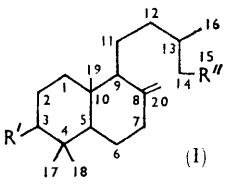
705. *New Diterpenes from* Araucaria imbricata.

By G. Chandra, J. Clark, J. McLean, P. L. Pauson, and J. Watson, with *an addendum* by R. I. Reed and (Miss) F. M. Tabrizi.

From a light-petroleum extract of the bark of *Araucaria imbricata* two new diterpenes have been isolated and characterised. These are labd-8(20)-ene-3β,15-diol (Ia) and 3β-hydroxylabd-8(20)-en-15-oic acid (Ic). The corresponding 15-aldehyde is also shown to be present.

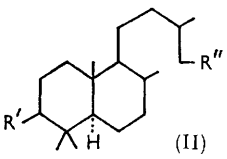
The *Araucaria* are a genus of coniferous trees found mainly in South America and Australia. They were introduced into Britain about two hundred years ago, and the hardier varieties withstand the winter. *A. imbricata* Pav. is grown for ornamental purposes and is popularly known as " monkey puzzle " or Chile pine.

Extraction of the powdered bark with light petroleum yields a gum. From the non-saponifiable portion of the latter a diol (Ia) is isolated along with an aldehyde (Ib) which is separated with Girard reagent T. The saponifiable fraction yields, after acidification, the



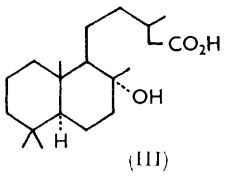
(I)

	R′	R″
a	OH	CH_2·OH
b	OH	CHO
c	OH	CO_2H
d	OAc	CH_2·OAc
e	OH	CO_2Me

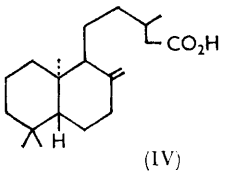


(II)

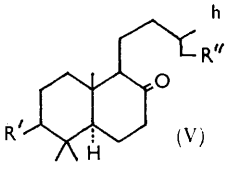
	R′	R″
a	OH	CH_2·OH
b	OAc	CH_2·OAc
c	OAc	CH_2·OH
d	OAc	CO_2H
e	OH	CO_2H
f	H	CO_2H
g	OH	CH_2·OAc
h	OH	CO_2Me



(III)

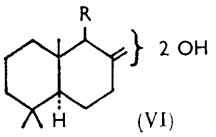


(IV)



(V)

	R′	R″
a	H	CO_2H
b	OH	CH_2·OH



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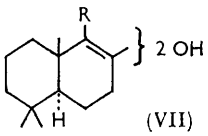

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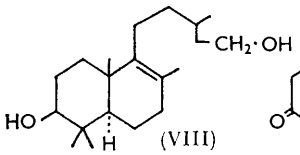
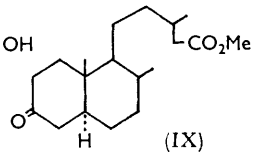


(VIII)



(IX)

acid (Ic), the three new compounds differing in the state of oxidation at C_{15}. Examination of the diol by infrared (i.r.) spectroscopy reveals the presence of hydroxyl, a vinylidene group, and a *gem*-dimethyl group, while ultraviolet (u.v.) absorption indicates the presence of an ethylenic bond.

Analysis and molecular-weight data are in agreement with the formula $C_{20}H_{36}O_2$, and a determination of active hydrogen (1·9 atoms per molecule) suggests that both oxygen atoms are present as hydroxyl groups. Hydrogenation of the diol (Ia) gives a saturated dihydro-diol (IIa) with the uptake of one mol. of hydrogen, while ozonolysis affords a

norketone, $C_{19}H_{34}O_3$, and formaldehyde, indicating the presence of a methylene function. Additional evidence for the presence of an olefinic linkage in the diol (Ia) comes from peracid oxidation with formation of a saturated epoxide, $C_{20}H_{36}O_3$. Furthermore, osmic acid reacts with the diol to give a tetraol, $C_{20}H_{38}O_4$, further oxidised by periodic acid to the norketone already obtained with ozone.

The foregoing facts suggest that the diol is a bicyclic compound containing an exocyclic methylene group and two hydroxyl groups. Acid-isomerisation of the diol (Ia) with sulphuric acid in methanol, furnishes an isomeric diol without the vinylidene group, and the absence of olefinic absorption in the i.r. spectrum suggests that the original double bond has moved into a tetrasubstituted position, as is typical of bicyclic terpenes containing an 8(20)-double bond.[1] The isomeric diol can be hydrogenated in acetic acid in presence of platinum to give the same dihydro-diol that is obtained from the natural diol, thus indicating that the isomerisation is not accompanied by any major structural change.

