleum gave a new diterpene diol whose structure has been elucidated. A tentative structure has been assigned to the diol and it has been shown to have a primary hydroxyl group at C_{16} and a secondary hydroxyl group at C_3.

Theoretical.

Some of the data given below has been supplied by Mr. J. Clark and is included with the author's work in order to give a complete picture of the problem.

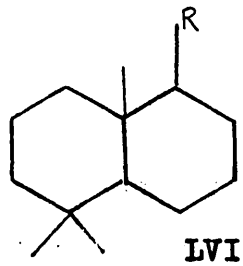
The neutral part of the light petroleum extract of the bark of Chile pine (Araucaria imbricata), when chromatographed on alumina, yielded a crystalline compound m.p. 114°, $[\alpha]_D$ + 29° showing bands in the infrared spectrum at 3635, 3440 $cm.^{-1}$ (hydroxyl group), 890 and 1640 $cm.^{-1}$ (vinylidene group) and a doublet at 1365 and 1385 $cm.^{-1}$ (gem dimethyl group). The ultraviolet spectrum showed absorption at 206 mμ ($\varepsilon_{max.}$ 5,000), indicating a double bond. On hydrogenation, the compound showed an uptake of hydrogen equivalent to one mole and active hydrogen determination gave a value of 1.92.

The molecular weight of the compound is about 320 and analysis of the compound and many of its derivatives indicated the molecular formula $C_{20}H_{36}O_2$. The molecular formula requires three double bond equivalents and, since one double bond is present, the system must be bicyclic.

The diol on acetylation furnished an oily diacetate which did not show any absorption in the hydroxyl region in the infrared spectrum. Thus the possibility of a tertiary hydroxyl

group is ruled out.

On dehydrogenation with selenium, the diol gave 1,2,5-trimethylnaphthalene, the most common dehydrogenation product of diterpenes like sclareol, manoöl, labdanolic and eperuic acids. The basic skeleton of the diol, hereafter called diol I, may be represented by (LVI) where R is the side chain of six carbon atoms usually present in such diterpenoids.

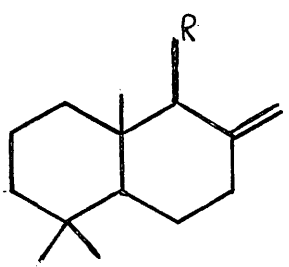


LVI

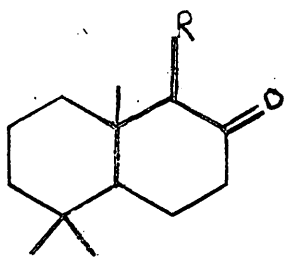
Ozonolysis of the diol I furnished formaldehyde and a norketone $C_{19}H_{34}O_3$ which confirmed the presence of a vinylidene group. The diol I, on treatment with methanolic sulphuric acid, gave an isomeric compound, diol II, which does not show bands at 890 and 1640 cm.$^{-1}$ in the infrared spectrum or the band for a trisubstituted double bond but still has absorption at 210 mμ. ($\varepsilon_{max.}$ 5,500) in the ultraviolet. The double bond has therefore shifted to a fully substituted position. Diol II, on hydrogenation, yields the same saturated compound as has been

obtained from diol I and it follows therefore that no skeletal rearrangement has taken place during acid isomerisation.

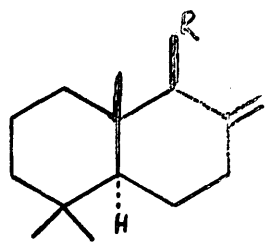
A comparison of the molecular rotation differences between the derivatives prepared from diol I with those from the dehydrated product of labdanolic acid i.e. labd-8,20-en-15-oic acid (XXXVI) shows that their magnitude and sign are similar in both cases and therefore the double bond is placed at $C_{8(20)}$, the side chain at C_9 is regarded as β-oriented, and, in the dihydrocompound, the methyl group at C_8 is also β-oriented. Hence, apart from the placing of functional groups, the skeletal structure (LVII) has been proposed where R stands for the side chain of six carbon atoms.



LVII



LVIII



LIX

The optical rotatory dispersion curve of the norketone (LVIII) shows a negative Cotton effect curve, resembling the corresponding labdanolic acid derivative. (The Cotton effect of the corresponding enamic acid derivative is positive).

The junction of the rings A and B is therefore trans and the stereochemistry normal. Hence the partial structure for the diol becomes (LIX).

Oxidation of the diol I or diol II by a number of reagents gave only gummy products from which no crystalline derivatives were obtained. The absence of αβ-unsaturated carbonyl absorption in the infrared spectrum of the oxidation products showed that the hydroxyl groups were not adjacent to the double bond. Also, the compound did not contain a vicinal glycol system as lead tetra-acetate did not produce cleavage. The spectral evidence indicated that one hydroxyl group is primary and the other secondary.

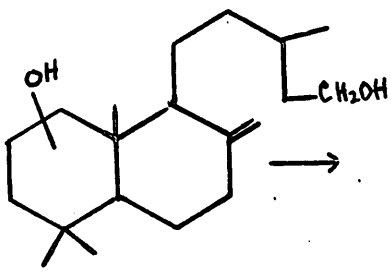
At this stage, the only problem left to be solved in the elucidation of the structure of Diol I was the placing of the hydroxyl groups and the experimental part of the thesis deals with this problem.

The diol I afforded a crystalline monoacetate by very mild hydrolysis of the diacetate and it was assumed that the hydroxyl group which was being hydrolysed was primary and was located at the end of the side chain, either at C_{18} or C_{19}, since positions C_{17}, C_{18} and C_{19} are somewhat hindered.

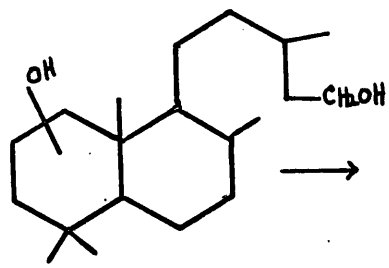
It was postulated that we might relate the dihydrodiol with a known compound, methyl dihydrocativate, by preferentially oxidising the primary hydroxyl group of the dihydrodiol-monoacetate (LXIII) to the acetoxy acid (LXIV). Hydrolysis of the acetoxy acid to the hydroxy-acid (LXV), followed by oxidation to the keto acid (LXVIa), methylation, and subsequent reduction, should give methyl dihydrocativate (LXVIIb).

The diol I (LX) on hydrogenation afforded the dihydrodiol (LXI) which was acetylated to furnish the diacetate (LXII). The diacetate, on partial hydrolysis, gave a crystalline mono-acetate (LXIII) which was oxidised by Kiliani mixture to give the acetoxy acid (LXIV). Hydrolysis of the acetoxy acid furnished a solid hydroxy-acid (LXV), which showed bands in the infrared spectrum at 3450 $cm.^{-1}$ and 1716 $cm.^{-1}$, characteristic of hydroxyl and carboxyl functions. The isolation of the hydroxy acid confirmed the belief that the hydroxyl group which had been preferentially oxidised was in fact primary.

