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METABOLITES OF THE POLYPORACEAE—I

NOVEL CONJUGATES OF POLYPORENIC ACID A FROM PIPTOPORUS BETULINUS[1]

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Abstract—Polyporenic acid A has been shown to occur in sporophores of *Piptoporus betulinus* mainly in the form of conjugates in which the 3-α-hydroxyl group is esterified, the acids involved being acetic, caproic, malonic and β-hydroxy-β-methylglutaric acids. The dibasic acid conjugates occur not only as the free acids but also partly as monomethyl esters.

IN SOME studies of naturally occurring triterpenes, doubt remains as to the natural form of these compounds owing to the once prevalent practice of saponifying the extracted material. The fungus *Piptoporus betulinus*, well known as a source of polyporenic acids A and C,[2] has, in the present work, been reexamined under conditions favourable to the isolation of triterpene conjugates.

Initially, investigations were carried out on the mixture obtained by extraction of the sporophores with cold methanol, followed by esterification with ethereal diazomethane, the components then being separated by a combination of gradient elution chromatography on silicic acid and preparative TLC. Following this procedure, appreciable amounts of methyl polyporenate A (I) were obtained, together with methyl polyporenate C (II),[3] and three new compounds.

The least polar product, $C_{34}H_{52}O_5$, m.p. 130–132°, showed IR bands corresponding to the presence of one OH group (3631 cm^{-1}), two ester groups (1739 cm^{-1}, ε 845) and an exocyclic methylene group (3080, 1640, and 890 cm^{-1}). It was readily recognized as a 3-α-ester of methyl polyporenate A from close similarities to methyl polyporenate A in IR and NMR spectra, and the mass spectra were almost entirely the same below 449 mass units.

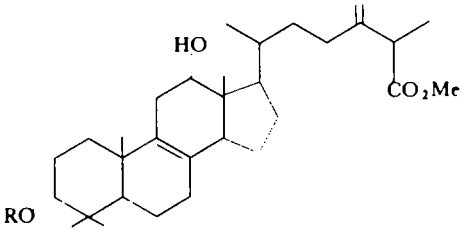
The presence of a singlet at 7·96 τ ($CH_3CO{\cdot}O$—) and of a diffuse triplet at 5·35 τ (H at C_3) suggested comparison with a sample of the acetate III, m.p. 136–137°, which was duly found to have virtually identical IR and NMR spectra. The discrepancy in m.p., which persisted throughout repeated efforts to further purify the isolated material, was traced to the presence of a small proportion of a related ester of the same polarity. Evidence that this was the corresponding caproate IV was provided firstly by the presence in the mass spectrum of a parent ion at *m/e* 598 (abundance

[1] Part of this work was presented at the I.U.P.A.C. Fourth International Symposium on the Chemistry of Natural Products, Stockholm, July 1966.

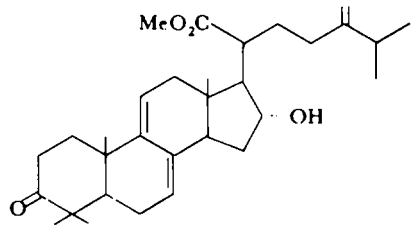
[2] R. G. Curtis, I. Heilbron, E. R. H. Jones, G. F. Woods, *J. Chem. Soc.* 457 (1953); T. G. Halsall, R. Hodges, E. R. H. Jones, *Ibid.* 3019 (1953).

[3] We are grateful to Dr. T. G. Halsall for authentic samples of methyl polyporenate A and methyl polyporenate C.

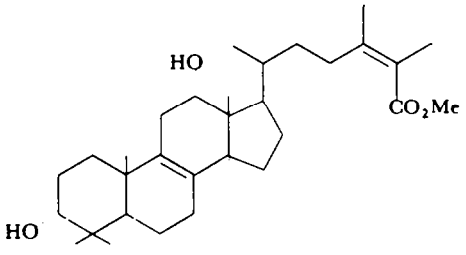
one eighth, relative to the parent molecular ion of the acetate, *m/e* 542) together with derived ions at *m/e* 583, 580, and 585 and secondly, by transesterification with sodium methoxide to give methyl acetate and methyl n-caproate, the latter being identified by GLC, together with methyl isopolyporenate A (V).[2] The shift of the βγ-double bond at C_{24} into conjugation with the ester group was reflected by the replacement