Dehydrogenation of the natural diol (Ia) provides valuable evidence concerning its structure. With palladised charcoal an oil is obtained which shows strong u.v. absorption at 2290 Å, suggestive of the presence of a 1,2,5-trialkylnaphthalene. Dehydrogenation with selenium at 350° again furnishes an oil which forms solid adducts with picric acid and with 1,3,5-trinitrobenzene. These are identical (i.r. spectra and mixed m. p.) with the corresponding adducts prepared from authentic 1,2,5-trimethylnaphthalene,[2] kindly supplied by Dr. K. Overton. Sclareol,[3,5] labdanolic acid,[1] eperuic acid,[4] and manoöl [5] give 1,2,5-trimethylnaphthalene on dehydrogenation, and it appears that the natural diol probably belongs to the group of bicyclic diterpenes typified by labdanolic acid (III) or eperuic acid (IV). Optical-rotatory dispersion data (kindly supplied by Professor W. Klyne) on the norketone (Vb) obtained by ozonolysis of the natural diol (Ia) shows a strong negative Cotton-effect curve (amplitude −132) similar to that exhibited by 8-oxo-20-norlabdan-15-oic acid (Va), and as a first assumption we place the ethylenic bond in the diol as 8(20). Confirmation for the assignment of this position is obtained by comparing molecular-rotation differences accompanying ozonolysis, hydrogenation, isomerisation, and hydroxylation of the diol (Ia) with analogous changes on labd-8(20)-en-15-oic acid. The Table summarises these results. It can be seen that the ΔM_D values are consistent with this location for the double bond, and part structures are suggested for the diol (VI) and for the isomeric diol (VII), the stereochemistry being as shown.

Conversion	ΔM_D
Labd-8(20)-en-15-oic acid [1] ⟶ 8-oxo-20-norlabdan-15-oic acid	−200
Diol (Ia) from *A. imbricata* ⟶ norketone (Vb)	−209
Labd-8(20)-en-15-oic acid [1] ⟶ labd-8-en-15-oic acid	+136
Diol (Ia) from *A. imbricata* ⟶ isomeric diol (VIII)	+154
Labd-8-en-15-oic acid [1] ⟶ labdanoic acid	−112
Isomerised diol (VIII) from *A. imbricata* ⟶ dihydro-diol (IIa)	−120
Labd-8-en-15-oic acid [1] ⟶ 8,9-dihydroxylabdan-15-oic acid	−218
Diol (Ia) from *A. imbricata* ⟶ tetraol	−191

The nature and location of the hydroxyl groups follow from the evidence presented in the sequel. Acetylation and benzoylation of the diol (Ia) result in the formation of oily esters which, from i.r. spectral evidence, do not contain hydroxyl groups; consequently the alcoholic functions in the diol (Ia) are primary or secondary. Partial hydrolysis of the oily diol diacetate (Id) and of the crystalline dihydro-diol diacetate (IIb) gives in each case a crystalline monoacetate. Oxidation of the dihydro-diol monoacetate (IIc) with

[1] Cocker and Halsall, *J.*, 1956, 4262.
[2] Heilbronner, Fröhlicher, and Plattner, *Helv. Chim. Acta*, 1949, **32**, 2479.
[3] Simonsen, " The Terpenes," Cambridge University Press, 1952, Vol. III, p. 360.
[4] King and Jones, *J.*, 1955, 658; Djerassi and Marshall, *Tetrahedron*, 1957, **1**, 238.
[5] Klyne, *J.*, 1953, 3072.

chromic acid in aqueous acetone furnishes a crystalline acetoxy-acid (IId) without loss of carbon, thus providing proof of the presence of a primary hydroxyl group in the diol (Ia).

The acetoxy-acid (IId) is hydrolysed with alkali to a crystalline hydroxy-acid (IIe) which, as its methyl ester is oxidised by chromium trioxide in pyridine to a non-crystalline keto-ester (IX), thus indicating the secondary nature of the second hydroxyl group in the diol (Ia). The same keto-ester (IX) is obtained directly from the dihydro-diol (IIa) by oxidation with chromic acid followed by esterification. Reduction of the keto-ester (IX) under Wolff–Kishner conditions gives dihydrocativic acid [1,6,7] (IIf) identified as the free acid, as the salt with 2-methyl-2-aminopropanol, and as the methyl ester by comparison with a specimen kindly supplied by Dr. T. G. Halsall. This conversion of the diol into dihydrocativic acid relates the stereochemistry of the diol to the labdane series and establishes the position of the primary hydroxyl group as 15.