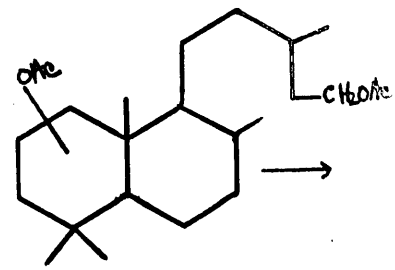
The hydroxy-acid was oxidised by chromium trioxide in pyridine to afford a noncrystalline ketoacid. The infrared spectrum of its ketoester showed no absorption in the hydroxyl region but a broad band at 1740 $cm.^{-1}$, with a shoulder at 1720 $cm.^{-1}$ was indicative of the presence of the carbonyl group. Neither



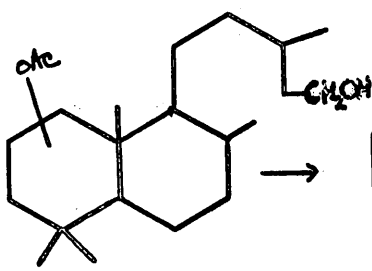
LX



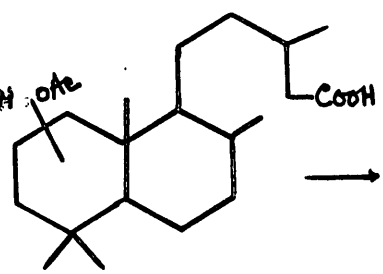
LXI



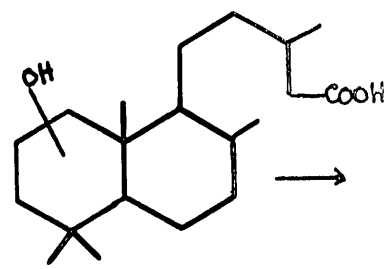
LXII



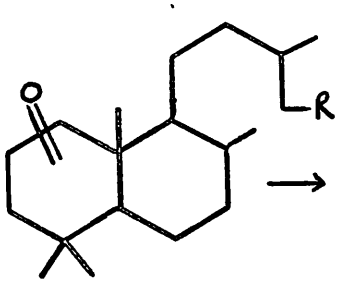
LXIII



LXIV

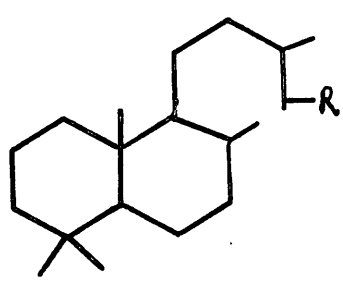


LXV



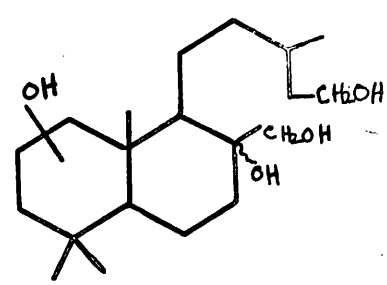
LXVI

(a) R = COOH
(b) R = COOMe



LXVII

(a) R = COOH
(b) R = COOMe



LXVIII

the ketoacid (LXVIa) nor the ketoester (LXVIb) gave a solid 2,4-dinitrophenylhydrazone, oxime, or semicarbazone. The hydroxyl group that has been oxidised to the ketone is of course secondary.

The ketoester (LXVIb) was reduced by the modified Wolff-Kishner[34] procedure to give a resinous acid (LXVIIa) which had a specific rotation of +22.5° (dihydrocativic acid has specific rotation +25.4°). Neither the acid nor its methyl ester could be obtained crystalline.

The same methyl ester (LXVIIb) was also obtained by the desulphurisation of the thioketal, prepared by treatment of the ketoester with ethane-dithiol. The thioketal was a gum and showed the presence of sulphur. The desulphurisation was done by Raney nickel in ethanol when the product obtained gave a negative test for sulphur. The infrared spectrum of the desulphurised product was identical with that of methyl dihydrocativate.

Another route to methyl dihydrocativate was investigated.[35] Tosylation of the methyl ester of the hydroxy-acid gave a gummy tosylate showing no absorption in the hydroxyl region of the infrared spectrum. The tosylated product was detosylated by

refluxing it with lithium aluminium hydride in tetrahydrofuran when the original starting material i.e. the dihydrodiol was obtained.

Since the hydroxy-acid did not lactonise, secondary hydroxyl positions γ and δ to the carboxyl group i.e. C_{11} and C_{12} can be eliminated. Since the tetrol (LXVIII), formed from the diol I with osmium tetroxide gave the norketone with lead tetra-acetate, the hydroxyl group is not at position C_7 and since the ketone did not dehydrate to form an $\alpha\beta$-unsaturated ketone, position C_8 for the second hydroxyl group is also eliminated. The only positions left for the secondary hydroxyl group are C_1, C_2 and C_3 and positions C_{16} and C_{18} for the primary hydroxyl group.

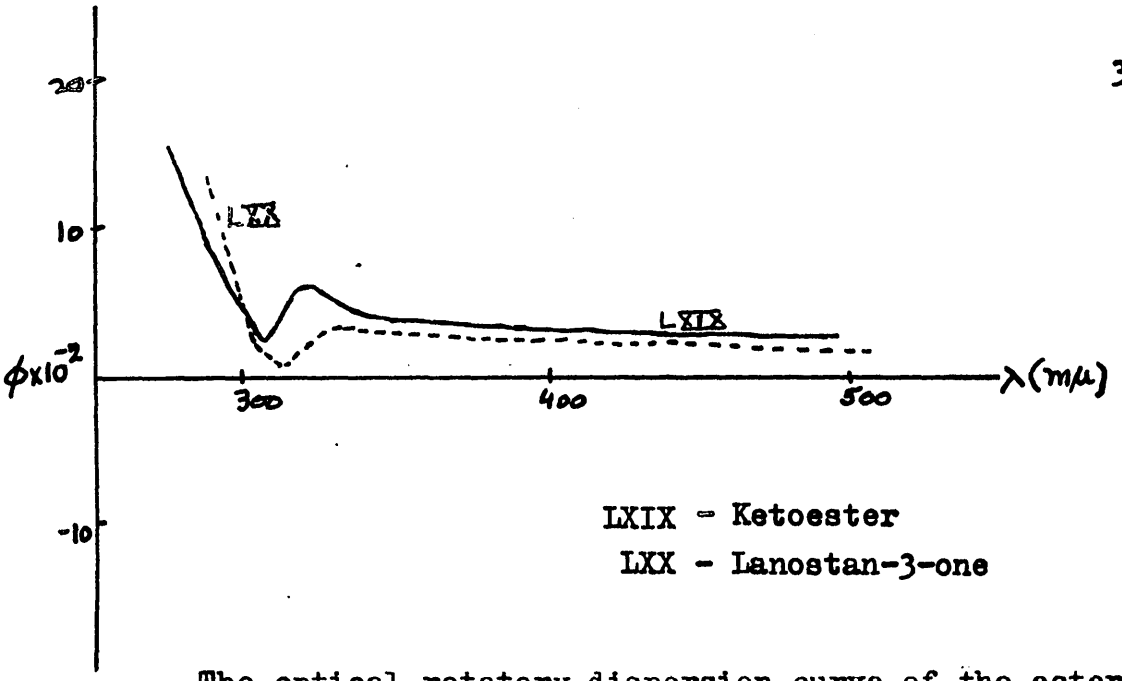
The mass spectra of diol I, diol II, dihydrodiol and the norketone show a ready elimination of 31 mass units which could be due to CH_2OH or OMe but only CH_2OH is present. The next important elimination is of 45 mass units, which must be due to $CH_2.CH_2OH$ and since this is very readily eliminated, the grouping $CH_2.CH_2OH$ must be joined at the branch in the side chain. The primary hydroxyl group must therefore be at C_{18} because a hydroxyl group attached to C_{16} would not give this pattern of cleavage.

