Lichens and Fungi. Part VII.[1] Extractives from the Lichen *Sticta mougeotiana* var. Dissecta Del

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Four new hopane triterpenes, 6α,7α,22-trihydroxyhopane, 6α-acetoxy-7α,22-dihydroxyhopane, 7α-acetoxy-6α,22-dihydroxyhopane, and 11β,22-dihydroxyhopane have been isolated from the hexane extractives of the lichen *Sticta mougeotiana*, together with n-heptadecane, pulvinic lactone, pulvinic acid, calycin, tenuiorin, and polyporic acid.

THE three new hopane * triterpenes 6α,7α,22-trihydroxyhopane (1), m.p. 230—231°, $[\alpha]_D^{20}$ +36·2°; 6α-acetoxy-7α,22-dihydroxyhopane (2), m.p. 198·5—199°, $[\alpha]_D^{20}$ +46·6°; and 7α-acetoxy-6α,22-dihydroxyhopane (3), m.p. 198—198·5°, $[\alpha]_D^{20}$ +22·2° have been isolated from the hexane extractives of the lichen *Sticta mougeotiana*.

Both compounds (2) and (3) gave the triol (1) on deacetylation; acetylation of the triol (1) gave a mixture of acetates (2) and (3). The α-glycol group in (1) was cleaved with periodic acid to give a dialdehyde [δ 9·95 (d, *J* 5 Hz, HC=O) and 9·85 (HC=O)]. Oxidation of the acetate (3) with Jones reagent gave the ketone (4) as the only product; oxidation of the acetate (2) with the same reagent gave the ketone (5) and the rearranged product (6). Deacetoxylation of the ketones (4) and (5) with calcium in liquid ammonia gave 6-oxo-22-hydroxyhopane [3] and 7-oxo-22-hydroxyhopane,[4] respectively, and established that compounds (1)—(3) were 22-hydroxyhopane derivatives oxygenated at C-6 and C-7.

The n.m.r. spectrum of the acetate (2) contained a one-proton doublet [δ 3·69 ($J_{ax,eq}$ 8 Hz, *H*C·OH)] and a one-proton quartet [δ 5·23 ($J_{ax,eq}$ 8 Hz, $J_{ax,ax}$ 12 Hz, *H*C·OAc)], which confirmed the 6α-acetoxy-7α-hydroxy-formulation. Similar confirmation of the 7α-acetoxy-6α-hydroxy formulation of (3) was provided by a one-proton doublet [δ 5·01 ($J_{ax,eq}$ 7·0 Hz, *H*C·OAc)] and a one-proton quartet [δ 3·70 ($J_{ax,eq}$ 7 Hz, $J_{ax,ax}$ 13 Hz, *H*C·OH)]. The coupling constants are consistent with the configurations assigned. The 14α-methyl signals of the acetates (2) and (3) are shifted downfield in comparison with 22-hydroxyhopane;[4] δ 1·15 in (3), deshielded by 0·17 p.p.m. by the axial 7-acetoxy-group, and δ 1·11 in (2), deshielded to a lesser extent by the smaller hydroxy-group.

The n.m.r. spectra of the ketones (4)—(6) were

* Pending clarification of the structure of hopane by *X*-Ray crystallography [2] it is assumed that the isopropyl side chain at C-21 has the α-orientation.

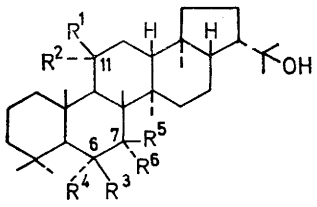
[1] Part VI, R. E. Corbett and R. A. J. Smith, *J. Chem. Soc.* (*C*), 1969, 44.

[2] I. Yosioka, T. Nakanishi, and I. Kitagawa, *Tetrahedron Letters*, 1968, 1485.

[3] D. H. R. Burton, P. de Mayo, and J. C. Orr, *J. Chem. Soc.*, 1958, 2239.

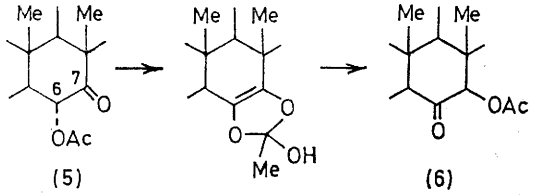
[4] R. E. Corbett and H. Young, *J. Chem. Soc.* (*C*), 1966, 1556.

consistent with the proposed structures. Ketone (5) [δ 5·52 (d, *J* 12 Hz, *HC*·OAc)], the oxidation product



	R^1	R^2	R^3	R^4	R^5	R^6
(1)	H	H	H	OH	H	OH
(2)	H	H	H	OAc	H	OH
(3)	H	H	H	OH	H	OAc
(4)	H	H	O		H	OAc
(5)	H	H	H	OAc	O	
(6)	H	H	O		OAc	H
(7)	OH	H	H	H	H	H
(8)	O		H	H	H	H
(9)	H	H	H	H	H	H
(10)	H	OH	H	H	H	H

of (2) was rearranged (Scheme 1) [5] under acidic conditions (alumina) to ketone (6) [δ 5·19 (*HC*·OAc)], which was epimeric at C-7 with ketone (4) [δ 5·25 (*HC*·OAc)], the oxidation product of (3), which could not be rearranged presumably because it enolised to C-5. The spectra of ketones (4) and (6) differ in the methyl region in the position of one peak only. The 14α-methyl resonance was at δ 0·89 in that of (6) and at δ 1·00 in that of (4). The downfield shift for (4) must be due to an α-acetoxy-group at C-7. The large coupling constant of the methine proton at C-6 in (5) is consistent with the equatorial (α) conformation of the acetoxy-group.