The position of the secondary hydroxyl group present in the diol (Ia) is established by monoacetylation of the dihydro-diol to the acetate (IIg) in which the primary hydroxyl group is preferentially acetylated. Dehydration of the monoacetate (IIg) with phosphorus oxychloride in pyridine at room temperature with subsequent ozonolysis of the product leads to the formation of acetone, which was identified by thin-layer chromatography of its 2,4-dinitrophenylhydrazone. The monoacetate (IIg) thus undergoes the retropinacolinic rearrangement associated with 3β-hydroxy-terpenoids [8] having a 4-*gem*-dimethyl group. Accordingly the secondary hydroxyl group is placed at position 3 in the equatorial (β) conformation, this location being confirmed also by mass-spectral studies [9] and the optical-rotatory dispersion curve [10] of the oxo-ester (IX). This curve shows a weak negative amplitude (−15) similar in sign and magnitude to the curves obtained from 4,4,9-trimethyldecal-3-one and lanostan-3-one,[11] and from the foregoing evidence we regard the diol as labd-8(20)-ene-3β,15-diol (Ia).

The acidic fraction isolated from the light-petroleum extract of the bark of *A. imbricata* was methylated and chromatographed on neutral alumina to furnish methyl 3β-hydroxylabd-8(20)-en-15-oate (Ie). Treatment of the ester with lithium aluminium hydride affords labd-8(20)-en-3β,15-diol identical in all respects with the diol isolated from the non-saponifiable portion of the bark extract. Hydrogenation of the unsaturated ester (Ie) in ethyl acetate in presence of platinum gives a product showing identity (i.r. spectra) with methyl 3β-hydroxylabdan-15-oate which had been prepared from the diol (Ia), *via* the hydroxy-acid (IIe) described above. However, when the saturated ester was prepared by catalytic hydrogenation of the unsaturated ester (Ie), it could not be induced to crystallise and probably consisted of a mixture of position 8 epimers. Reduction of the mixture with lithium aluminium hydride affords labdan-3β,15-diol (IIa) identical with a sample obtained by hydrogenation of the unsaturated diol (Ia).

The non-saponifiable portion of the petroleum extract of the bark contained an aldehydic component which was separated from the extract by means of Girard reagent T. We formulate the aldehyde as 3β-hydroxy-15-oxolabd-8(20)-ene (Ib). Attempts to form crystalline carbonyl derivatives were unsuccessful but treatment with lithium aluminium hydride gives the unsaturated diol (Ia).

Experimental

Rotations were determined for chloroform solutions at room temperature, u.v. spectra for ethanol solutions and i.r. spectra for Nujol mulls unless otherwise stated. Light petroleum refers to the fraction of b. p. 60—80°.

[6] Halsall and Moyle, *J.*, 1960, 1324.
[7] Grant and Zeiss, *J. Amer. Chem. Soc.*, 1954, **76**, 5001; 1957, **79**, 1201.
[8] Ruzicka, Montavon, and Jeger, *Helv. Chim. Acta*, 1948, **31**, 819.
[9] See addendum.
[10] We are indebted to Professor W. Klyne for the measurements and interpretation of the optical-rotatory dispersion curves.
[11] Djerassi and Marshall, *J. Amer. Chem. Soc.*, 1958, **80**, 3986.