Also, the fact that the infrared spectrum of the methyl ester (LXVIIb) is identical with that of methyl dihydrocativate and that the specific rotation of the acid (LXVIIa) agrees within experimental error with that of dihydrocativic acid supports the position assigned to the primary hydroxyl group at C_{15}.

The mass spectra of diol I and dihydrodiol showed a ready elimination of the grouping $C_8H_{12}O$ which is a very common type of fission encountered in diterpenes. This would agree with the location of a hydroxyl group in ring A at positions C_1, C_2 or C_3. C_1 is an unlikely position on biogenetic grounds, moreover a substituent at C_1 would lead to a crowded molecule unlikely to exhibit abundant parent molecular ions and since these are apparent, positions C_2 and C_3 are more likely.

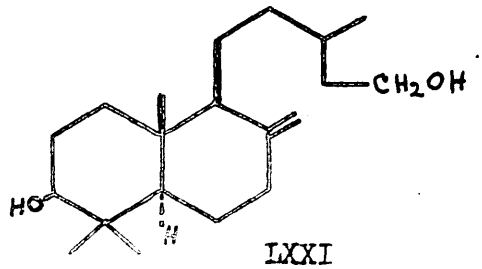
The mass spectrum of the ketoester shows that the loss of methyl groups is less marked and this would be expected if a carbonyl group occupied a position adjacent to the gem dimethyl group.

Evidence in support of position C_3 for the secondary hydroxyl group came from optical rotatory dispersion measurements.



LXIX - Ketoester
LXX - Lanostan-3-one

The optical rotatory dispersion curve of the ester with a carbonyl group at C_2 would be expected to exhibit a positive Cotton effect curve (amplitude +70) and the ester with a carbonyl function at C_3 should exhibit a small negative Cotton effect curve (amplitude about -15). The optical rotatory dispersion curve of the ketoester (LXVIb) shows a small negative Cotton effect curve (LXIX) [amplitude - 14] and very closely resembles that of lanostan-3-one (LXX) and therefore the secondary hydroxyl group is placed at C_3. The diol I can now be represented by (LXXI).



LXXI

EXPERIMENTAL

All melting points are uncorrected. Specific rotations were determined in chloroform solution unless otherwise stated using a one decimeter tube at room temperature. Ultraviolet spectra were determined in ethanol solution and infrared spectra in Nujol mulls unless otherwise stated. Grade II alumina was used for chromatography and light petroleum refers to the fraction of b.p. 60-80°.

Light Petroleum Extraction of Bark.

The crushed bark of Araucaria imbricata (6 lb) was extracted continuously with hot light petroleum for 24 hr. and the extract (92 g.) was saponified with 10% methanolic potassium hydroxide (900 ml.) under reflux for 4 hr. Working up in the usual way through ether gave the nonsaponifiable matter (22.1 g.) which was dissolved in hot ethyl acetate (150 ml.) and left overnight when ceryl alcohol separated out. This was filtered off (6.1 g.) and the remaining nonsaponifiable portion (16 g.) chromatographed on alumina (480 g.) from benzene, benzene-ether (1:1), benzene-ether (1:4), ether, ether containing methanol (1%) and ether containing methanol (5%).

The following fractions were collected:

Fraction number	Eluant	Weight (g)
1 - 2	benzene	0.30
3 - 6	benzene-ether (1:1)	1.00
7—15	benzene-ether (1:4)	6.11
16—26	ether	2.19
27—32	ether-methanol (1%)	3.36
33—37	ether-methanol (5%)	0.84
		13.80

β-sitosterol.

Fractions 16-26, eluted by ether gave β-sitosterol (0.9 g.) as plates from chloroform-methanol, m.p. and mixed m.p. 136-137°, $[\alpha]_D$ -36°. Infrared comparison with an authentic sample confirmed identity.

Diol I.

Fractions 25-30, eluted by ether-methanol (1%) on crystallisation from chloroform-light petroleum gave diol I (1.02 g.) as needles, m.p. 114°, $[\alpha]_D$ +29°, showing infrared absorption bands at 3650 and 3440 $cm.^{-1}$ (hydroxyl), 1640 and 890 $cm.^{-1}$ (vinylidene) and doublet at 1385 and 1365 $cm.^{-1}$ (gem dimethyl), $\lambda_{max.}$ 206 mμ (ϵ, 5,000).

Hydrogenation of diol I.

Diol I (1.5 g.) was dissolved in ethyl acetate* (75 ml.) and hydrogenated in presence of freshly reduced platinum oxide, and was shaken overnight when 75 ml. hydrogen was absorbed. The solution was filtered through a sintered glass funnel and taken to dryness. The residue on crystallisation from

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* The ethyl acetate used for hydrogenation was washed successively with 5% sodium carbonate and water, dried over anhydrous calcium chloride (2 hr.) and distilled.

chloroform-light petroleum gave dihydrodiol (1.1 g.) as needles, m.p. 122-123°, $[\alpha]_D$ +39° (c,1.0), $\nu_{max.}$ 3640 cm.$^{-1}$ (hydroxyl). The compound showed no absorption in the ultraviolet.

(Found: C,77.6; H,12.5; $C_{20}H_{38}O_2$ requires C,77.4; H,12.4%).

Acetylation of the dihydrodiol.

The dihydrodiol (0.95 g.) in pyridine (5 ml.) was acetylated with acetic anhydride (5 ml.) on a steam bath for 2 hr. The product was worked up in the usual way to give the gummy acetate (1.1 g.). This was dissolved in benzene and filtered through alumina. Crystallisation from aqueous methanol gave the diacetate as needles, m.p. 78°, $[\alpha]_D$ +24°, (c,1.0) $\nu_{max.}$ 1730, 1242 and 1260 cm.$^{-1}$ (acetate), no absorption in the ultraviolet.