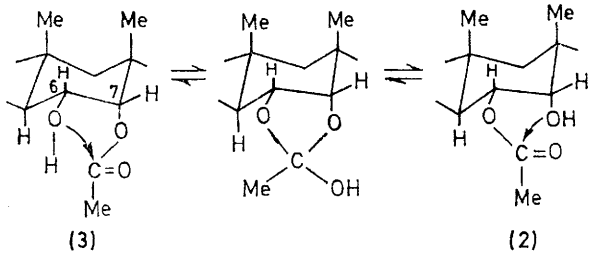


SCHEME 1

Purification of the acetates (2) and (3) was difficult because they equilibrated on the usual adsorbents. Pure (2) or pure (3) adsorbed on acidic or basic alumina gave an approximately equimolar mixture of (2) and (3). The acetates (2) and (3) could be obtained pure by repeated preparative layer chromatography (p.l.c.) on silica gel if the bands were removed immediately the chromatogram had been run, followed by fractional crystallisation from ethanol–water (8 : 2). Analytical t.l.c. showed the presence of both (2) and (3) in the crude hexane extract of the lichen. This is the first report of an acetyl rearrangement involving hydroxy-functions at C-6 and C-7. It presumably proceeds through a cyclic intermediate (Scheme 2),[6] and requires the depicted geometry for the hydroxy-groups.

[5] P. de Mayo, 'Molecular Rearrangements,' vol. 2, Interscience, New York, 1964, pp. 763—766.

6α,7α,22-trihydroxyhopane was synthesised from 22-hydroxy-7-oxohopane, by acetoxylation with lead tetra-acetate followed by reduction of the 6α-acetoxy-22-hydroxyhopane with lithium aluminium hydride. The product was identical with compound (1). The acetoxylation reaction gave an almost equimolar mixture of the

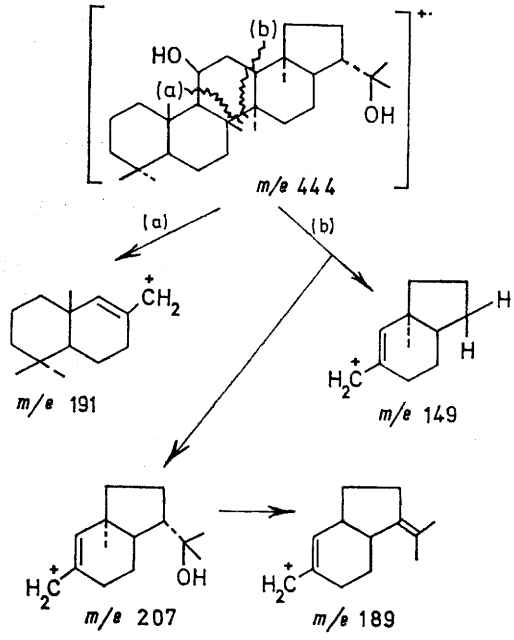


SCHEME 2

6α-acetoxy-compound and 7β-acetoxy-6-oxohopane. This is not surprising in view of the acidic conditions required for the reaction.

A fourth triterpene, which was assigned the structure 11β,22-dihydroxyhopane (7), was also isolated from the neutral extractives. Oxidation of (7) with ruthenium tetroxide to the oxo-derivative (8) followed by Wolff–Kishner reduction gave 22-hydroxyhopane (9).

The mass spectra of compounds (7) and (8) and of deuteriated (8) were consistent with the proposed formulations. Previous studies [4] of the mass spectra of hopane and its derivatives have shown that two types of cleavage predominate, designated (a) and (b) in Scheme 3. The pronounced peak at *m/e* 191 (*m** 82·5)



SCHEME 3

could only arise from a fragment which included rings A and B and its observation precluded the attachment of

[6] L. F. Fieser and M. Fieser, 'Steroids,' Reinhold, New York, 1959, pp. 229—230.

any oxygen function to these rings. The peak at *m/e* 207 and the 'daughter' peak at *m/e* 189 (*m** 172·6) established that there was only the C-22 tertiary hydroxy-function and no other oxygen function associated with rings D and E. Thus any other oxygen functions in compounds (7) and (8) could only be at C-11 or C-12. Deuteriation of the hydroxy-ketone (8) under forcing conditions led to the incorporation of three deuterium atoms. In the mass spectrum of the deuteriated ketone there was a peak at *m/e* 192 which was absent in that of the unlabelled (8). This could only mean that a deuterium atom had been incorporated at the 9α-position, and established conclusively that there was an oxo-function at C-11, and that deuteriation had given [9α,12α,12β-$^2H_3$]-22-hydroxy-11-oxohopane.