Labd-8(20)-*ene*-3β,15-*diol* (Ia).—Dry crushed bark (13 lb.) was extracted continuously with light petroleum for 20 hr. and the extract (200 g.) was hydrolysed by refluxing with methanolic potassium hydroxide (10%; 500 ml.) for 1·5 hr. Working up in the usual way through ether gave the non-saponifiable matter (41 g.). This was dissolved in ethyl acetate and left overnight, whereupon ceryl alcohol (8 g.) separated, m. p. 79° (from chloroform–methanol) (Found: C, 81·45; H, 14·2. $C_{26}H_{34}O$ requires C, 81·6; H, 14·25%). (The acetate had m. p. 63°.) The mother-liquors on evaporation yielded a gum (32 g.) which was chromatographed on alumina (1 kg.) from benzene. Development of the column with benzene, benzene–ether, and ether gave β-sitosterol (2·0 g.) m. p. 139°, $[\alpha]_D$ −36°, identified by mixed m. p. and i.r. spectral comparison with an authentic sample. Continued elution with ether–methanol (1%) furnished a mixture of alcohols from which β-sitosterol was removed by crystallisation from methanol. Removal of methanol and crystallisation of the residue from light-petroleum gave *labd*-8(20)-*ene*-3β,15-*diol* (2·8 g.) as blades, m. p. 114°, $[\alpha]_D$ +29° (*c*, 0·6); $\nu_{max.}$ 3665 and 3440 cm.$^{-1}$ (OH), 1640 and 890 cm.$^{-1}$ (vinylidene), 1385 and 1365 cm.$^{-1}$ (*gem*-dimethyl), $\lambda_{max.}$ 2060 Å (ε, 5000) [Found: C, 78·0; H, 11·8%; *M* (Rast), 320; active hydrogen, 1·92 atoms. $C_{20}H_{36}O_2$ requires C, 77·9; H, 11·8%; *M*, 308]. Acetylation of the diol (Ia) in pyridine with acetic anhydride at 100° (1 hr.) and extraction through ether gave an oily diacetate (free of OH-absorption). Partial hydrolysis of the latter (0·13 g.) in methanol (25 ml.) containing anhydrous sodium carbonate (0·16 g.) dissolved in the minimum quantity of water at room temperature (24 hr.) gave, after ether-extraction and chromatography of the extract from benzene–ether (3 : 1) on alumina (3 g.), *labd*-8(20)-*ene*-3β,15 *diol* 3-*monoacetate* (0·07 g.), needles, (from light petroleum) m. p. 69°, $[\alpha]_D$ +35° (*c*, 0·5) (Found: C, 75·1; H, 10·9. $C_{22}H_{38}O_3$ requires C, 75·4; H, 10·9%); $\lambda_{max.}$ 2060 Å (ε 5000); $\nu_{max.}$ 3520 cm.$^{-1}$ (OH), 1721 and 1276 cm.$^{-1}$ (OAc), 1645 and 889 cm.$^{-1}$ (vinylidene group).

8α,20-*Epoxylabdane*-3β,15-*diol*.—The diol (Ia) (0·1 g.) in ether (10 ml.) was added to monoperphthalic acid in ether (10 ml.; 0·34M) and left at 0° (72 hr.). The mixture was poured on crushed ice (50 g.) containing ethanol (25 ml.), sodium dithionite (1·5 g.), and sodium hydroxide (25 ml.; 1N), and the ether was removed at room temperature. The aqueous alkaline phase was extracted (ether), and the extracts washed and dried (Na_2SO_4). Removal of ether and crystallisation of the residue from light petroleum gave 8α,20-*epoxylabdane*-3β,15-*diol* as prisms, m. p. 116—117°, $[\alpha]_D$ +8·5° (c, 0·5) (Found: C, 74·4; H, 11·4. $C_{20}H_{36}O_3$ requires C, 74·0; H, 11·2%). The compound was transparent to u.v. light and did not show vinylidene absorption in the i.r. spectrum.

8-*Oxo*-20-*norlabdane*-3β,15-*diol* (Vb).—(1) Ozone was passed through a solution of labd-8(20)-ene-3β,15-diol (0·15 g.) in chloroform (25 ml.) at −70° for 30 min. When the solution had reached room temperature, acetic acid (4 ml.) was added and then zinc dust (0·4 g.) with stirring (30 min.). The excess of zinc was removed and the filtrate washed and evaporated to furnish a green gum which was chromatographed on alumina (4 g.) to give 8-*oxo*-20-*norlabdane*-3β,15-*diol* as plates (from light petroleum), m. p. 107·5°, $[\alpha]_D$ −39·7° (*c*, 0·5) (Found: C, 73·3; H, 11·2. $C_{19}H_{34}O_3$ requires C, 73·5; H, 11·0%); $\nu_{max.}$ 3320 cm.$^{-1}$ (hydroxyl) and 1695 cm.$^{-1}$ (ketone). The aqueous washings were treated with a saturated solution of dimedone at room temperature (24 hr.). Crystallisation of the solid product from ethanol afforded formaldehyde dimedone, identical (m. p. and i.r. spectra) with an authentic specimen.