(Found: C,71.6; H,10.7; $C_{24}H_{42}O_4 \cdot \frac{1}{2} CH_3OH$ requires C,71.7; H,10.7%).

Partial hydrolysis of the diacetate.

The diacetate (1.09 g.) was taken up in methanol (200 ml.) and hydrolysed with one equivalent of anhydrous sodium carbonate (0.146 g.) dissolved in the minimum amount of

distilled water. The reaction mixture was left at room temperature for 24 hr., diluted with water, and the methanol distilled off under reduced pressure. Extraction with ether, washing and drying (sodium sulphate) gave a crude product (0.91 g.) which was purified by chromatography on alumina (30 g.) from light petroleum, light petroleum-benzene (1:1), benzene, benzene-ether (9:1), ether and ether containing 1% methanol. Twelve fractions (each 60 ml.) were collected.

Fractions 3-9, eluted by benzene and benzene-ether (9:1) on crystallisation from light petroleum gave the monoacetate (0.5 g.) as needles, m.p. 79-80°, $[\alpha]_D$ +26.4°, (c,1.0) $\nu_{max.}$ 3540 cm.$^{-1}$ (hydroxyl), 1725 and 1270 cm.$^{-1}$ (acetate).

(Found: C,74.8; H,11.5; $C_{22}H_{40}O_3$ requires C,75.0; H,11.4%).

Oxidation of the monoacetate.

The monoacetate (0.41 g.) was dissolved in acetone (20 ml.) and Kiliani mixture[x] (1.4 ml.) was added dropwise

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[x] Kiliani mixture. Sodium dichromate (17.62 g.) in concentrated H_2SO_4 (15 ml.) made up to 100 ml. with water.

in course of 0.5 hr. with stirring. The reaction mixture was stirred for a further 1.5 hr. and then poured into an excess of water. This was extracted with ether and the ether extract washed successively with water, aqueous sodium carbonate (5%) and water and worked in the usual way to give the neutral oxidation product (0.03 g.).

The alkaline washings were acidified with dilute hydrochloric acid, extracted with ether and worked in the usual manner to give the acetoxy acid as needles (from aqueous acetone), m.p. 93-94°, $[\alpha]_D$ +17° (c,1.0), $\nu_{max.}$ 1735 and 1280 cm.$^{-1}$ (acetate) and 1710 cm.$^{-1}$ (carboxyl).
(Found: C,72.0; H,10.7; $C_{22}H_{38}O_4$ requires
C,72.1; H,10.5%).

Cyclohexylamine salt of acetoxy acid.

The acetoxy acid (0.07 g.) in ethyl acetate (2 ml.) was treated with cyclohexylamine (0.03 ml.), warmed on a steam bath and on cooling for 24 hr. at 0° gave the cyclohexylamine salt as blades (from chloroform-ethyl acetate), m.p. 96-105°, $[\alpha]_D$ +10° (c,0.5), no absorption in the carboxyl region in the infrared spectrum.
(Found: C,72.9; H,10.9; N,3.3; $C_{28}H_{51}O_4N$ requires
C,72.2; H,11.0; N,3.0%).

Hydrolysis of the acetoxy acid.

The acetoxy acid (0.3 g.) was hydrolysed with 5% methanolic potassium hydroxide (10 ml.) for 2 hr. under reflux. This was diluted with excess of water and acidified with dilute hydrochloric acid. Isolation of the product through ether gave the hydroxy-acid (0.24 g.) as needles (from chloroform-light petroleum), m.p. 154-156°, $[\alpha]_D$ +20° (c,1.0), $\nu_{max.}$ 3450 (hydroxyl) and 1716 cm.$^{-1}$ (carboxyl).

(Found: C,73.7; H,11.0; $C_{20}H_{36}O_3$ requires C,74.0; H,11.2%).

Methylation of hydroxy-acid.

The hydroxy-acid (0.22 g.) in ether (5 ml.) was treated with an excess of ethereal diazomethane and the mixture left at room temperature for 24 hr. Isolation of the product in the usual manner through ether gave the methyl ester of hydroxy-acid (0.23 g.) as needles (from light petroleum), m.p. 74°, $[\alpha]_D$ +29° (c,0.5), $\nu_{max.}$ 3540 cm.$^{-1}$ (hydroxyl), 1735 cm.$^{-1}$ (ester) and doublet at 1360 and 1380 cm.$^{-1}$ (gem dimethyl).

(Found: C,74.9; H,11.7; $C_{21}H_{38}O_3$ requires C,74.5; H,11.3%).

Oxidation of hydroxy methyl ester.

A solution of the hydroxy methyl ester (0.28 g.) in pyridine (5 ml.) was added to chromium trioxide-pyridine complex [prepared by adding chromium trioxide (0.3 g.) to pyridine (5 ml.)] and the mixture left at room temperature for 24 hr. Methanol (5 ml.) was added to destroy the excess of chromium trioxide complex and after dilution with water, the solution was extracted in the usual manner with ether. Removal of ether under reduced pressure gave an oily residue (0.24 g.) which was purified by chromatography on neutral alumina (1.5 g.) to yield the ketoester (0.14 g.). No hydroxyl absorption in the infrared spectrum and a broad band at 1740 $cm.^{-1}$ with a shoulder at 1720 $cm.^{-1}$ (carbonyl).

Wolff-Kishner reduction of the ketoester.

The ketoester (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 180-185°) for 5 hr. The excess hydrazine was distilled off and the reaction mixture refluxed for 5 hr. (external temperature 225-230°). The product after cooling was diluted with water, acidified with dilute hydrochloric acid and extracted with ether. It was worked in the usual manner to give dihydro-

cativic acid (0.05 g.) which failed to crystallise.

The acid (0.05 g.) was esterified with ethereal diazomethane (5 ml.), worked up in the usual way and chromatographed on neutral alumina (1.5 g.). Elution with light petroleum gave a fraction (0.028 g.) which failed to crystallise. $\nu_{max.}$ 1716 cm.$^{-1}$ This was hydrolysed back to dihydrocativic acid $[\alpha]_D$ +22.5° (c,0.4); Grant and Zeiss[5] give $[\alpha]_D$ +25.4°.

Preparation of the thioketal from the ketoester.

The ketoester (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 and washed with sodium carbonate solution (5%) to remove excess of ethanedithiol. The product was isolated in the usual way and purified by filtration through alumina (2 g.) to give a clear gum which contained sulphur.

Desulphurisation of thioketal.

The clear gum (0.05 g.) in absolute alcohol (20 ml.) was refluxed with Raney nickel (0.5 g.) for 10 hr. The Raney nickel was filtered off, and the product evaporated to dryness to yield a gum (0.04 g.) which was further purified

by filtration through alumina. The resinous product failed to crystallise but its infrared spectrum was identical to that of an authentic sample of methyl dihydrocativate.