It remained to define the configuration of the C-11 hydroxy-group in structure (7). The carbinol proton of the secondary hydroxy-group produced an ill-defined multiplet in the ^1H n.m.r. spectrum at δ 3·87. The half-band width of 7·5 Hz was consistent with an 11β- (axial) configuration for the hydroxy-group. The epimeric alcohol (10) was obtained as the minor product from the lithium aluminium hydride reduction of compound (8). The lower R_F value (t.l.c.) and the diamagnetic shift of the carbinol proton resonance in the ^1H n.m.r. spectrum to δ 3·70 (multiplet) with increased half-band width (12·5 Hz) confirmed that the *epi*-diol was 11α,22-dihydroxyhopane, the equatorial epimer.

The methyl signals in the ^1H n.m.r. spectra of compound (7) and its derivatives were assigned (Table) by methods similar to those used for other 22-hydroxyhopane derivatives,[1] but these methods did not permit the 4β-, 4α-, and 10β-methyl signals to be distinguished. However, Cheung and Williamson,[7] in the course of a study of derivatives of ursane, oleanane, and lupane, have recently published substituent increments which make the complete assignment of the methyl signals in these compounds possible. Hopane has the same conformation for rings A, B, and C as the three triterpenes mentioned, and the chemical shifts of methyl groups associated with rings A and B in the four groups of triterpenes should be almost identical and subject to the same substituent effects. The chemical shifts of the ring A methyl groups in the examples quoted are at progressively lower field in the order 4β > 4α > 10β and this is the order in which they are assigned in 22-hydroxyhopane. The large downfield shifts of the 10β- (20 Hz) and 8β- (21 Hz) methyl signals from structure (9) to structure (7) confirm the 1,3-diaxial relationship of both the C-8 and C-10 methyl groups with the C-11 hydroxy-group.

Chemical shifts (δ) of methyl groups (p.p.m. from tetramethylsilane)

	4β	4α	10β	8β	14α	18α
(9) 22-Hydroxyhopane	0·79	0·83	0·85	0·96	0·96	0·77
(7) 11β,22-Dihydroxyhopane	0·80	0·86	1·05	1·17	0·99	0·78
(8) 22-Hydroxy-11-oxohopane	0·80	0·83	1·04	1·17	0·99	0·75

A fraction that travelled with the solvent front through the alumina column used to separate the neutral components was shown (analysis and i.r. and n.m.r. spectra) to be a hydrocarbon and was identified as n-heptadecane by g.l.c.

Separation of the acidic fraction from the hexane extraction of the lichen by p.l.c. on silica gel gave pulvinic lactone (3·1% of the total acidic fraction), calycin (12·5%), tenuiorin (24·0%), pulvinic acid (32·6%), and polyporic acid (19·3%). The identifications of pulvinic lactone, calycin, pulvinic acid, and polyporic acid rest on the preparation of appropriate derivatives and direct comparison with authentic specimens. An authentic specimen of tenuiorin could not be obtained; its identification depends on its colour reactions and the preparation of a derivative.

EXPERIMENTAL

Experimental procedures are as described in Part VI.[1]

Extraction of the Lichen.—The lichen was collected from the Lower Manorburn Dam, Central Otago, in January 1968. Samples collected from various other localities and at different times of the year yielded the same compounds.

Air-dried lichen (1·23 kg) was ground into a fine powder in a Wiley mill and extracted (Soxhlet) with hexane (16 l) for 80 h. The pale yellow precipitate (7·2 g) which separated was filtered off; concentration of the hexane solution (5 l) gave more precipitate (5 g). Evaporation of the solvent gave a dark yellow-brown residue (35 g). T.l.c. (1 : 1 H–E) showed only one compound in the precipitates, but the residue was a complex mixture.

The residue (21·9 g) from the filtrate of the hexane extract was dissolved in chloroform and extracted with 2M-sodium hydroxide. The chloroform layer contained the neutral compounds. The alkaline layer contained the acids.

The solid precipitate (12·2 g.) (from ethanol) gave 6α,7α,22-*trihydroxyhopane* (1), which after sublimation (160° at 0·01 mmHg) had m.p. 230—231°, $[\alpha]^{20}$ +36·2° (*c* 0·24 in pyridine), $\nu_{max.}$ 3350br cm^{-1} (OH), δ 0·77 (3H), 0·90 (3H), 1·01 (3H), 1·06 (6H), 1·18 (3H), 1·20 (6H), and 3·61 (2H, m, total splitting 32 Hz) p.p.m.; δ (pyridine) 0·94 (3H), 1·09 (3H), 1·15 (3H), 1·27 (3H), 1·31 (3H), 1·37 (3H), 1·47 (6H), 3·80 and 3·89 (1H, d), and 3·81, 3·89, 4·00, and 4·08 (1H, q) p.p.m. (Found: C, 78·3; H, 11·4. $C_{30}H_{52}O_3$ requires C, 78·2; H, 11·4%).