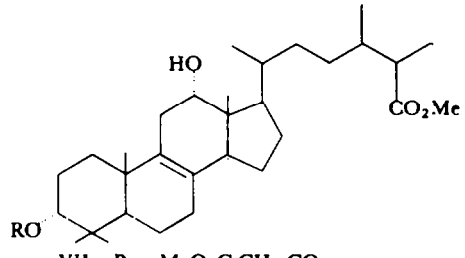
I. R = H
III. R = Ac—
IV. R = $Me(CH_2)_4CO$—
VI. R = $MeO_2C{\cdot}CH_2{\cdot}CO$—
IX. R = $MeO_2C{\cdot}CH_2{\cdot}CMe(OH){\cdot}CH_2{\cdot}CO$—



II



V



VII. R = $MeO_2C{\cdot}CH_2{\cdot}CO$—
VIII. R = H
X. R = $MeO_2C{\cdot}CH_2{\cdot}CMe(OH){\cdot}CH_2{\cdot}CO$—

of the doublet- quartet system (J = 7·2 c/s) at 8·76 τ and 6·90 τ due to the Me and hydrogen at C_{25} in the acyloxy methyl polyporenates A by a 6H singlet at 8·20 τ in the product, corresponding to two vinylic Me groups.

The other two compounds isolated also showed the spectral properties of a 3α-ester of I. In the mass spectrum of the less polar of these, $C_{36}H_{56}O_7$ (VI), elimination of the acyl grouping was associated with losses of 118 mass units from the parent and (M − 33) ions (cf. Table 1) and was identified as a carbomethoxyacetyl grouping by the presence in the NMR of singlets at 6·36 τ and 6·76 τ due to MeO— and $CH_2(CO{\cdot}O—)_2$ respectively and by the twin absorption bands at 1756 and 1736 cm^{-1}. These values were shown to be typical of malonates by comparison with those shown by dialkyl malonates and by the 3β-carbomethoxyacetyl derivative of cholesterol (cf. Experimental). The complexity of the CO absorption of malonates has been ascribed[4] to a combination of vibrational coupling and rotational isomerism.

[4] R. A. Abramovitch, *Canad. J. Chem.* 37, 1146 (1959).

TABLE 1. MASS SPECTRA OF ESTERS OF POLYPORENIC ACID A AND ITS DIHYDRO DERIVATIVE

Mol. wt (z) of $R.CH_2CO_2H$	*m/e* of principal ions > 390*						
	Acetate III 60	Malonate VI 118	Malonate VII 118	Glutarate IX 176	Glutarate X 176	Monomethyl ester XIII 176	
M	542(38)[a,b]	600(3)[b,c]	602(14)[b,c]				
M − 15	527(7)[a,d]	585(1)[d]					
M − 18	524(3)[b]	582(4)[b]	584(8)[b]	640(83)[e,f]	642(100)[e,f]	626(23)[f]	582(92)[e]
M − 33	509(100)[a,e,h]	567(34)[d,h]	569(100)[d,h]	625(13)[e,h]	627(20)[e,h]	611(5)[h]	567(17)[e,h]
M − z	482(6)	482(14)[f]	484(4)[f]	482(7)	484(5)	468(6)	424(13)
M − (15 + z)	467(12)[a,i]	467(100)[i]	469(17)[i]	467(36)[i]	469(10)[i]	453(18)[i]	409(42)[i]
M − (18 + z)	464(2)	464(3)	466(4)	464(9)[f]	466(12)[f]	450(8)[f]	406(13)
M − (33 + z)	449(62)[h,i]	449(82)[h,i]	451(92)[h,i]	449(100)[h,i,j,k]	451(92)[h,i,j,k]	435(41)[h,i,m*]	391(100)[h,i,l]
M − (33 + 74)				551(15)[j]	553(5)[j]		493(12)
M − (33 + 116)				509(14)[k]	511(5)[k]		451(20)
Other	465(6)	465(13)	467(40)	465(21)	467(40)	451(20)	407(35)

* % Relative abundances are given in parenthesis; metastable ions are present corresponding to the transition between ions in a given compound with the same superscript (*a* to *k*).