(2) The unsaturated diol (Ia) (0·3 g.) in pyridine (5 ml.) was treated with osmium tetraoxide (0·3 g.) in dry ether (5 ml.). After 6 days in the dark, the solution was refluxed (1 hr.) with lithium aluminium hydride, and the excess of hydride destroyed with crushed ice. Extraction with ether in the usual manner gave *labdane*-3β,8α,15,20-*tetraol*, needles (from light petroleum), m. p. 138—139°, $[\alpha]_D$ −17° (*c*, 0·5) (Found: C, 70·0; H, 11·3. $C_{20}H_{38}O_4$ requires C, 70·1; H, 11·2%). The compound was transparent to u.v. light and did not show double-bond absorption in the i.r. spectrum. The tetraol (0·1 g.) in methanol (10 ml.) was treated with periodic acid (0·15 g.) in water (8 ml.) and left at room temperature (3 days). Water (50 ml.) was added and most of the methanol removed *in vacuo*. Extraction of the aqueous solution with chloroform afforded a gum (0·1 g.) which was chromatographed on alumina (3 g.) to give 8-oxo-20-norlabdane-3β,15-diol (Vb), m. p. and mixed m. p. (with the specimen obtained above) 107°. Treatment of the aqueous solution with dimedone again afforded formaldehyde dimedone, m. p. 191°.

Dehydrogenation of labd-8(20)-*ene*-3β,15-*diol* (Ia).—The diol (Ia) (1 g.) was heated with selenium (1 g.) at 300—350° (6 hr.). Continuous extraction of the residue with ether gave a

yellow oil (0·3 g.), which was chromatographed on alumina (30 g.) from light petroleum. Elution with the same solvent yielded a fraction ($\lambda_{max.}$ 2290 Å, $E_{1\,cm.}^{1\cdot0\%}$ 2200) which appeared to be mainly 1,2,5-trimethylnaphthalene. A portion (0·01 g.) was converted into the picrate, orange-coloured needles (from ethanol); m. p. and mixed m. p. with an authentic sample, 134—136°. Another portion (0·01 g.) was converted into the 1,3,5-trinitrobenzene adduct, yellow needles (from ethanol); m. p. and mixed m. p. 153—155°. Infrared spectral comparison with an authentic sample showed complete identity.

Labdane-3β,15-*diol* (IIa).—The unsaturated diol (Ia) (0·11 g.) in ethyl acetate (20 ml.) was shaken with hydrogen in the presence of platinum (from 0·12 g. of PtO_2) for 4 hr. and worked up in the usual way to give *labdane*-3β,15-*diol* (IIa) as blades (0·11 g.; from light petroleum), m. p. 122—124°, $[\alpha]_D$ +39° (*c*, 1·0) (Found: C, 77·6; H, 12·45. $C_{20}H_{38}O_2$ requires C, 77·4; H, 12·3%). The compound showed no absorption in the u.v. spectrum. Acetylation of the saturated diol (IIa) with acetic anhydride in pyridine at 100° (1 hr.) gave *labdane*-3β,15-*diol* 3,15-*diacetate* (IIb), needles (from aqueous methanol), m. p. 78°, $[\alpha]_D$ +24° (*c*, 1·0) (Found: C, 71·55; H, 10·7. $C_{24}H_{42}O_4,\frac{1}{2}CH_3{\cdot}OH$ requires C, 71·7; H, 10·7%). Partial hydrolysis of the diacetate (IIb) (1·4 g.) in methanol (250 ml.) with sodium carbonate (0·19 g.) in water (3 ml.) for 24 hr. at room temperature and subsequent extraction with ether gave a gum. Chromatography on alumina (30 g.) and elution with benzene–ether (4 : 1) furnished *labdane*-3β,15-*diol* 3-*acetate* (IIc), needles (0·8 g.; from light petroleum), m. p. 78—79°, $[\alpha]_D$ +26·4° (*c*, 1·0) (Found: C, 74·8; H, 11·5. $C_{22}H_{40}O_3$ requires C, 74·95; H, 11·4%); $\nu_{max.}$ 3480 cm.$^{-1}$ (hydroxyl); 1715 and 1270 cm.$^{-1}$ (acetate). Monoacetylation of the saturated diol (IIa) (0·31 g.) was done in pyridine (5 ml.) by adding acetic anhydride (0·027 g.) in pyridine (4 ml.) dropwise with stirring over 3 hr. Extraction with ether and chromatography of the extract (0·34 g.) on alumina (10 g.) gave *labdane*-3β,15-*diol* 15-*acetate* (IIg.) as needles (from light petroleum), m. p. 81°, $[\alpha]_D$ +26·4° (*c*, 1·0) (Found: C, 74·5; H, 11·1. $C_{22}H_{40}O_3$ requires C, 74·95; H, 11·4%); $\nu_{max.}$ 3540 cm.$^{-1}$ (hydroxyl), 1720 and 1270 cm.$^{-1}$ (acetate).