6α,7α-*Diacetoxy*-22-*hydroxyhopane*.—The triol (90 mg) in chloroform (15 ml) was heated under reflux for 20 h with dimethylaniline (4 ml) and acetyl chloride (3 ml). Work-up in the usual way gave a solid (90 mg), which was separated by p.l.c. with E–H (1 : 9) into 6α,7α-*diacetoxy*-22-*hydroxyhopane* (50 mg) and dehydration products (40 mg). 6α,7α-Diacetoxy-22-hydroxyhopane had m.p. 222° (85% ethanol–water), $\nu_{max.}$ 3420 (OH), 1730 (OAc), 1240 (OAc), and 1020 (C–O) cm^{-1}, δ 0·83 (3H), 0·86 (3H), 0·95 (3H), 0·99 (3H), 1·00 (3H), 1·19 (6H), 1·26 (3H), 1·96 (3H), 2·02 (3H), and 5·16, 5·24, 5·31, 5·43, and 5·51 (2H, m) p.p.m. (Found: C, 75·2; H, 10·3. $C_{34}H_{56}O_5$ requires C, 75·0; H, 10·4%).

6α-*Acetoxy*-7α,22-*dihydroxyhopane* (2) *and* 7α-*Acetoxy*-6α,22-*dihydroxyhopane* (3).—The triol (1) (3·0 g) in pyridine

[7] H. T. Cheung and D. G. Williamson, *Tetrahedron*, 1969, **25**, 119.

(150 ml) and acetic anhydride (150 ml) was kept at room temperature for 72 h. Work-up in the usual way gave a solid (2·9 g) which was adsorbed from hexane on alumina [90 g; deactivated with water (2·7 ml)]. Elution with E–H (1 : 4; 3 l) gave a mixture (2·8 g) of acetates (2) and (3). These were separated by the following procedure. P.l.c. plates were lightly loaded (0·70 g. per 1 m plate) and run once with E–H (7 : 3), and the two resulting bands were cut out and eluted. These partially purified products were reapplied separately to plates and the procedure was repeated twice. The pure monoacetates were then obtained by fractional crystallisation from ethanol–water (4 : 1), each crop being recrystallised again. The lower R_F compound, 6α-*acetoxy*-7α,22-*dihydroxyhopane* (2), had m.p. 198·5—199°; $[\alpha]_D^{20}$ +46·6° (*c* 0·075 in $CHCl_3$), $\nu_{max.}$ 3580 (OH), 3410 (OH), 1710 (OAc), and 1265 (OAc) cm^{-1}; δ 0·77 (3H), 0·86 (3H), 0·94 (3H), 1·02 (3H), 1·05 (3H), 1·11 (3H), 1·17 (3H), 1·21 (3H), 2·08 (3H), 3·66 and 3·74 (1H, d), and 5·13, 5·22, 5·25, and 5·33 (1H, q) p.p.m. (Found: C, 76·3; H, 10·7. $C_{32}H_{54}O$ requires C, 76·4; H, 10·8%). The higher R_F compound, 7α-*acetoxy*-6α,22-*dihydroxyhopane* (3), had m.p. 198—198·5°, $[\alpha]^{20}$ +22·2° (*c* 0·140 in $CHCl_3$), $\nu_{max.}$ 3560 (OH), 3500 (OH), 1695 (OAc), and 1265 (OAc) cm^{-1}, δ 0·76 (3H), 0·91 (3H), 1·00 (3H), 1·07 (3H), 1·15 (9H), 1·19 (3H), 2·05 (3H), 3·59, 3·66, 3·73, and 3·80 (1H, q), and 4·98 and 5·05 (1H, d) p.p.m. (Found: C, 76·4; H, 10·8. $C_{32}H_{54}O_4$ requires C, 76·4; H, 10·8%).

6α,7α,22-*Triacetoxyhopane*.—The dihydroxy-monoacetates (100 mg) and toluene-*p*-sulphonic acid (37 mg) in isopropenyl acetate (5·65 ml) were stirred at room temperature for 24 h. Work-up in the usual way gave a complex mixture of products (t.l.c.), including some resulting from dehydration under the acidic conditions employed. Purification by p.l.c. with E–H (1 : 1) gave, at intermediate R_F, 6α,7α,22-triacetoxyhopane (30 mg), m.p. 245° (85% ethanol–water), $\nu_{max.}$ 1723 (OAc), 1255 (OAc), and 1230 (OAc) cm^{-1}, δ 0·78 (3H), 0·87 (3H), 0·99 (3H), 1·10 (3H), 1·20 (3H), 1·27 (3H), 1·43 (3H), 1·49 (3H), 1·94 (6H), 2·00 (3H), and 5·14, 5·24, 5·31, 5·43, and 5·51 (2H, m) p.p.m. ($C_{36}H_{58}O_6$ requires C, 73·7; H, 9·9. Found: C, 77·6; H, 10·2. $C_{34}H_{54}O_4$ requires C, 77·6; H, 10·3%). The conditions for the preparation of an analytical sample (drying at 100° *in vacuo*) must have caused deacetoxylation at the 22-position.