Final structure proof was provided by transesterification to methyl isopolyporenate A (V) and dimethyl malonate, the latter being identified by GLC. The dihydro derivative VII, obtained by catalytic reduction, showed a very similar cracking pattern (cf. Table 1) but lacked terminal methylene peaks in the NMR and IR. Transesterification in this case gave methyl 24(28)-dihydropolyporenate A (VIII).

In an analogous manner, the most polar ester $C_{39}H_{62}O_8$ was shown to be the γ-carbomethoxy-β-hydroxy-β-methylbutyrate IX. The acyl grouping in this case gave rise in the NMR to three singlets at 6·34 τ, 7·40 τ and 8·70 τ due respectively to the MeO group, two methylene groups adjacent to carboxylate functions and to a Me group geminal to a OH group. In carbon tetrachloride solution, the ester CO groups absorb at 1742 and 1715 cm^{-1}, one apparently being intramolecularly hydrogen bonded to the OH group which appears at 3528 cm^{-1}, the intensity relative to the 12-OH band (ν_{max} 3638 cm^{-1}) being unchanged by dilution. Methanolysis of the dihydro derivative X gave VIII together with dimethyl β-hydroxy-β-methylglutarate which was identified by GLC.

The mass spectrum of the relatively nonvolatile β-hydroxy-β-methylglutarate conjugate X differed from that of any of the other conjugates (cf. Table 1), in that the parent ion could not be detected, the ion of highest mass occurring at *m/e* 640 corresponding to the loss of H_2O from the parent ion. Apparently this loss does not involve the tertiary OH group of the glutarate system since abundant ions appear, corresponding to losses of 74, 116 and 176 mass units respectively from the (M − 33) ion, i.e. *m/e* 625, these evidently resulting from McLafferty rearrangements[5] involving the carbomethoxy-β-hydroxy-β-methylbutyrate system.[6] The presence of a metastable ion corresponding to the transition $625^+ \rightarrow 449^+$ is particularly significant since it suggests the elimination of the whole hydroxymethylglutarate moeity as a unit.

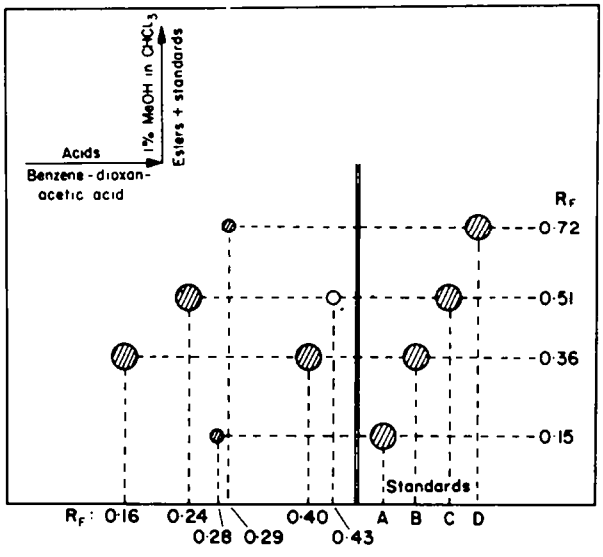
The natural form of the conjugates was later found by examination of a methanolic extract of the original mixture of acids. Fig. 1 shows the result of a special two dimensional thin layer chromatogram. After elution in the x direction, using benzene–dioxan–acetic acid ("B.D.A.") (35:5:1), the acids were esterified *in situ* with ethereal diazomethane and then eluted as Me esters in the *y* direction with 1% methanol in chloroform using samples of the Me esters previously isolated as standards. This located polyporenic acid A and 3-acetylpolyporenic acid A as the acids of R_f (B.D.A.) 0·28 and 0·29 respectively. It also revealed that the hydroxymethylglutarate IX and the malonate VI were each formed by methylation of two distinct acidic compounds. These were isolated after chromatography on silicic acid. The two more polar compounds (R_f in B.D.A. 0·16 and 0·24) were obtained as crystalline solids which were shown to be XI and XII, the dicarboxylic acids corresponding to IX and VI, since they lacked MeO resonances in the NMR but afforded the known dimethyl esters on treatment with diazomethane.