Tosylation of the methyl ester of the hydroxy acid.

The hydroxy methyl ester (0.13 g.) in pyridine (2 ml.) was tosylated with p-tosylchloride (0.45 g.) dissolved in pyridine (2 ml.) and left at 5° for 24 hr. This was diluted with water, extracted with benzene, washed, dried (sodium sulphate), concentrated, cooled and filtered through a column of alumina (5 g.) to give a gum (0.17 g.).

The tosylated product gave a positive test for sulphur $\nu_{max.}$ 1725, 1600, 1450, 1349 and 1170 $cm.^{-1}$ (indicative of a tosyl ester).

Attempted detosylation with lithium aluminium hydride.

The tosylate (0.1 g.) in tetrahydrofuran (5 ml.) was refluxed with lithium aluminium hydride (0.15 g.) in tetrahydrofuran (15 ml.) for 12 hr. The excess of lithium aluminium hydride was destroyed by adding small pieces of ice and the product isolated in the usual way through ether. Crystallisation from chloroform-light petroleum gave dihydro-

diol as needles and its identity was established by mixed m.p. and infrared comparison with an authentic sample.

S E C T I O N II(a)

Extractives from the Woods of Dipterocarpus Crinitus and Araucaria Brasiliensis.

The benzene extract of the wood of *Dipterocarpus crinitus* has yielded betulin while that of *Araucaria brasiliensis* has not given any terpenic constituent. Both the extracts gave β-sitosterol and ceryl alcohol.

The Dipterocarpaceae are an important family of tropical trees growing abundantly in southeast Asia particularly India, Ceylon, Indonesia, Malaya, and East Indies and are characterized by secretion of resins and oleoresins. These exudations have often economic importance, gurjun being obtained from Dipterocarpaceae, dammar resins from Shorea and Hopea, and borneol from Dryobalanops.

Glimmann[36] first examined the dammar resins systematically. Mills[37,38] later carried out exhaustive investigations on the chemistry of dammar resins and found hydroxydammarenone-II as one of the constituents of the neutral part, which had been first isolated by van Itallie[39] from the balsams of D. hasseltii and D. trinervis. King et al[40] isolated the same triterpene in several varieties of Dipterocarpus woods and showed hydroxydammarenone-II to be identical with dipterocarpol isolated by Ourisson et al[41,42] and McLean and Watts[43] from Dipterocarpus species D. dyeri, D. alatus, D. intricatus, D. atrocarpifolius and D. verrucosis, and D. grandiflorus respectively.

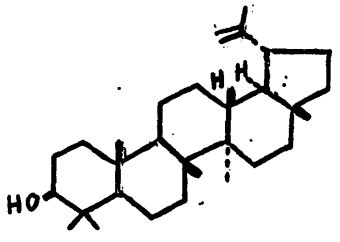
The extraction of Araucaria imbricata bark had given a new diterpene diol and since Parana pine (Araucaria brasiliensis) belongs to the same species and was readily

available, it was decided to investigate the constituents of the latter in the hope that it might be a more convenient source of the diol, but the only products isolated were ceryl alcohol and β-sitosterol.

Some reactions of betulin.

Dipterocarpus crinitus has yielded betulin as the major constituent of the neutral part of the benzene extract and, therefore, some reactions of betulin are given in the following pages.

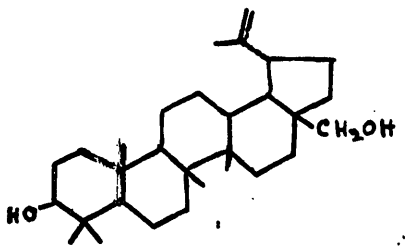
Betulin belongs to the pentacyclic group of triterpenoids, subgroup lupeol. Lupeol itself has been assigned the structure (LXXII).[44-48]



LXXII

Betulin (LXXIII) forms a diacetate and a dibenzoate showing the presence of two alcoholic oxygen functions. The diacetate on partial hydrolysis gives a monoacetate which on

oxidation with chromic acid gives an aldehyde and on further oxidation an acetyl-acid, identical with the acetate of the naturally occurring betulinic acid. These products contain the same number of carbon atoms indicating that the hydroxyl group is primary.



LXXIII

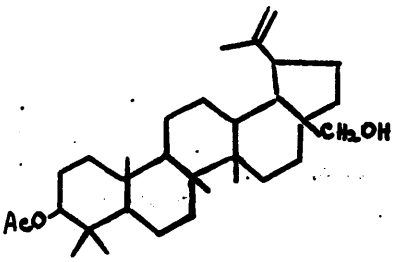
The semicarbazone of the aldehyde on treatment with ethanolic sodium ethoxide gives lupeol (LXXII) showing that betulin differs from lupeol only in having a primary hydroxyl group instead of a methyl group.

The monoacetate of betulin was converted into the acetate benzoate, thence into acetate phenylcarbamate which on mild hydrolysis gave the monophenylcarbamate. The monophenylcarbamate, on oxidation gave a ketone, betulone phenylcarbamate, whose oxime on Wolff-Kishner reduction yielded desoxybetulin showing that the less reactive hydroxyl group is secondary.

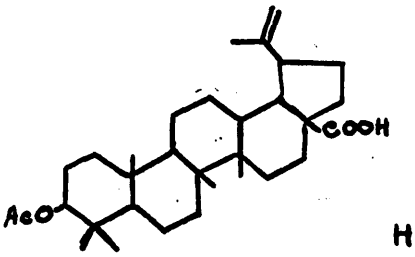
The presence of a double bond was indicated by colouration with tetranitromethane. The diacetate on hydrogenation gave dihydrobetulin diacetate indicating that the double bond was more reactive than that of α- and β-amyrins. It was later shown that the double bond is present as an isopropenyl group and that ring E is five membered.

Relationship with moradiol.

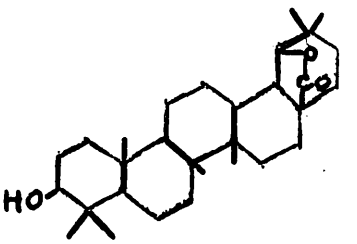
Jones, Meakins and co-workers[49-52] thereafter established the relationship between betulin and moradiol whose structure was known. Betulin monoacetate (LXXIV) on chromic acid oxidation gave acetyl betulinic acid (LXXV) which, under the influence of acid, was converted into a hydroxy-lactone (LXXVI) and then oxidised to a ketolactone (LXXVII). This ketolactone on reduction with lithium aluminium hydride gave a triol which afforded a diacetate (LXXVIII) on mild acetylation. The diacetate, on dehydration with phosphorus oxychloride in pyridine, gave moradiol acetate (LXXIX) which, on hydrolysis, afforded moradiol (LXXX).