Dehydration of Labdane-3β,15-*diol* 15-*Acetate* (IIg).—Phosphorus oxychloride (2·5 ml.) was added dropwise with stirring to the monoacetate (IIg) (0·13 g.) in pyridine (7·5 ml.) and the mixture heated to 100° (0·5 hr.) before being kept at room temperature for 24 hr. Extraction with ether and chromatography on alumina furnished an oil (0·03 g.) which was devoid of hydroxyl absorption in the i.r. spectrum. The oil (0·03 g.) in carbon tetrachloride (at −5°) was treated with ozone (10%) for 0·5 hr. Water (10 ml.) and zinc dust (0·1 g.) were added and the solution was distilled. The first few drops of distillate were collected in a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid and the precipitate which formed was extracted with ether. Comparison of the R_F value (0·28) of the product with that of acetone 2,4-dinitrophenylhydrazone from benzene solution on thin-layer silica-gel plates established the identity of the 2,4-dinitrophenylhydrazones.

Acid Isomerisation of Labd-8(20)-*ene*-3β,15-*diol* (Ia).—The diol (Ia) (0·2 g.) in methanol (5 ml.) was refluxed with methanolic sulphuric acid (6·7%; 25 ml.) for 3 hr. The solvent was reduced to quarter bulk *in vacuo* at room temperature, diluted with water, and extracted with ether in the usual way. Crystallisation of the product from light petroleum afforded *labd*-8-*ene*-3β,15-*diol* (VIII) as blades, m. p. 121°, $[\alpha]_D$ +78° (*c*, 0·5) (Found: C, 77·6; H, 11·9. $C_{20}H_{36}O_2$ requires C, 77·9; H, 11·8%); $\lambda_{max.}$ 2100 Å (ε 5500). Infrared absorption showed the absence of vinylidene. Acetylation and benzoylation gave oily diesters. Hydrogenation of the diol (VIII) (0·5 g.) in acetic acid (25 ml.) in the presence of platinum and working up in the usual way through ether gave labdane-3β,15-diol, identical (m. p. and i.r. spectra) with the saturated diol (IIa) above.

Reactions of Labd-8-*ene*-3β,15-*diol with Osmium Tetraoxide and Perbenzoic Acid.*—The isomeric diol (VIII) in pyridine was treated with osmium tetraoxide in dry ether as described above for the diol (Ia). After treatment with lithium aluminium hydride and working up through ether, an impure tetraol was obtained. This was acetylated and chromatographed on alumina, and the acetate hydrolysed with sodium hydroxide in ethanol to give labdane-3β,8α,9α,15-tetraol [12] as needles (from light petroleum), m. p. 120—121°, $[\alpha]_D$ +3° (*c*, 0·5) (Found: C, 70·4; H, 11·3. $C_{20}H_{38}O_4$ requires C, 70·1; H, 11·2%). The tetraol did not absorb u.v. light.

Epoxidation of the isomeric diol (VIII) was carried out as described above for the diol (Ia)

[12] We prefer the α-configuration for the epoxide and for the hydroxyl groups introduced by osmium tetraoxide as attack is likely to occur from the less-hindered α-face of the molecule.

to give a gum which crystallised after 3 months. Recrystallisation from light petroleum afforded 8α,9α-*epoxylabdane*-3β,15-*diol* as plates, m. p. 88°, $[\alpha]_D$ +56° (*c*, 0·3) (Found: C, 73·6; H, 10·9. $C_{20}H_{36}O_3$ requires C, 74·0; H, 11·2%). The compound did not show selective absorption of u.v. light.

Methyl 3β-*Hydroxylabd*-8(20)-*en*-15-*oate* (Ie).—The alkaline solution which remained after the removal of the non-saponifiable material from the bark extract (above) was acidified with hydrochloric acid. The precipitated acid was extracted with ether to give a gum, a sample (21 g.) of which was esterified with an excess of diazomethane. Chromatography of the resulting esters on alumina (500 g.) gave *methyl* 3β-*hydroxylabd*-8(20)-*en*-15-*oate* (Ie), b. p. 200°/0·5 mm., n_D 1·5073, $[\alpha]_D$ +52° (*c*, 0·5) (Found: C, 74·6; H, 10·5. $C_{21}H_{36}O_3$ requires C, 74·95; H, 10·8%); $\nu_{max.}$ 3500 cm.$^{-1}$ (hydroxyl), 1735 cm.$^{-1}$ (ester), 1650 and 890 cm.$^{-1}$ (vinylidene group). The methyl ester (0·23 g.) in ether (50 ml.) and lithium aluminium hydride (0·1 g.) were refluxed (1 hr.) at 100°. Isolation of the product in the normal manner gave labd-8(20)-ene-3β,15-diol (Ia) in 95% yield, identical with the compound described above. Hydrogenation of the unsaturated ester (Ie) in ethyl acetate in the presence of platinum gave an oily mixture of 8-epimers of methyl 3β-hydroxylabdan-15-oate, identical (i.r. spectra) with a crystalline specimen prepared from labdane-3β,15-diol 3-acetate (below). The mixed epimeric esters (0·5 g.) in ether (50 ml.) were refluxed with lithium aluminium hydride (1 hr.). Isolation of the product in the usual way gave labdane-3β,15-diol (IIa), identical (m. p. and i.r. spectra) with the product described above.