The acid of R_f 0·40 which was obtained only as a gum, also afforded the hydroxymethylglutarate IX on treatment with diazomethane. From its NMR spectrum, which was very similar to that of the dicarboxylic acid XI except for the presence of a 3H singlet at 6·30 τ, it was evident that this compound was a monomethyl ester of XI. Its mass spectrum (cf. Table 1) shows two series of ions differing by 14 and (14 + 44)

[5] Cf. F. W. McLafferty, *Determination of Organic Structures by Physical Methods* Vol. 2. Academic Press, N.Y. (1962).

[6] The composition of these ions were confirmed by mass measurements.

mass units respectively from the corresponding ions in the spectrum of the hydroxymethylglutarate IX. The ion of highest mass again corresponds to (M − 18). The spectrum also indicates that the MeO group is located in the conjugating acid moiety since several abundant ions are present whose formation involves the elimination of γ-carbomethoxy-β-hydroxy-β-methylbutyric acid (mol wt 176) or of neutral



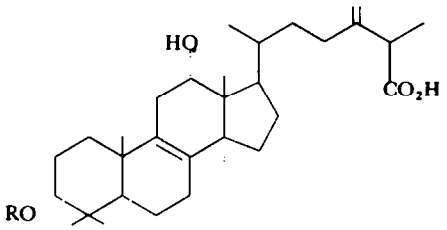
A. Methyl polyporenate A (I)
B. Methyl 3-(4-carbomethoxy-3-hydroxy-3-methylbutyryl)-polyporenate A (IX).
C. Methyl 3-carbomethoxyacetylpolyporenate A (VI).
D. Methyl 3-acetylpolyporenate A (III).

FIG. 1 Two dimensional TLC of the acids from *P. betulinus*.

fragments presumed to be parts thereof, namely MeO·C(OH)=CH_2 (mass 74) and $MeO_2C{\cdot}CH_2{\cdot}CO{\cdot}Me$ (mass 116). Moreover, metastable ions are present confirming the occurrence of transitions corresponding to the loss of 176 mass units from the M − 33 and M − (33 + 44) ions.

This monomethyl ester (i.e. XIII) and XI occurred in approximately equal amounts whereas the malonate conjugate appeared to occur mainly as the free dicarboxylic acid XII. The trace component of R_f 0·43, although not characterized, afforded VI on methylation with diazomethane and had the R_f to be expected for a monomethyl ester of VI. In order to eliminate the possibility that the monomethyl esters were artefacts arising from the use of methanol during work up procedure, the isolation of XIII from a sporophore was repeated using ethanol, the unchanged nature of the alkoxyl group being confirmed by NMR.

The fact that the major triterpene acid of *Piptoporus betulinus* is almost entirely conjugated with such biologically important molecules as malonic and β-hydroxy-β-methylglutaric acids strongly suggests that the conjugates may fulfil some significant function in the sporophore. Some studies relevant to this aspect are in progress.



XI. R = $HO_2C{\cdot}CH_2{\cdot}CMe(OH){\cdot}CH_2{\cdot}CO$—
XII. R = $HO_2C{\cdot}CH_2{\cdot}CO$—
XIII. R = $MeO_2C{\cdot}CH_2CMe(OH){\cdot}CH_2{\cdot}CO$—
XIV. R = $MeO_2C{\cdot}CH_2{\cdot}CO$—

EXPERIMENTAL

Fractionation of the triterpene acids from sporophores of P. betulinus *as their methyl esters.* Fresh sporophores of *P. betulinus* (1 Kg) were blended with cold MeOH and the filtered extract evaporated. The residue, after defatting with boiling light petroleum (b.p. 40–60°) was esterified at 0° with an excess of ethereal diazomethane. The ether soluble fraction of the product (75·5 g) was adsorbed on silica and eluted with light petroleum (2 l.) to which AcOEt acetate (2 l.) was being continuously added. This divided the less polar esters, R_f 0·72, 0·62 and 0·51 from the more polar esters R_f 0·36 and 0·15.[7]