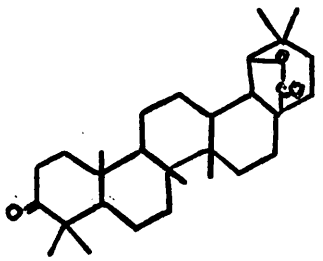
LXXIV



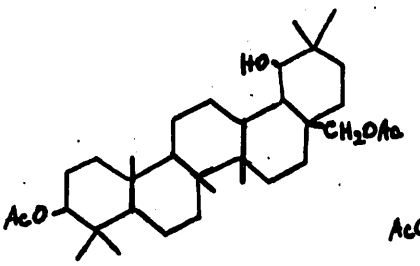
LXXV



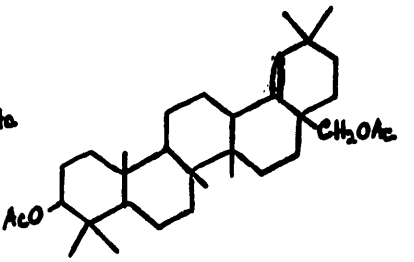
LXXVI



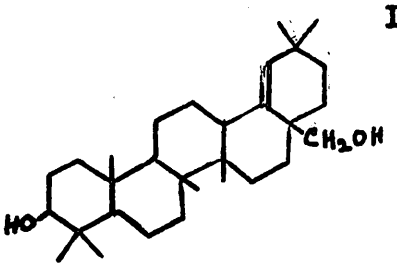
LXXVII



LXXVIII



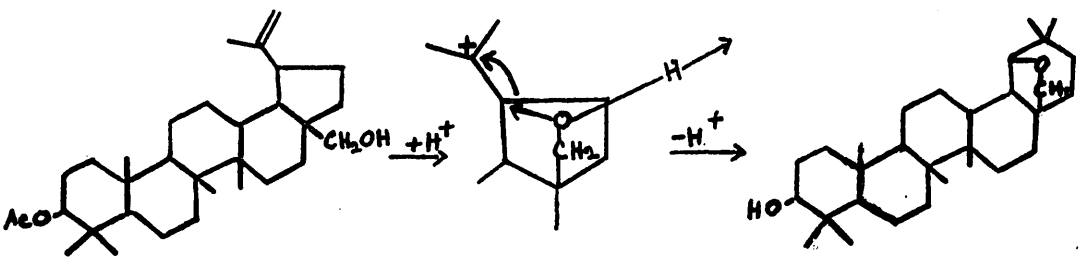
LXXIX



LXXX

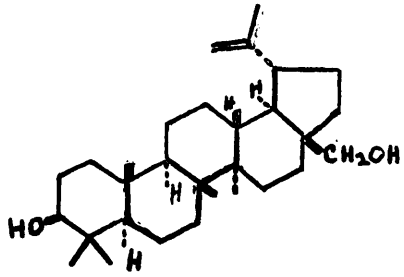
The above reactions showed that the primary hydroxyl group of betulin is at the same position as the carboxyl group of oleanolic acid i.e. C_{17}. This also proved that the ring junction at D and E is trans.

Betulin, when heated with 90% formic acid, gave a monoformate, which, on hydrolysis, afforded a saturated monhydric alcohol, allobetulin, isomeric with betulin. The formation of allobetulin involves the interaction of the primary hydroxyl group with the isopropenyl group because betulin-3-monoacetate is isomerised to allobetulin, while the corresponding dihydro-compound is not isomerised. The isomerisation involves the formation of the carbonium ion (LXXXI) which undergoes ring enlargement and loss of a proton to give allobetulin (LXXXII). Betulin is represented by

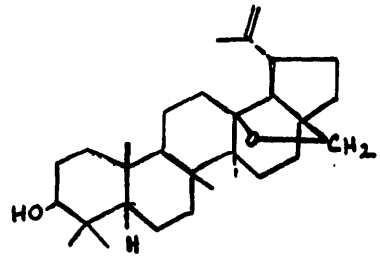


LXXIV LXXXI LXXXII

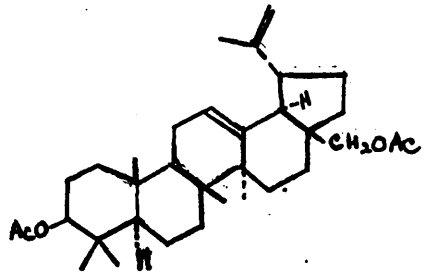
the stereochemical formula (LXXXIII). Recently[83] it has been shown that dehydrogenation of betulin with mercuric acetate results in the formation of the cyclic ether (LXXXIV) whereas betulin diacetate under similar conditions forms the nonconjugated diene (LXXXV).



LXXXIII



LXXXIV



LXXXV

EXPERIMENTAL

All melting points are uncorrected. Specific rotations were determined in chloroform solution at room temperature. Ultraviolet absorption spectra were measured in ethanol solution and infrared spectra in Nujol mulls (unless otherwise stated). Grade II alumina and light petroleum (b.p. 60-80°) were used for chromatography.

Benzene extraction of wood.

The wood (4 lb.) of *Dipterocarpus crinitus* in the form of shavings was extracted continuously with hot benzene for 30 hr. and the extract (62 g.) was saponified with 10% methanolic potassium hydroxide (600 ml.) under reflux for 6 hr. The hydrolysis liquor was diluted with much water and extracted with ether to give the nonsaponifiable fraction A (19.5 g.).

The soap solution was acidified with dilute hydrochloric acid and worked up to yield solid acid (3.9 g.) m.p. 74-75° which appears to be saturated fatty acids.

Treatment of nonsaponifiable fraction (A).

The nonsaponifiable portion was taken up in hot benzene and on standing overnight deposited a solid which gave a positive Liebermann-Burchard test for triterpenes. The remaining benzene solution was chromatographed on alumina (550 g.) using benzene, benzene-ether, ether and ether-methanol as eluants. The chromatogram is summarised below:

Fraction Number	Eluant	Weight (g.)	Characteristics
1 - 9	benzene	3.67	pale yellow oil
10 - 23	benzene-ether	3.39	pale yellow oil

Fraction Number	Eluant	Weight (g.)	Characteristics
24-35	benzene-ether (7:3)	2.08	gum
36-45	benzene-ether (1:1)	1.06	gum
46-52	ether	0.42	-
53-57	ether-methanol (1%)	0.08	-
58-77	ether-methanol (5%)	3.16	dark brown gum

Fraction insoluble in benzene.

The fraction insoluble in benzene (1.4 g.) on crystallisation from methanol gave betulin, as needles, m.p. 244-248°, $[\alpha]_D$ +14° (c,1.0), showing infrared absorption bands at 3390 $cm.^{-1}$ (hydroxyl) and 1647 and 885 $cm.^{-1}$ (vinylidene). Infrared comparison with an authentic sample confirmed identity.

