

The Chemistry of the Triterpenes and Related Compounds.
 Part XXVI.* *The Nature of Polyporenic Acid B.*

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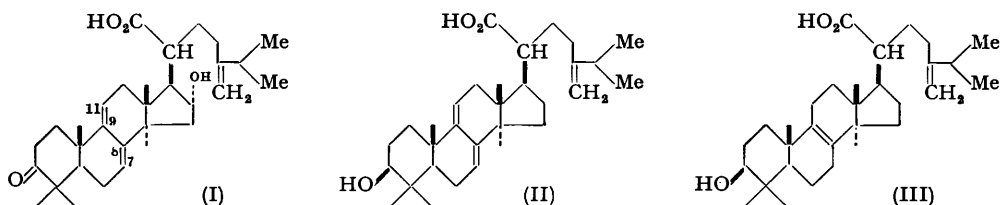
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Polyporenic acid B (Cross, Eliot, Heilbron, and Jones, *J.*, 1940, 632) has been shown to be a mixture of 3β : 16α -dihydroxyeburico-8 : 24(28)-dien-21-oic acid and the corresponding dehydro-acid. It is accompanied in *Polyporus betulinus* by the 3β -monoacetates of these acids.

IN 1939, Cross, Eliot, Heilbron, and Jones (*J.*, 1940, 632) isolated three acids, polyporenic acids A, B, and C, from the birch-tree fungus, *Polyporus betulinus* Fr. It was suggested that these acids might be triterpenoids and for polyporenic acid B a formula $C_{30}H_{48}O_4$ or $C_{31}H_{50}O_4$ was put forward on the basis of analyses of the acid, m. p. 300—310 (decomp.), and its methyl ester, m. p. 160°. A Zerewitinoff determination on the ester indicated that acid B was a dihydroxy-acid. The work was abandoned in 1940 and it is only recently that it has become possible to examine polyporenic acid B in detail.

Cross *et al.* (*loc. cit.*) indicated that acid B accompanied polyporenic acid C in the ether-acetone extract of fungus which had already been extracted with alcohol to remove polyporenic acid A. With the elucidation of the structure of acid C (I) (Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548; Bowers, Halsall, and Sayer, *J.*, 1954, 3070), and the knowledge that it reacted with Girard's reagent, it was possible to isolate acid B by methylating with ethereal diazomethane the acetone extract of the fungus from which polyporenic acid A had been removed by ethanol extraction, and then subjecting it to a Girard separation. The non-ketonic fraction was chromatographed on alumina and two esters were isolated. One was a methyl dihydroxy-ester ($C_{32}H_{52}O_4$) which formed needles, m. p. 168—171°, from methanol. This is obviously methyl polyporeenate B. The other ester was a monoacetoxy-monohydroxy-derivative ($C_{34}H_{54}O_5$) which could be hydrolysed to methyl polyporeenate B.

Both esters on spectroscopic examination showed absorption at 2360, 2430, and 2520 Å, corresponding to the presence of 40% (dihydroxy-ester) and 15% (monoacetoxy-mono-hydroxy-ester), respectively, of the typical 7 : 9(11)-diene chromophore such as is found in polyporenic acid C (I) and dehydroeburicoic acid (II) (Gascoigne, Robertson, and