Isolation of methyl polyporenate C (I). A part (8·3 g) of the mixture of less polar esters obtained as above was adsorbed on to silicic acid (480 g) and eluted with light petroleum (1·5 l) to which was continuously added 50% AcOEt–light petroleum (1·5 l.). Early fractions gave a mixture of the esters R_f 0·72 and 0·62 from which the latter, methyl polyporenate C,[8] was obtained by crystallization from light petroleum–AcOEt as needles (0·99 g), m.p. 193–194°. ν_{max}^{KBr} 3086, 3030, 1734, 1711, 1642, 890 cm^{-1}. $\nu_{max}^{CCl_4}$ 3623 (ε77, $\Delta\nu_{\frac{1}{2}}$16), 1736 (ε580, $\Delta\nu_{\frac{1}{2}}$18), 1713 cm^{-1} (ε569, $\Delta\nu_{\frac{1}{2}}$14). λ_{max}^{MeOH} 237 mμ (ε13,500), 243 mμ (ε15,300), 251 mμ (ε10,600).

NMR (CCl_4): 4·55 τ (olefinic H at C_7 and C_{11}, 2H, t, J = 5·6 c/s), 5·25 τ ($CH_2{=}C\lessdot$, d, J = 3 c/s, 5·90 τ (H *gem* to OH at C_{16}, m), 6·28 τ (MeO—, 3H, s). R.D. (MeOH, c = 0·556) ϕ_{263} + 15,400, ϕ_{300} + 198. (Found: C, 77·2; H, 9·8; OMe, 6·6; M^+ 496. Calc. for $C_{32}H_{48}O_4$: C, 77·4; H, 9·7; OMe, 6·3%; M.W. 496).

Isolation of methyl polyporenate A 3-*acetate*/3-n-*caproate mixture.* The mother liquors from the crystallization of methyl polyporenate C contained ca. 50% of a mixture of two esters with R_f 0·72. Preparative TLC of the mixture on silica using fourfold elution with 50% benzene in chloroform, followed by successive crystallizations from light petroleum and aqueous MeOH gave the mixture of esters (59 mg), m.p. 130–132°. ν_{max}^{KBr} 3629, 3080, 1738, 1640, 1249, 890 cm^{-1}. $\nu_{max}^{CCl_4}$ 3631 (ε55, $\Delta\nu_{\frac{1}{2}}$16), 1739 (ε845, $\Delta\nu_{\frac{1}{2}}$23), 3090, 1645 cm^{-1}.

UV: no absorption above 220 mμ. NMR (CCl_4): 5·10 τ ($CH_2{=}C\lessdot$, 2H, m), 5·35 τ (H at C_3, 1H, m), 6·04 τ (H *gem* to OH at C_{12}, 1H, d, J = 7·2 c/s), 6·33 τ (MeO—, 3H, s), 8·72 τ (Me at C_{25}, 3H, d, J = 7·2 c/s).

The above m.p. was unchanged after further crystallizations from aqueous MeOH or repeated TLC on silica. The mixture was identical in R_f and almost identical in IR and NMR with a sample of methyl 3-acetylpolyporenate A, m.p. 136–137°, prepared by acetylation of methyl polyporenate A. Comparison of the mass spectrum of the pure acetate with that of the isolated sample showed extra peaks in the latter at *m/e* 598 (abundance ca. 10% of the parent ion of III at *m/e* 542), 585, 580, 565.

The isolated mixture (181 mg) was refluxed with 0·043 M methanolic MeONa (10 ml) for 13 hr and then poured in 6N HCl (10 ml). The product, obtained by ether extraction, was examined by GLC on a 10% P.E.G.A. column using a Perkin-Elmer F11 chromatograph, at a flow rate of 32 ml/min N_2 and with the

UV and IR spectra were determined on Unicam SP 500 and SP 100 spectrophotometers respectively, NMR spectra on a Perkin-Elmer 60 mc instrument and mass spectra on an A.E.I. MS9 double focusing spectrometer.

[7] *p*-Aminoazobenzene, R_f 0·67 and *p*-hydroxyazobenzene, R_f 0·35 were used as internal standards, with 1% MeOH in $CHCl_3$ as eluent.