Oxidation of Labdane-3β,15-*diol* 3-*Acetate* (IIc).—Kiliani's [13] chromic acid (4·26 ml.) was added dropwise to the monoacetate (IIc) (1·21 g.) in acetone (20 ml.) with stirring, which was continued for 1 hr. after the oxidant had been added. The product was isolated with ether and the extract washed with 2N-sodium carbonate. Acidification of the carbonate extract gave 3β-*acetoxylabdan*-15-*oic acid* (IId), needles (from aqueous acetone), m. p. 94°, $[\alpha]_D$ +17° (*c*, 1·0) (Found: C, 72·0; H, 10·7. $C_{22}H_{38}O_4$ requires C, 72·1; H, 10·45%). Hydrolysis of the acetoxy-acid (IId) (0·62 g.) with methanolic potassium hydroxide (5%; 50 ml.) at 100° for 1 hr. followed by removal of methanol *in vacuo* with simultaneous addition of water, gave, on acidification of the alkaline solution, 3β-*hydroxylabdan*-15-*oic acid* (IIe), needles (from light petroleum) m. p. 154—156°, $[\alpha]_D$ +20° (*c*, 1·0) (Found: C, 73·7; H, 11·25. $C_{20}H_{36}O_3$ requires C, 74·0; H, 11·2%); $\nu_{max.}$ 3450 cm.$^{-1}$ (hydroxyl) and 1716 cm.$^{-1}$ (carboxyl). Methylation of the hydroxy-acid (IIe) in ether with diazomethane (24 hr.) gave *methyl* 3β-*hydroxylabdan*-15-*oate* (IIh) as needles, m. p. 74°, $[\alpha]_D$ +29° (*c*, 0·5) (Found: C, 74·9; H, 11·6. $C_{21}H_{38}O_3$ requires C, 74·5; H, 11·4%); $\nu_{max.}$ 3540 cm.$^{-1}$ (hydroxyl) and 1735 cm.$^{-1}$ (ester).

Dihydrocativic Acid (IIf).—Labdane-3β,15-diol (6 g.) in acetone (150 ml.) was oxidised by Kiliani's chromic acid [13] (43·5 ml.) as described above for the 3-monoacetate (IIc). Isolation of the acidic oxidation product gave 3-oxolabdan-15-oic acid (4 g.) as an oil which was esterified with diazomethane to give methyl 3-oxolabdan-15-oate (IX), identical (i.r. spectra) with a specimen prepared by oxidation of methyl 3β-hydroxylabdan-15-oate with the chromium trioxide – pyridine complex. A mixture of the oxo-ester (IX) (4 g.), potassium hydroxide (12 g.), and hydrazine hydrate (98%, 18 ml.) in diethylene glycol (100 ml.) was refluxed in an oil-bath (external temperature 180—185°) for 5 hr. The excess of hydrazine was removed by distillation, and the reaction mixture refluxed for a further 5 hr. (bath temperature 225—230°). The cooled solution was acidified and extracted with ether, and the gummy extract was esterified with diazomethane. Chromatography of the ester in light petroleum on alumina (80 g.) gave methyl dihydrocativate as needles (from methanol at −60°), m. p. and mixed m. p. with an authentic sample 41°, $[\alpha]_D$ +28·2° (*c*, 1·0). The ester (IX) was hydrolysed with methanolic potassium hydroxide (10%) and the acidic fraction worked up in the usual way to give dihydrocativic acid (IIf), which was crystallised from methanol in small amounts on a glass plate, m. p. 69°, $[\alpha]_D$ +24·5° (*c*, 1·0) (Grant and Zeiss [7] give m. p. 71°, $[\alpha]_D$ +25·4°). The salt with 2-methyl-2-aminopropanol (from ethyl acetate) had m. p. 125°, $[\alpha]_D$ +21·3° (Grant and Zeiss [7] give m. p. 127°, $[\alpha]_D$ +22°).