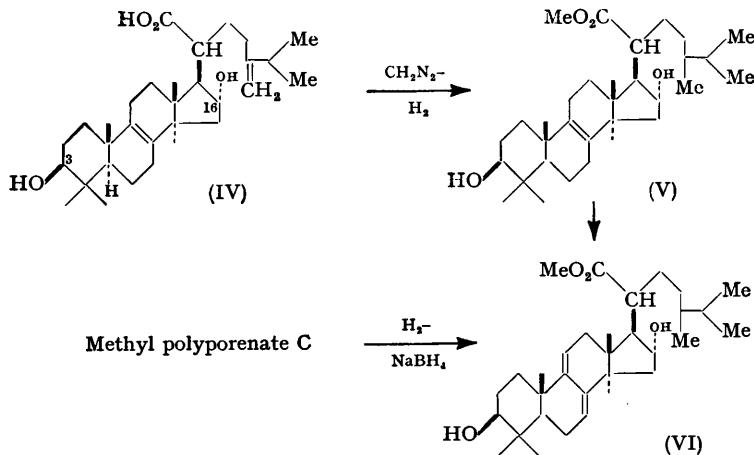


Simes, *J.*, 1953, 1830). These workers have found that extraction of certain fungi leads to the isolation of practically inseparable mixtures of eburicoic (III) (Holker, Powell, Robertson, Simes, Wright, and Gascoigne, *J.*, 1953, 2422) and dehydroeburicoic (II) acids. It therefore appeared likely that "methyl polyporeenate B" was a mixture of the esters of a similar pair of acids. Simple transformations of mixtures of this type do not usually bring about much change in the percentage of dehydro-derivative present [cf. the reactions of a mixture of α -elemolic acid and its dehydro-derivative (Halsall, Meakins, and Swayne, *J.*, 1953, 4139)]. A number of simple reactions with the "methyl polyporeenate B" obtained as described above were therefore carried out.

The infra-red spectrum of "methyl polyporeenate B" determined in carbon tetrachloride had bands at 3635, 1737, and 1640 and 894 cm^{-1} indicative of hydroxyl, methoxycarbonyl,

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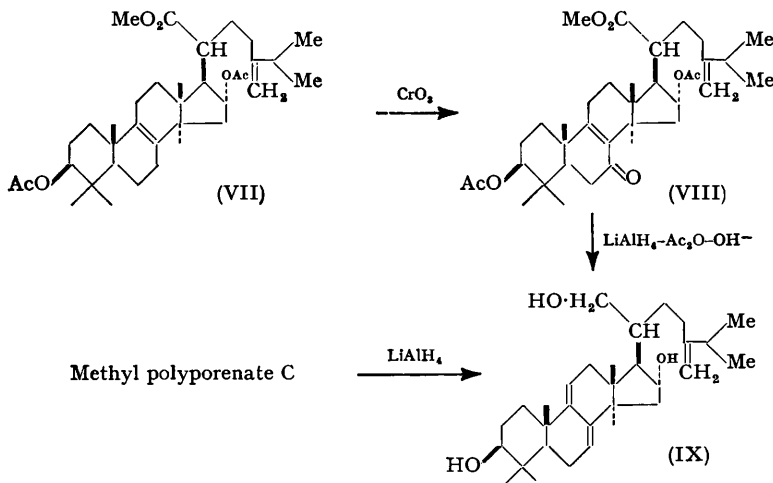
and vinylidene groups, respectively. Acetylation of the ester gave a diacetate which was also obtained by acetylation of the methyl ester monoacetate described above. The light absorptions of the two samples of diacetate were very similar ($\epsilon_{2430\text{Å}} = 3930$ and 3740 respectively). Oxidation of the monoacetate gave a mono-ketone which exhibited, in carbon tetrachloride, a very strong band at 1737 cm^{-1} but no band at *ca.* 1705 cm^{-1} . This indicates that the oxygen atom of the keto-group and hence of the original free hydroxyl group must be attached to a five-membered ring. Oxidation of "methyl polyporene B" itself gave a diketone which, in carbon tetrachloride, exhibited bands at 895 , 1714 , and 1740 cm^{-1} . These are assigned respectively to a vinylidene group, a keto-group in a six-membered ring and the methoxycarbonyl group, and a keto-group in a five-membered ring. These results suggested that "polyporenic acid B" might be very closely related to polyporenic acid C (I) and be a mixture of (IV) and its dehydro-derivative. This was proved to be correct as follows:



"Methyl polyporene B" was hydrogenated to the dihydro-derivative (cf. V) and this was converted into the corresponding dehydro-compound by oxidation with monophtalic acid and treatment of the resulting oxide in boiling ethanol with concentrated hydrochloric acid for two hours. Modifications of this method have been used to convert dihydrolanosteryl acetate (Birchenough and McGhie, *J.*, 1949, 2038), *O*-acetylidhydroeburicoic acid (Lahey and Strasser, *J.*, 1951, 873), and dihydroeuphyl acetate (Barbour, Bennett, and Warren, *J.*, 1951, 2540) into their corresponding dehydro-derivatives. The dehydro-derivative from "methyl dihydropolyporene B" was identical with methyl 3 β :16 α -dihydroxyeburico-7:9(11)-dien-21-oate (VI) obtained from methyl polyporene C by hydrogenation and reduction of the 3-oxo-group with sodium borohydride (Bowers, Halsall, Jones, and Lemin, *loc. cit.*). This interconversion proves the structure of the main component of "polyporenic acid B" apart from the position of the vinylidene group. To locate this group the diacetate of "methyl polyporene B" (cf. VII) was oxidised to the 7-oxo-derivative (cf. VIII). This was reduced with lithium aluminium hydride and the product heated with acetic anhydride and then hydrolysed with methanolic potassium hydroxide to give 3 β :16 α :21-trihydroxyeburico-7:9(11):24(28)-triene (IX) which was identical with an authentic sample prepared by the reduction of polyporenic acid C with lithium aluminium hydride (Bowers, Halsall, and Sayer, *loc. cit.*). Similar methods for the formation of the conjugated diene system have been used in the eburicoic acid (Gascoigne, Robertson, and Simes, *loc. cit.*) and the lanosterol (Cavalla and McGhie, *J.*, 1951, 744) series.

These results show that "polyporenic acid B," as isolated from the fungus, is a mixture of 3 β :16 α -dihydroxyeburico-8:24(28)-dien-21-oic acid (IV) and 3 β :16 α -dihydroxyeburico-7:9(11):24(28)-trien-21-oic acid. The "monoacetate" isolated must be a mixture of the corresponding 3 β -monoacetates since oxidation as described above shows that the

free hydroxyl group is attached to a five-membered ring. The isolation of the β -acetate of a fungal acid has been previously reported, both Lahey and Strasser (*J.*, 1951, 873)



and Gascoigne, Holker, Ralph, and Robertson (*J.*, 1951, 2346) having isolated eburicoic acid β -monoacetate from *P. anthracophilus* Cooke grown naturally on *Eucalyptus regnans*.

EXPERIMENTAL

Rotations were determined in chloroform at room temperature unless otherwise stated. M.p.s were determined on a Kofler block and are corrected. The alumina used for chromatography, unless otherwise stated, was Peter Spence grade 'H' and had been deactivated with 5% of 10% acetic acid solution. Light petroleum refers to the fraction with b. p. 60–80°. Ultra-violet spectra were determined in ethanol.

Isolation from Polyporus betulinus Fr. of Polyporenic Acid B and its β -Monoacetate as their Methyl Esters.—Minced fungus (ca. 5 kg.), which had already been extracted twice with ethanol to remove polypropenic acid A, was extracted with acetone. The extract was methylated with ethereal diazomethane and then separated into ketonic and non-ketonic fractions by use of Girard's reagent T, the former fraction containing methyl polypropenate C. The non-ketonic fraction (50 g.) was adsorbed from light petroleum (200 c.c.) on alumina (1000 g.) which had been deactivated with aqueous acetic acid (10%; 100 g.). Elution with light petroleum (1000 c.c.; 1000 c.c.; 1500 c.c.) and with benzene (500 c.c.; 2500 c.c.) gave the following five fractions: (i) orange oil (12.3 g.), (ii) white gummy solid (4.4 g.), (iii) yellow gum (4.0 g.), (iv) brown gum (12.4 g.), and (v) brown gum (5.9 g.). Fraction (ii) was washed with light petroleum and crystallised from methanol, giving needles (1.2 g.), m. p. 173–178°. Fractions (iii) and (iv) were crystallised from methanol, giving ergosterol (0.3 g. and 1.2 g.), m. p. 142–148°. The mother liquors from fractions (iii) and (iv), and fraction (v) were combined and rechromatographed on alumina (600 g.) to give, with the solvents indicated, the following brown gums: (1) with benzene (250 c.c.) (0.7 g.), (2) with benzene (750 c.c.) (1.3 g.), (3) with benzene (750 c.c.) (0.7 g.), (4) with benzene-ether (9:1; 750 c.c.) (2.7 g.), (5) with benzene-ether (9:1; 1750 c.c.) (2.5 g.), and (6) with benzene-ether (1:1; 500 c.c.) (1.5 g.).