[8] Identity with an authentic sample of methyl polyporenate C kindly provided by Dr. T. G. Halsall was established by m.p., mixed m.p., TLC and mass spectra.

temp increasing after 8 min at 50° by 3°/min to 90°. This gave, apart from the solvent peak, a single peak of retention time 16·0 min. In separate runs, methyl n-caproate had retention time 16·1 min and a mixture of the samples gave a single well defined peak at 16·0 min. TLC of the non volatile product of this reaction showed only one spot with R_f identical to that of methyl isopolyporenate A (see below).

Isolation of methyl carbomethoxyacetyl polyporenate A (VI). Later fractions from the column affording mixtures of II, III and IV contained the *malonate* VI, R_f 0·51. This crystallized from AcOEt–light petroleum in needles (1·5 g), m.p. 88–89°. ν_{max}^{KBr} 3602, 3090, 1755, 1736, 1645, 1236, 890 cm^{-1}. $\nu_{max}^{CCl_4}$ 3630 (ε 60, $\Delta\nu_{\frac{1}{2}}$ 17), 1756 (ε 705, $\Delta\nu_{\frac{1}{2}}$ 16), 1736 (ε 1228, $\Delta\nu_{\frac{1}{2}}$ 19), 3090, 1646 cm^{-1}. R.D. (MeOH, c = 0·433) Φ_{227} +3060, Φ_{233} +4230, Φ_{300} +300. NMR (CCl_4): singlets at 6·32 τ (3H) and 6·70 τ (2H) due respectively to the MeO and methylene of the carbomethoxyacetate group. (Found: C, 71·7; H, 9·5; M^+ at m/e 600. $C_{36}H_{56}O_7$ requires: C, 72·0; H, 9·4; M.W. 600).

Conversion of VI *to methyl isopolyporenate* (V) *and dimethyl malonate.* The malonate VI (318 mg) in MeOH (8 ml) was added to methanolic MeONa prepared from Na (50 mg) and dry MeOH (10 ml). After refluxing for 16 hr, the mixture was added to chilled conc HCl (20 ml) and extracted with ether. The crude product was purified by preparative TLC on rhodamine G treated silica using 2% MeOH in chloroform as eluent, followed by treatment with charcoal and crystallization from aqueous MeOH giving colourless needles (52 mg), m.p. 158–163° (lit. m.p. 163–165°). ν_{max}^{KBr} 2838, 1708, 1633 cm^{-1}. $\nu_{max}^{CCl_4}$ 3637 (ε 92, $\Delta\nu_{\frac{1}{2}}$ 22), 1718 (ε 430, $\Delta\nu_{\frac{1}{2}}$ 18). λ_{max}^{MeOH} 226 mμ (ε 8900). NMR (CCl_4): 6·04 τ (H at C_{12}, br. d, J = 7 c/s), 6·30 τ (MeO—, 3H, s), 6·63 τ (H at C_3, t, J = 3 c/s), 8·2 τ (Me at C_{24} and C_{25}, 6H, s).

In a separate experiment, the ethereal soln of methanolysis products was carefully concentrated by fractional distillation and then examined by GLC (10% P.E.G.A. column, Pye-Argon chromatograph at 75°, 26 ml/min of argon). A single peak of retention time 20·9 min was observed, and this could not be separated from that produced by dimethyl malonate.

3-Carbomethoxyacetyl chloesterol. A soln of cholesterol (1 g) in dry pyridine (4 ml) and anhyd ether (10 ml) was added dropwise during 1 hr to a soln of carbomethoxyacetyl chloride (6 g) in dry ether (10 ml), and stirred for a further 2 hr. After addition of water (25 ml), the soln was washed with 6N HCl (6 × 25 ml), dried and evaporated to give an oily solid. Chromatography on silica (100 g) using 5% ether in light petroleum as eluent afforded *3-carbomethoxyacetylcholesterol* (1·16 g, 92%) which crystallized from light petroleum–AcOEt as needles, m.p. 108–108·5°. ν_{max}^{KBr} 1765, 1736 cm^{-1}. $\nu_{max}^{CCl_4}$ 1759 (ε 570, $\Delta\nu_{\frac{1}{2}}$ 17), 1739 cm^{-1} (ε 697, $\Delta\nu_{\frac{1}{2}}$ 17). NMR (CCl_4): 5·40 τ (H at C_3, m), 6·25 τ (MeO—, 3H, s), 6·79 τ (—OCO·CH_2·CO·O—, 2H, s). (Found: C, 76·5; H, 10·5; M^+ at m/e 486. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·35%; M.W. 486).