15-*Oxolabd*-8(20)-*en*-3β-*ol* (Ib).—The non-saponifiable material from the light-petroleum extract of the bark was taken up in ethyl acetate and ceryl alcohol and β-sitosterol removed as described earlier. The resultant gum was then chromatographed on alumina. From the fraction (10 g.) eluted with ether–methanol (99 : 1), the carbonyl component was isolated by

[13] Kiliani and Merck, *Chem. Ber.*, 1901, **34**, 3564.

refluxing (1 hr.) in dry ethanol (80 ml.) and acetic acid (10 ml.) containing Girard reagent T, (7 g.). Ethylene glycol (80 ml.) was then added and the mixture extracted with ether to give an extract containing the diol (Ia). The glycol solution was diluted with much 1N-hydrochloric acid and set aside (1 hr.). Isolation through ether gave 15-oxolabd-8(20)-en-3β-ol, as an oil; $\nu_{max.}$ 3400 cm.$^{-1}$ (hydroxyl), 2730 and 1715 cm.$^{-1}$ (aldehyde), 1645 and 895 cm.$^{-1}$ (vinylidene). Attempts to form crystalline carbonyl derivatives were unsuccessful, but reduction of the aldehyde with lithium aluminium hydride in ether afforded labd-8(20)-en-3β,15-diol.

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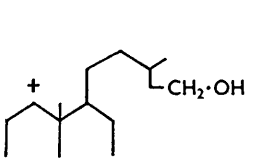
Addendum

By R. I. Reed and (Miss) F. M. Tabrizi.

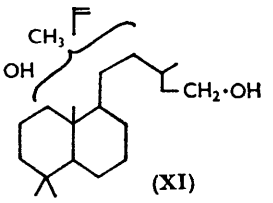
The mass spectra of compounds I(a), II(a), V(b), VIII, and IX have been examined with the following conclusions.

The spectra are characteristic of those diterpenes which possess two rings and a side-chain.[14,15] The masses of the molecular ions correspond to the formula $C_{20}H_{36}O_2$ for I(a), requiring three double-bond equivalents which are present as two rings and one double-bond. The parent molecular ions of all the compounds eliminate thirty-one units as an entity, and this must be either methoxyl or hydroxymethylene. Other evidence shows that the latter is the functional group.

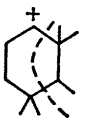
A further ready elimination of forty-five mass units (C_2H_5O) also occurs. Since a hydroxymethylene group must be terminal, the fragment is probably $-CH_2{\cdot}CH_2{\cdot}OH$, which is attached at a branch in a carbon chain. Consideration of the commonly-occurring diterpene structures suggests a probable partial formula of the form (X). The ready loss of methyl from all the compounds examined indicates the presence of a *gem*-dimethyl group, characteristic of a bicyclic terpenoid, and leads to the part structure (XI).



(X)



(XI)



(XII)

Previous studies [16–18] have shown that one common form of fission in these classes of compound is as represented in (XII) and the very abundant ion $m/e = 100$ ($C_6H_{12}O^+$), which is, moreover, the base peak of the spectrum, corresponds to this type of fragmentation. Therefore, the second hydroxyl is on ring A. Comparison of the spectra of I(a), II(a), V(b), and VIII with the ester (IX) seems to indicate that the loss of methyl is more pronounced when ring A carries a hydroxyl rather than an oxo-group. Hydroxyl groups favour fission of the nearest non-adjacent bond,[19] and this indicates that the substituent is on carbon atom 1 or 3. Position 1 is unlikely both biogenetically and from general considerations of the cracking-pattern, and position 3 is preferred. As the structure of ring A is now determined, the remaining carbon atom and double bond must be associated with ring B.

[14] Reed, A.S.T.M. Committee E-14 on Mass Spectrometry, Atlantic City, July 1960.
[15] Reed, unpublished observations.
[16] Reed, *J.*, 1958, 3432.
[17] Friedland, Lane, Longman, Train, and O'Neal, *Analyt. Chem.* 1959, **31**, 169.
[18] Genge, *Analyt. Chem.*, 1959, **31**, 1750.
[19] Beynon, "Mass Spectrometry and its Applications to Organic Chemistry," Elsevier, London, 1960, p. 350.

Ozonolysis of (Ia) yields a norketone (Vb) which proves the existence of an *exo*-methylene group on ring B. Moreover the fragment ion, $m/e = 101$ ($C_6H_{13}O$), corresponding to the removal of the side-chain, is more favoured in (Ia) than in (Vb) and the location of the methylene group at position 8 would be consistent with this observation, since an allylic bond is broken, and we arrive at structure (Ia) for the natural diol.

THE ROYAL COLLEGE OF SCIENCE AND TECHNOLOGY, GLASGOW C.1.
THE UNIVERSITY, GLASGOW W.2.
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