Cleavage of 6α,7α,22-*Trihydroxyhopane* (3).—The triol (30 mg) in ethanol (100 ml) was treated with periodic acid (30 mg) in water. The mixture was left at room temperature for 1 h. Work-up gave a crude product (25 mg). P.l.c. (1 : 1 H–E) gave 22-*hydroxy*-6,7-*secohopanedial* (20 mg), which after sublimation (95° at 0·01 mmHg) had m.p. 147°, $\nu_{max.}$ 3500 (OH) and 1700 (C=O) cm^{-1}, δ 0·80 (3H), 0·89 (3H), 0·98 (3H), 1·10 (3H), 1·14 (3H), 1·16 (3H), 1·20 (3H), 1·28 (3H), 9·98 and 9·93 (1H, d), and 9·85 (1H, s) p.p.m. (Found: C, 78·9; H, 10·7. $C_{30}H_{50}O_3$ requires C, 78·6; H, 10·9%).

Acid-induced Rearrangement of 7α-*Acetoxy*-6α,22-*dihydroxyhopane* (3).—7α-Acetoxy-6α,22-dihydroxyhopane (30 mg) was adsorbed from hexane on Woelm acidic alumina (30 g; grade I) for 24 h. T.l.c. and integral analysis of the n.m.r. spectrum of the eluted product showed it to be an approximately equimolar mixture of 7α-acetoxy-6α,22-dihydroxyhopane and 6α-acetoxy-7α,22-dihydroxyhopane.

Acid-induced Rearrangement of 6α-*Acetoxy*-7α,22-*dihydroxyhopane* (2).—Similar acidification also gave a mixture of the two dihydroxy-monoacetates.

Alkali-induced Rearrangement of 7α-*Acetoxy*-6α,22-*dihydroxyhopane* (3).—7α-Acetoxy-6α,22-dihydroxyhopane (20 mg) was adsorbed from benzene on Woelm alkaline alumina (20 g; grade I) for 24 h. T.l.c. and integral analysis of the n.m.r. spectrum of the eluted product showed it to be an approximately equimolar mixture of the two dihydroxy-monoacetates.

Alkali-induced Rearrangement of 6α-*Acetoxy*-7α,22-*dihydroxyhopane* (2).—Similar basification also gave a mixture of the two dihydroxy-monoacetates.

Reduction of 6α-*Acetoxy*-7α,22-*dihydroxyhopane* (2).—6α-Acetoxy-7α,22-dihydroxyhopane (70 mg) in dry ether (50 ml) was heated under reflux with excess of lithium aluminium hydride for 2 h. Work-up gave 6α,7α,22-trihydroxyhopane (60 mg), which was purified by sublimation (160° at 0·01 mmHg) and was identical (m.p., mixed m.p., i.r. spectrum, and t.l.c.) with the original triol.

Reduction of 7α-*Acetoxy*-6α,22-*dihydroxyhopane* (3).—7α-Acetoxy-6α,22-dihydroxyhopane (50 mg) in dry ether (30 ml) was heated under reflux with excess of lithium aluminium hydride for 2 h. Work-up gave 6α,7α,22-trihydroxyhopane (50 mg), which was purified by sublimation (160° at 0·01 mmHg) and was identical (m.p., mixed m.p., i.r. spectrum, and t.l.c.) with the original triol.

Oxidation of 6α-*Acetoxy*-7α,22-*dihydroxyhopane* (2).—6α-Acetoxy-7α,22-dihydroxyhopane (60 mg) in AnalaR acetone (20 ml) was stirred while Jones reagent (0·2 ml) was added dropwise until a permanent orange colour resulted. After a further 2 min stirring the crude product (60 mg) was isolated in the usual way. Multiple p.l.c. (×8) with E–H (3 : 7) gave, in order of increasing R_F value, unchanged starting material (10 mg), 6α-*acetoxy*-22-*hydroxy*-7-*oxohopane* (5) (30 mg), and 7β-*acetoxy*-22-*hydroxy*-6-*oxohopane* (6) (20 mg). The ketone (5) had m.p. 229° (from hexane), $\nu_{max.}$ 3530 (OH), 1730 (C=O), 1705 (OAc), and 1255 (OAc) cm^{-1}, δ 0·77 (3H), 0·89 (3H), 1·04 (3H), 1·07 (3H), 1·15 (3H), 1·17 (3H), 1·21 (3H), 1·31 (3H), 2·16 (3H), and 5·46 and 5·58 (1H, d) p.p.m., $\lambda_{max.}$ (EtOH) 282 nm (ε 30) (Found: C, 76·8; H, 10·4. $C_{32}H_{54}O_4$ requires C, 76·8; H, 10·4%). The ketone (6) had m.p. 260° (ethanol–water), $\nu_{max.}$ 3450 (OH), 1735 (C=O), 1705 (OAc), and 1230 (OAc) cm^{-1}, δ 0·79 (3H), 0·89 (6H), 1·18 (3H), 1·23 (6H), 1·26 (6H), 2·09 (3H), and 5·19 (1H) p.p.m., $\lambda_{max.}$ (EtOH) 280 nm (ε 30) (Found: C, 76·6; H, 10·4. $C_{32}H_{52}O_4$ requires C, 76·8; H, 10·4%).