Preparation of betulin diacetate.

Betulin (0.16 g.) in pyridine (2 ml.) was acetylated with acetic anhydride (2 ml.) on a steam bath for 3 hr. The product was worked in the usual way to give the crude acetate which was dissolved in light petroleum and filtered through alumina. Removal of light petroleum gave betulin diacetate (0.06 g.) as needles, from methanol, m.p. and mixed

m.p. 218-220°, $[\alpha]_D$ +22° (c,1.0). Infrared comparison with an authentic sample confirmed identity.

Fractions 1-9 Ceryl alcohol.

These fractions, eluted with benzene gave ceryl alcohol m.p. and mixed m.p. 78-79°. Infrared comparison with an authentic sample confirmed identity.

Fractions 10-23 β-sitosterol.

These fractions, eluted with benzene-ether (9:1) gave β-sitosterol as plates from chloroform-methanol, m.p. and mixed m.p. 136-137°, $[\alpha]_D$ -34° (c,1.0). Infrared comparison with an authentic specimen confirmed identity.

β-sitosteryl acetate.

Acetylation of β-sitosterol (0.04 g.) in pyridine-acetic anhydride and working up as usual gave β-sitosteryl acetate (0.02 g.) as blades from chloroform-methanol, m.p. and mixed m.p. 125-126°, $[\alpha]_D$ -34° (c,1.0).

Fractions 24-25 Betulin.

These fractions, eluted with benzene-ether (7:3) gave impure betulin as needles from methanol, m.p. 245-247°. It was acetylated in the usual way and chromatographed on

alumina to give betulin diacetate, as needles from methanol m.p. and mixed m.p. 218-220°, $[\alpha]_D$ +22.5 (c,1.0). Infrared comparison with an authentic specimen confirmed identity.

Fractions 61-64.

These fractions, eluted with ether containing 5% methanol failed to yield a crystalline material. The infrared spectrum in chloroform showed bands at 3390 $cm.^{-1}$ (hydroxyl) and 1720 $cm.^{-1}$ (carbonyl) but attempts to obtain crystalline derivatives by acetylation and by formation of the usual carbonyl derivatives failed.

Fractions 66-67.

These fractions, eluted with ether containing 5% methanol gave a crystalline compound (0.1 g.) as needles from chloroform-light petroleum, m.p. 151-152°, showing strong hydroxyl absorption in the infrared (3340 $cm.^{-1}$). There was no absorption in the ultraviolet.

(Found: C,74.3; H,10.7; $C_{20}H_{34}O_3$ requires C,74.5; H,10.6%).

Preparation of the acetate.

The above compound was acetylated in the usual manner to give a diacetate as needles from light petroleum, m.p. 98-99°.

(Found: C,71.2; H,9.8; $C_{24}H_{38}O_8$ requires C,70.9; H,9.4%).

There was too little of the material to permit further investigation.

Benzene extraction of Araucaria brasiliensis.

The wood shavings (5 lb.) of Parana pine (Araucaria brasiliensis) were extracted continuously with hot benzene for 24 hr. and the extract (36 g.) was saponified with 10% methanolic potassium hydroxide (350 ml.) for 4 hr. under reflux. Working up in the usual way through ether gave the nonsaponifiable portion (4.5 g.) which was dissolved in benzene-light petroleum (7:3) and chromatographed on alumina (150 g.). The following fractions were collected.

Fraction number	Eluant	Weight (g.)
1 - 7	benzene-light petroleum (7:3)	1.00
8 - 14	benzene	1.05
15 - 23	benzene-ether (1:1)	0.60
24 - 27	ether	0.67
28 - 35	ether-methanol (5%)	0.22
36 - 41	ethyl acetate	0.49
		4.03

Ceryl alcohol.

Fractions 5-6, eluted with benzene and light petroleum (7:3) gave ceryl alcohol, m.p. and mixed m.p. 78-79°. Infrared comparison with an authentic sample confirmed identity.

β-sitosterol.

Fractions 10-12, eluted with ether gave β-sitosterol as plates from chloroform-methanol, m.p. and mixed m.p. 135-136°, $[\alpha]_D$ -34° (c,1.0). Infrared comparison with an authentic specimen confirmed identity.

Fractions 25-27.

These fractions, eluted with ether gave a crystalline compound as blades from chloroform-light petroleum, m.p. 180-190°, $[\alpha]_D$ -60° (c,1.2), infrared absorption bands at 3278 $cm.^{-1}$ (hydroxyl). The ultraviolet spectrum showed absorption at 206 mμ. (double bond).

The compound gave a positive Liebermann-Burchard test for steroids and when tested on a silica gel chromatoplate against β-sitosterol, showed a much lower R_f value, indicating the presence of more than one hydroxyl group.

The compound was acetylated in the usual manner, but no crystalline acetate could be isolated and further investigation was abandoned due to lack of material.

S E C T I O N II(b)

Thin-Layer Chromatography of Triterpenes.

It has been found that triterpenes having different functional groups, for example, alcohols, ketones and esters can be separated by thin-layer chromatography but individual members of the same class can not be separated. Triterpene alcohols can be separated from steroid alcohols.

Introduction.

There does not appear to be any reference to the separation and identification of triterpenes by thin-layer chromatography and it was therefore decided to investigate the possibilities of this new technique as a means of separating and identifying triterpene alcohols, ketones and esters. If successful, this method should prove useful in a rapid preliminary examination of the nonsaponifiable portion of plant extracts.

Kirchner, Miller and Keller[54-56] were the first to describe the technique of adsorption microchromatography for the separation and identification of terpenes. Reitsema[57,58] and Stahl[59,60] modified the method and a review on the application of chromatoplates and chromatostrips for the separation of various organic compounds has been published by Demole.[61]

The separations on these chromatostrips are largely effected by the degree of activation of the adsorbent as well as its thickness. These factors can be kept constant by the use of an apparatus which can produce layers of uniform thickness and by drying the plates under standard conditions. Results reproducible within $\pm$ 0.05 R_f values have been obtained.

Furukawa[62] and Stahl[63] have recently studied the factors affecting the R_f values in stripchromatography viz. particle size of silicic acid, thickness of layers, water content of adsorbent films and border effect.

Thin-layer chromatography is a rapid method for checking solvents and adsorbents for use in preparative chromatography. It also permits the characterisation of various constituents in a single microchromatogram using very small quantities of mixture. The chromatoplates are most suitable for lipid soluble materials of moderate polarity, the time required for development being short (30-40 minutes) and the adsorbent can withstand strong detecting agents. Sometimes microchemical reactions can also be carried out on these plates for example, oxidation, reduction and preparation of derivatives which give a more satisfactory identification of compounds than is possible on the basis of R_f values alone. The use of chromatoplates also offers a rapid method for checking the homogeneity of a particular fraction during column chromatography. Columns are in fact less sensitive than chromatoplates due to diffusion bands in the former.