Preparation and methanolysis of methyl 3-carbomethoxyacetyl-24,28-dihydropolyporenate A (VII). The malonate VI (131 mg) in EtOH (10 ml) was hydrogenated using PtO_2 (32 mg) until absorption was complete (2 hr). After filtration through glass paper, evaporation gave the *dihydro derivative* VII, which crystallized from aqueous MeOH in colourless needles (98 mg), m.p. 140–141°. ν_{max}^{KBr} 3560, 1756, 1736, 1262 cm^{-1}. (Found: C, 71·4; H, 9·5; M^+ at m/e 602. $C_{36}H_{58}O_7$ requires: C, 71·7; H, 9·7%; M.W. 602).

Methanolysis of VII (128 mg) using the previously described procedure gave VIII (50 mg), m.p. 139–141°, undepressed on admixture with a sample, m.p. 140–142° prepared from polyporenic acid A. The samples were also identical in IR, NMR and R_f.

Isolation of methyl 3-(4-carbomethoxy-3-hydroxy-3-methylbutyryl) polyporenate A IX *and of methyl polyporenate A* (I). A portion (28 g) of the mixture obtained as described above containing the more polar esters, R_f 0·36 and 0·15 was adsorbed on alumina (Woelm, Grade IV, neutral) and eluted with light petroleum (1·5 l) to which was being added continuously AcOEt (1·5 l). This gave fractions in which these esters were concentrated but did not effect a separation. Preparative TLC of a part (3·5 g) on Rhodamine G treated silica using threefold elution with 2% MeOH in Chf gave the pure esters. IX, R_f 0·36, after treatment with charcoal and crystallization from light petroleum–AcOEt, formed needles (900 mg), m.p. 79–81°. ν_{max}^{KBr} 3084, 1742, 1726, 1705, 1647, 892 cm^{-1}. $\nu_{max}^{CCl_4}$ 3638 (ε 56, $\Delta\nu_{\frac{1}{2}}$ 14), 3528 (ε 56, $\Delta\nu_{\frac{1}{2}}$ 80), 1742 (ε 925, $\Delta\nu_{\frac{1}{2}}$ 20), 1715 (ε 388 sh), 3090, 1646 cm^{-1}. NMR (CCl_4): ester group giving rise to resonances at 5·30 τ (H at C_3, 1H, t, J = 2·4 c/s), 6·32 τ (MeO—, 3H, s), 7·40 τ (—CH_2CO·O—, 4H, s), 8·70 τ (Me—$\underset{|}{\overset{|}{C}}$—O—, 3H, s). M^+ at m/e 658 undetected, first ion in mass spectrum at m/e 640 (M— 18). (Found: C, 71·2; H, 9·3. $C_{39}H_{62}O_8$ requires: C, 71·1; H, 9·5%).

Methanolysis of IX (420 mg) under the previously described conditions gave after purification of the product, V (100 mg), m.p. 165° (lit. m.p. 163–165°), indistinguishable in IR, NMR and R_f from the sample

obtained previously. The product was also examined by GLC on a four foot column with 2% Versamide 900 as stationary phase, using a Perkin-Elmer F11 chromatograph, at a flow rate of nitrogen of 38 ml/min, and with the temp increasing after 5 min at 80° by 5°/min to 200°. Samples of the product and of dimethyl 3-hydroxy-3-methylglutarate, either separately or together, gave a single well defined peak (retention time 16·3 ± 0·3 min).