Oxidation of 7α-*Acetoxy*-6α,22-*dihydroxyhopane* (3).—Oxidation of 7α-acetoxy-6α,22-dihydroxyhopane (60 mg) with Jones reagent gave a crude product (60 mg). P.l.c. with E–H (1 : 1) gave 7α-acetoxy-22-hydroxy-6-oxohopane (4) (40 mg), m.p. 256—256·5 (from hexane), $\nu_{max.}$ 3510 (OH), 1720 (C=O), 1700 (OAc), and 1205 (OAc) cm^{-1}, δ 0·80 (3H), 0·90 (3H), 1·00 (3H), 1·16 (3H), 1·21 (3H), 1·24 (3H), 1·26 (6H), 2·09 (3H), and 5·25 (1H) p.p.m., $\lambda_{max.}$ (EtOH) 290 nm (ε 30) (Found: C, 76·8; H, 10·4. $C_{32}H_{52}O_4$ requires C, 76·8; H, 10·4%).

Attempted Acid-induced Epimerisation of 7α-*Acetoxy*-22-*hydroxy*-6-*oxohopane* (4).—(*a*) 7α-Acetoxy-22-hydroxy-6-oxohopane (45 mg) was adsorbed from hexane on Woelm acidic alumina (20 g; grade I) for 24 h. Elution with hexane gave unchanged starting material [δ 5·25 (s) p.p.m.].

(*b*) 7α-Acetoxy-22-hydroxy-6-oxohopane (160 mg) and freshly distilled propionic acid (40 ml) were refluxed for 24 h. Work-up gave unchanged starting material [δ 5·25 (s) p.p.m.].

Attempted Alkali-induced Epimerisation of 7α-*Acetoxy*-22-

hydroxy-6-oxohopane (4).—7α-Acetoxy-22-hydroxy-6-oxohopane (40 mg) was adsorbed from hexane on Woelm alkaline alumina (20 g; grade I) for 24 h. Elution with hexane gave unchanged starting material [δ 5·25 (s) p.p.m.].

Acid-induced Rearrangement of 6α-Acetoxy-22-hydroxy-7-oxohopane (5).—6α-Acetoxy-22-hydroxy-7-oxohopane (70 mg) was adsorbed from hexane on Woelm acidic alumina (30 g; grade I) for 24 h. Elution with hexane gave a solid (65 mg) which was separated by multiple p.l.c. (×6) with E–H (3 : 7) into, in order of increasing R_F value, 6α-acetoxy-22-hydroxy-6-oxohopane (30 mg) and 7β-acetoxy-22-hydroxy-6-oxohopane (6) (35 mg), identical (m.p., mixed m.p., and n.m.r. spectrum) with authentic samples.

Attempted Alkali-induced Rearrangement of 6α-Acetoxy-22-hydroxy-7-oxohopane.— 6α-Acetoxy-22-hydroxy-7-oxohopane (15 mg) was adsorbed from hexane on Woelm alkaline alumina (20 g; grade I) for 24 h. Elution with hexane gave only unchanged starting material [δ 5·46 and 5·58 (d) p.p.m.].

22-Hydroxy-6-oxohopane.—An excess of calcium (60 mg) was added to liquid ammonia and the resulting solution was stirred vigorously for 5 min. A solution of 7α-acetoxy-22-hydroxy-6-oxohopane (40 mg) in toluene (5 ml) was added dropwise and the mixture was stirred for 20 min. Ammonium chloride was then added to destroy the excess of calcium. Work-up gave a crude product (20 mg) which after p.l.c. with E–H (1 : 1) gave 22-hydroxy-6-oxohopane, m.p. 239—241° (hexane), identical (mixed m.p., i.r. and n.m.r. spectra, and t.l.c.) with an authentic sample (Found: C, 81·2; H, 11·4. Calc. for $C_{30}H_{50}O_2$: C, 81·2; H, 11·4%).

22-Hydroxy-7-oxohopane.—Similar reduction of 6α-acetoxy-22-hydroxy-7-oxohopane (40 mg) with calcium in liquid ammonia gave 22-hydroxy-7-oxohopane (25 mg), m.p. 255° (hexane), identical (mixed m.p., i.r. and n.m.r. spectra, and t.l.c.) with an authentic sample.

Acetoxylation of 22-Hydroxy-7-oxohopane.—22-hydroxy-7-oxohopane (130 mg) and lead tetra-acetate (185 mg) in glacial acetic acid (50 ml) were heated under reflux for 10 h. Work-up gave a crude solid (120 mg) which was purified by p.l.c. with E–H (1 : 1). This gave three major products and a number of minor products. The major products were unchanged starting material (50 mg), identified by m.p., mixed m.p., and t.l.c.; and 6α-acetoxy-22-hydroxy-7-oxohopane (5) (25 mg), m.p. 229°, and 7β-acetoxy-22-hydroxy-6-oxohopane (6) (30 mg), m.p. 260°, identical (mixed m.p. and t.l.c.) with authentic samples.