Discussion of results.

It was noticed that in some pure solvents, for example, ethyl acetate (Table I) the compounds tested moved approximately the same distance while with chloroform (Table I) although different compounds moved through different distances, the distances moved were not sufficiently great to enable accurate R_f values to be calculated hence, mixed solvents for example, benzene containing 1% methanol or n-hexane containing ethyl acetate (20%) were used (Table II).

The experiments show that monohydric triterpene alcohols have fairly constant R_f values in a particular solvent. This also holds for members of other classes shown in Table II. Consequently members of a class cannot be separated from one another by thin-layer chromatography but monohydric alcohols may be separated from dihydric alcohols by this method; thus lupeol and betulin are readily separated (Table IIIa) whereas α-amyrin and cycloeucalenol can not be separated and attempts lead to mixed spots appearing in the chromatostrips even when a wide range of solvents was employed (Table IV).

Monohydric steroids have R_f values which are considerably lower than that of monohydric triterpene alcohols and higher

than dihydric triterpene alcohols and consequently can be separated from the latter (Table IIIb). Similarly, steroid ketones have R_f values which are lower than those of triterpene ketones and separation can be effected (Table IIIc)

From the R_f values (Table II), it appears that the polarity of triterpenes follow the order to be expected namely carboxylic acids, dihydric alcohols, monohydric alcohols, ketones and esters in descending order of polarity.

EXPERIMENTAL

Preparation of chromatoplates.

30 g. silica gel (E. Merck for thin-layer chromatography) was shaken with distilled water (60 ml.) for 30 seconds, poured in the sprayer, and the plates (5 x 20 cm.) sprayed with the emulsion immediately. The sprayer gives a fairly uniform layer of thickness (250-300 μg.). The plates were dried in an air oven at 105-110° for four hours and kept in a desiccator until ready for use.

Solvents used.

Benzene, ether and n-hexane were dried over sodium wire; ethyl acetate was washed with a solution of sodium carbonate, then with water, dried over anhydrous calcium chloride and distilled; chloroform was washed with water, dried over anhydrous calcium chloride and distilled, methanol and ethanol were dried over magnesium alkoxide and distilled.

Development of the chromatogram.

The plates were marked 1.5 cm. from one end and a solution of a few μg. of the compound under investigation in light petroleum or benzene was applied on the marked spot, by means of a melting point tube, keeping the distance between each spot at least 1 cm. In this way three spots

were placed on one plate (5 cm. x 20 cm.). The marked plate was dipped into the developing solvent (50 ml.) in a battery jar such that the marked spots are just above the solvent level. The jar contained a filter paper soaked in the same solvent and kept on the inner side of the jar to check the border effect.

The solvent front moved a distance of 10-12 cms. in 30-45 minutes after which the solvent front was marked. The plate was removed from the jar, dried over a hot plate to evaporate the solvent, cooled in a desiccator and tested for the movement of spots.

Detection of spots.

The plates were sprayed with a 20% solution of antimony pentachloride (redistilled) in redistilled carbon tetrachloride which served to locate the compounds as brownish pink spots. Initial difficulties arising from the choking of the sprayer nozzle were overcome by applying the reagent with a fine dropping tube. The R_f values have been calculated in the usual way.

Table I.

Rf values of betulin and lupeol.

Solvent	Distance travelled by solvent front (cm.)	Lupeol (cm.)	Rf value	Betulin (cm.)	Rf value
1. Chloroform	10.5	2.5	0.23	0.5	0.047
2. Benzene	11.0	0.6	0.054	<1	-
3. Ethyl acetate	12.0	7.4	0.61	8.1	0.68

Table II.

Rf values of triterpenes and steroids.

	Solvent benzene-methanol (1%)	Solvent n-Hexane-ethyl acetate (20%)
I. Monohydric alcohols.		
1. α-Amyrin	0.37-0.40	0.40
2. β-Amyrin	0.39-0.44	0.44-0.46
3. Germanicol	0.39-0.42	0.41
4. Lupeol	0.36-0.40	0.41
5. Tirucallol	0.39	-
6. Cycloeucalenol	0.37-0.38	-
7. Lanost-7-en-3β-ol	0.33-0.34	-
8. Allobetulin	0.30	0.40
9. Dipterocarpol	0.34-0.38	0.37

	Solvent benzene-methanol (1%)	Solvent n-Hexane-ethyl acetate (20%)
II. Dihydric alcohols.		
1. Betulin	0.18-0.20	0.19-0.20
2. Erythrodiol	0.19	0.21
3. Brein	0.16	-
III. Ketones.		
1. α-Amyrenone	0.62-0.65	0.62
2. δ-Amyrenone	0.65-0.66	-
3. Lupenone	0.67	-
4. Cycloeucalenone	0.68	-
5. Euphenone	0.68	-
IV. Esters of monohydric alcohols.		
1. Euphenyl acetate	0.72-0.74	-
2. α-Amyrin acetate	0.70	-
3. δ-Amyrin acetate	0.66	-
V. Carboxylic acids		
1. β-Boswellic acid	0.04	-
2. Oleanolic acid	0.05	-
VI. Steroids		
1. β-Sitosterol	0.26-0.27	0.24-0.26
2. Cholestanone	0.46-0.48	-

Table III

Separation of mixtures containing monohydric and dihydric alcohols.

(a)

Solvent	R_f values				Remarks
	Lupeol	Betulin	β-Amyrin	Erythrodiol	
1. Ethyl acetate	0.65-0.66	0.64-0.68	-	-	No separation.
2. Benzene-methanol (5%)	0.51	0.26	-	-	Separated.
3. Benzene-methanol (1%)	0.42	0.18	0.44	0.22	Readily separated.
4. n-Hexane-ethyl acetate (20%)	0.44	0.19	0.46	0.20	Readily separated.

(b) **Separation of a mixture containing sterol and triterpene alcohol.**

	β-Sitosterol	Betulin	β-Amyrin	
Benzene-methanol (1%)	0.27	0.19	0.39	Readily separated.

(c) **Separation of a mixture of steroid and triterpene ketone.**

	Cholestanone	α-Amyrenone	
Benzene-methanol (1%)	0.48	0.62	Readily separated.

Table IV.

Separation of α-amyrin and cycloeucalenol.

Solvent system	Rf value		
	α-Amyrin	Cycloeucalenol	Mixture
1. n-Hexane-ethyl acetate (20%)	0.40	0.36	mixed spot.
2. n-Hexane-ethyl acetate (25%)	0.44	0.38	mixed spot
3. n-Hexane-ethyl acetate (40%)	0.56	0.51	mixed spot
4. n-Hexane-ethyl acetate (70%)	0.58	0.54	mixed spot
5. Benzene-methanol (2%)	0.41	0.35	mixed spot
6. Benzene-ethyl acetate-ethanol			
45 10 3	0.68	0.64	mixed spot

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