Compound I, R_f 0·15, isolated as described above, after treatment with charcoal, crystallized from aqueous MeOH as needles (720 mg), m.p. 148–149° (lit. m.p. 148·5–149·5°). ν_{max}^{KBr} 3090, 1742, 1727, 1648, 900 cm^{-1}. $\nu_{max}^{CCl_4}$ 3639 (ε 93, $\Delta\nu_{\frac{1}{2}}$ 18), 1744 (ε 510, $\Delta\nu_{\frac{1}{2}}$ 23), 3090, 1648 cm^{-1}. λ_{max}^{MeOH}: No absorption above 220 mμ. NMR (CCl_4): 5·10 τ (CH_2=C<, 2H, m), 6·04 τ (H *gem* to OH at C_{12}, 1H, br. d, J = 7·2 c/s), 6·35 τ (MeO—, 3H, s), 6·63 τ (H at C_3, 1H, m), 6·90 τ (H at C_{25}, 1H, q, J = 7 c/s), 8·75 τ (Me at C_{25}, 3H, d, J = 7 c/s). (Found: C, 76·5; H, 10·4; M^+ at *m/e* 500. Calc. for $C_{32}H_{52}O_4$: C, 76·75; H, 10·5%; M.W. 500). Identity with an authentic sample of methyl polyporenate A kindly supplied by Dr. T. G. Halsall was established by IR, NMR TLC and mixed m.p.

Methyl 3-(4-*carbomethoxy*-3-*hydroxy*-3-*methylbutyryl*)-24,28-*dihydropolyporenate A* (X). Compound IX (834 mg) in EtOH (25 ml) was hydrogenated using PtO_2 (157 mg) as catalyst. The *dihydro derivative* X crystallized from light petroleum as prisms (810 mg), m.p. 91–93°. ν_{max}^{KBr} 1734, 1700 sh. M^+ at *m/e* 660 undetected, first ion in mass spectrum at *m/e* 642 (M − 18). (Found: C, 71·2; H, 9·7. $C_{39}H_{64}O_8$ requires: C, 70·9; H, 9·7%).

Methanolysis of this dihydro compound (280 mg) using the previously described procedure, afforded, after purification of the product, VIII, (60 mg), m.p. 139–141°. Identity with an authentic sample was confirmed by mixed m.p., IR, NMR, and TLC.

Fractionation of the free triterpenoid acids from sporophores of P. betulinus. A portion (2·6 g) of the material extracted with MeOH from a finely divided sporophore of *P. betulinus* was adsorbed on a column of silicic acid (300 g), and eluted first with Chf and then with 1% MeOH in Chf. TLC on silica using benzene–dioxan–AcOH (35:5:1) as eluent was used to allow grouping of the column fractions.

Isolation of 3-(4-*carboxy*-3-*hydroxy*-3-*methylbutyryl*)*polyporenic acid A* (XI). The later fractions from the above column afforded XI, the most polar product, R_f 0·16, which crystallized from light petroleum–AcOEt as needles (120 mg), m.p. 165–166°. ν_{max}^{Chf} 3620, 3518, 1742, 1710 cm^{-1}. (Found: C, 70·2; H, 9·5. $C_{37}H_{58}O_8$ requires: C, 70·4; H, 9·3%). The product obtained by methylation of XI had R_f identical to the ester IX.

A compound R_f 0·32, which was also eluted from the above column with Chf, was obtained as a colourless gum. ν_{max}^{Chf} 1736, 1710 cm^{-1}. NMR ($CDCl_3$); almost identical to that of the above diacid XI but showing an additional singlet (3H) at 6·30 τ. M^+ at *m/e* 644 undetected, first ion in mass spectrum at *m/e* 626. Again the product obtained by methylation was indistinguishable from the ester IX on thin layers.

Isolation of 3-*carboxyacetylpolyporenic acid A* (XII). Elution of the above column with 1% MeOH in Chf gave the *acid malonate* XII, R_f 0·24, which crystallized in needles (200 mg), m.p. 184–185°. ν_{max}^{Chf} 3614 (ε 49, $\Delta\nu_{\frac{1}{2}}$ 30), 3505 (ε 59, $\Delta\nu_{\frac{1}{2}}$ 54), 1763 (ε 262), 1734 (ε 565), 1710 cm^{-1} (ε 785, $\Delta\nu_{\frac{1}{2}}$ 28). (Found: C, 71·3; H, 9·55. $C_{34}H_{52}O_7$ requires: C, 71·3; H, 9·15%). The product obtained by methylation with ethereal diazomethane had R_f identical with that of the malonate VI.

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