6α,7α,22-Trihydroxyhopane (1).—6α-Acetoxy-22-hydroxy-7-oxohopane (25 mg) and a slight excess of lithium aluminium hydride in dry ether (25 ml) were refluxed for 3 h. Work-up gave a solid which t.l.c. (E) showed to be one compound only. Sublimation at 160° and 0·01 mmHg gave crystals of 6α,7α,22-trihydroxyhopane (1), m.p. 231°, identical (mixed m.p., i.r. spectrum, and t.l.c.) with an authentic sample (Found: C, 78·5; H, 11·3. $C_{30}H_{52}O_3$ requires C, 78·2; H, 11·4%).

Additional Neutral Compounds.—The neutral chloroform layer of the mother liquor residue of the hexane extraction was washed with water and evaporated, yielding a yellow-brown solid (20 g). The solid (14 g) was adsorbed from the hexane on alumina (600 g). The column was eluted with H, and then H–E mixtures. Fractions (100 ml) were collected and combined according to their t.l.c. properties. Four major components, n-heptadecane (0·500 g), 11β,22-dihydroxyhopane (0·400 g), and 6α-acetoxy-7α,22-dihydroxyhopane and 7α-acetoxy-6α,22-dihydroxyhopane (6·08 g total) were eluted from the column in that order. Minor components, which included 6α,7α,22-trihydroxyhopane (m.p., mixed m.p., i.r. spectrum, and t.l.c. identical with those of an authentic sample), were not further investigated because of their small amounts.

11β,22-Dihydroxyhopane (7) gave needles, m.p. 216—217° (decomp.) (from ethanol–water), $[\alpha]_D^{20}$ +27·1° (*c* 0·019 in $CHCl_3$), $\nu_{max.}$ 3300br (OH) cm^{-1}, δ 0·78 (3H), 0·80 (3H), 0·86 (3H), 0·99 (3H), 1·05 (3H), 1·17 (3H), 1·18 (3H), 1·21 (3H), and 3·87 (1H, m, half-band width 7·5 Hz) p.p.m. (Found: C, 81·2; H, 11·9. $C_{30}H_{52}O_2$ requires C, 81·1; H, 11·7%).

Ruthenium Tetroxide Solution.—Ruthenium dioxide (40 mg) was suspended in carbon tetrachloride (50 ml) and a solution of sodium periodate (3·2 g) in water (50 ml) was added. The mixture was stirred for 1 h at 0°. The black dioxide gradually dissolved as it was converted into the tetroxide. The clear yellow organic layer was filtered through glass wool to remove insoluble material. The resulting solution was stored in an ice-chest.

22-Hydroxy-11-oxohopane (8).—(*a*) 11β,22-dihydroxyhopane (60 mg) dissolved in AnalaR carbon tetrachloride (30 ml) was covered with water (10 ml). Ruthenium tetroxide solution (6 ml) was added, and the mixture was stirred for 10 min. Excess of tetroxide was destroyed with propan-2-ol (1 ml). Filtration of the mixture and evaporation of the carbon tetrachloride layer gave the oxidation product. P.l.c. with E–H (3 : 7) gave *22-hydroxy-11-oxohopane* (8) (40 mg), m.p. 249° (from hexane), $\nu_{max.}$ (KBr) 3530 (OH), and 1680 (C=O) cm^{-1}, δ 0·75 (3H), 0·80 (3H), 0·83 (3H), 0·99 (3H), 1·04 (3H), 1·17 (3H), 1·19 (3H), 1·21 (3H), 2·16, 2·30, 2·52, and 2·66 (1H, q, J_{AB} 14 Hz), and 2·32 (1H, q) p.p.m.; c.d. $\Delta\varepsilon_{303}$ −2·68 (MeOH); o.r.d. $[\phi]_{323}$ −4800, $[\phi]_{276}$ +7950, *a* −128 (MeOH) (Found: C, 81·5; H, 11·6. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·4%).

22-Hydroxyhopane.—22-Hydroxy-11-oxohopane (8) (25 mg) in hexane was added to freshly distilled diethylene glycol (15 ml) which had been treated with sodium (0·150 g). As the temperature was increased to 180° the hexane was distilled off. Anhydrous hydrazine was added until the solution was refluxing freely at 180° (solution temperature). After 24 h the temperature was raised to 210° by distilling off some of the hydrazine. After a further 24 h at 210° the mixture was cooled, neutralised with acid, and worked up in the usual way. Following filtration through Woelm neutral alumina (20 g), the product was purified by p.l.c. with E–H (1 : 1). Crystallisation from hexane gave 22-hydroxyhopane (9) (20 mg), m.p. and mixed m.p. identical with those of an authentic sample.

[9α,12α,12β-$^2H_3$]-22-Hydroxy-11-oxohopane.—Sodium (60 mg) was dissolved in dueterioethanol (2 ml) and the resulting solution was added to 22-hydroxy-11-oxohopane (10 mg) in deuterium oxide (0·5 ml). After refluxing for 4 days, distilled water (1 drop) was added; evaporation of the solvent then left a solid which was purified by p.l.c. with E–H (7 : 3). Sublimation at 160° and 0·01 mmHg gave *[9α,12α,12β-$^2H_3$]-22-hydroxy-11-oxohopane*, *m/e* 445 (M^+).

11α,22-Dihydroxyhopane (10).—A solution of 22-hydroxy-11-oxohopane (9) (40 mg) in dry ether (20 ml) containing a slight excess of lithium aluminium hydride was heated under reflux for 3 h. Work-up followed by p.l.c. with E–H (7 : 3) gave two compounds. Crystallisation from

ethanol of the higher R_F isomer gave 11β,22-dihydroxyhopane (30 mg), which was identified by comparison (m.p., mixed m.p., n.m.r. spectrum, and t.l.c.) with an authentic sample. Crystallisation from ethanol of the lower R_F isomer gave *11α,22-dihydroxyhopane* (10) (8 mg), m.p. 220°, δ 3·70 (1H, m, half-band width 12·5 Hz) (Found: C, 80·7; H, 11·6. $C_{30}H_{52}O_2$ requires C, 81·1; H, 11·7%).

n-Heptadecane.—Purification by p.l.c. with H gave a compound (0·300 g), semi-solid at room temperature, δ 0·88 (6H) and 1·26 (30H) p.p.m. Preparation of an analytical sample was attempted by distillation at 70° and 0·01 mmHg (Found: C, 85·6; H, 14·5. Calc. for $C_{17}H_{36}$: C, 85·0; H, 15·0%). It was examined by g.l.c. in a column of 5% (w/w) Apiezon L on Chromosorb P (100—200 mesh) packed in copper tubing (6 ft × 3/16 in o.d.) coiled after packing. The column temperature was 165°, the carrier gas nitrogen, and the flow rate 60 ml min^{-1}. Dodecane and octadecane were used as standards; the retention time measured relative to dodecane was 8·0. The retention times of the three hydrocarbons plotted on a log scale against carbon numbers gave a straight line.

7α-Acetoxy-6α,22-dihydroxyhopane (3) *and 6α-Acetoxy-7α,22-dihydroxyhopane* (2).—These were separated as already described and identified by comparison (m.p., mixed m.p., i.r. and n.m.r. spectra, and t.l.c.) with authentic samples.

The Acid Fraction.—The alkaline aqueous layer of the chloroform extraction of the mother liquor residue was treated with dil. sulphuric acid, yielding a solid (0·49 g) and a filtrate, which, on extraction with ether, gave more solid (1·36 g). These solids were identical qualitatively (t.l.c.). P.l.c. with toluene–glacial acetic acid (4 : 1) of the solid (0·960 g) gave, in order of decreasing R_F value, pulvinic lactone (0·030 g), calycin (0·120 g), tenuiorin (0·230 g), pulvinic acid (0·320 g), and polyporic acid (0·185 g).

Pulvinic lactone gave yellow needles, m.p. and mixed m.p. 224° (from benzene). Ethyl pulvinate, prepared by heating the lactone under reflux in ethanol, had m.p. and mixed m.p. 128°.

Further purification of calycin by p.l.c. with toluene–glacial acetic acid (4 : 1) gave an orange solid, which crystallised in orange-red needles from ethanol; m.p. and mixed m.p. 248°. Calycin gave calycin acetate when heated under reflux with acetic anhydride–pyridine; m.p. and mixed m.p. 178° (from ethanol).

Purification of tenuiorin by p.l.c. with toluene–glacial acetic acid (4 : 1) and crystallisation from benzene gave needles, m.p. 179—180° (lit.,[8] 180° with solidification and remelting at 238°: the latter phenomenon was not observed with this specimen) (Found: C, 62·4; H, 5·0. Calc. for $C_{26}H_{24}O_{10}$: C, 62·9; H, 4·9%). The following colour reactions were observed; the colour in brackets refers to authentic tenuiorin.[8] Ethanolic iron(III) chloride: negative (negative); aqueous sodium hydroxide (2M), sodium carbonate (2M), sodium hydrogen carbonate (2M): a pink colouration in each case; conc. sulphuric acid: negative. Triacetyltenuiorin prepared with acetic anhydride–pyridine had m.p. 195° (from acetone) (lit.,[8] 195) (Found: C, 61·9; H, 4·5. Calc. for $C_{32}H_{30}O_{13}$: C, 61·7; H, 4·8%).

Purification of pulvinic acid by p.l.c. with toluene–glacial acetic acid (4 : 1) gave a yellow solid, m.p. and mixed m.p. 214—216° (from ethanol). Pulvinic lactone prepared by heating the acid under reflux with acetic anhydride had m.p. and mixed m.p. 222° (from benzene).

Purification of polyporic acid by p.l.c. with absolute ethanol gave a brown solid, which gave purple crystals, m.p. and mixed m.p. 307° (from acetone). Polyporic acid diacetate, prepared by heating polyporic acid with acetic anhydride–glacial acetic (1 : 1), crystallised in yellow needles from benzene and had m.p. and mixed m.p. 211°.

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