Fraction (2) was crystallised from methanol, giving the *monoacetate* of "methyl polypropenate B" as needles (0.8 g.), m. p. 175–180°, raised by further crystallisations from methanol to 180–184°, $[\alpha]_D^{25} +39^\circ$ (c, 1.1) (Found: C, 75.45; H, 10.2. $C_{34}H_{54}O_5$ requires C, 75.25; H, 10.05. $C_{34}H_{52}O_5$ requires C, 75.5; H, 9.7%). Light absorption: Max., 2360, 2430, and 2520 Å; $\epsilon = 2160, 2420, \text{ and } 1640$.

Fraction (5) was crystallised from methanol, giving "methyl polypropenate B" as needles (1.5 g.), m. p. 160–165°, raised by further crystallisations from methanol to 168–171°, $[\alpha]_D^{25} +28^\circ$ (c, 1.01) (Found: C, 76.85; H, 10.15. Calc. for $C_{32}H_{52}O_4$: C, 76.75; H, 10.45. Calc. for $C_{32}H_{50}O_4$: C, 77.05; H, 10.1%). Light absorption: Max., 2360, 2430, and 2520 Å; $\epsilon = 5250, 6500, \text{ and } 3550$.

Hydrolysis of fraction (2) and also fraction (ii) from the first chromatogram with methanolic

potassium hydroxide yielded "methyl polyporene B," m. p. 160—165°, $[\alpha]_D + 27^\circ$ (*c.* 0.97). Fraction (ii) is therefore the monoacetate.

The mother liquor from fraction (2), and fractions (3) and (4) contained a mixture of "methyl polyporene B" and its monoacetate. Hydrolysis of these fractions with 5% methanolic potassium hydroxide for 16 hr. at 20°, followed by purification of the product, yielded "methyl polyporene B" (1 g.).

Acetylation of "Methyl Polyporene B."—The ester (m. p. 160—165°; 0.6 g.) was treated with acetic anhydride (10 c.c.) and pyridine (10 c.c.) at 100° for 4 hr. The product was crystallised several times from methanol, giving the *diacetate* as needles (0.4 g.), m. p. 159—161°, $[\alpha]_D + 10.5^\circ$ (*c.* 0.99) (Found: C, 73.9; H, 9.8. $C_{36}H_{56}O_6$ requires C, 73.95; H, 9.65. $C_{36}H_{54}O_6$ requires C, 74.2; H, 9.35%). Light absorption: Max., 2360, 2430, and 2510 Å; $\epsilon = 3420, 3930, \text{ and } 2580$.

Acetylation of "Methyl Polyporene B Monoacetate" Isolated from the Fungus.—The monoacetate (0.4 g.) was treated with acetic anhydride (5 c.c.) and pyridine (5 c.c.) at 100° for 4 hr. The product was adsorbed on alumina (20 g.) and eluted with benzene (300 c.c.), giving the diacetate as needles (250 mg.) (from methanol), m. p. 155—158° undepressed on admixture with a sample prepared from "methyl polyporene B," $[\alpha]_D + 10^\circ$ (*c.* 1.02). Light absorption: Max., 2360, 2430, and 2510 Å; $\epsilon = 3300, 3740, \text{ and } 2340$.

Oxidation of "Methyl Polyporene B Monoacetate" Isolated from the Fungus.—An aqueous sulphuric acid solution of chromic acid (6*N*; 0.6 c.c.) was added to a solution of the monoacetate (200 mg.) in acetone (10 c.c.). The mixture was immediately diluted with water and extracted with ether. The extract was adsorbed from benzene on alumina (10 g.). Elution with benzene (200 c.c.) gave the *monoketone* as needles (from methanol) (150 mg.), m. p. 175—177°, $[\alpha]_D - 42^\circ$ (*c.* 1.0) (Found: C, 75.35; H, 9.7. $C_{34}H_{52}O_5$ requires C, 75.5; H, 9.7. $C_{34}H_{50}O_5$ requires C, 75.8; H, 9.35%). Light absorption: Max., 2360 and 2430 Å; inflexion, 2510 Å; $\epsilon = 2540, \text{ and } 2020$.

Oxidation of "Methyl Polyporene B."—"Methyl polyporene B" (100 mg.) was oxidised as in the experiment described above. The product was adsorbed from benzene on alumina (5 g.) and eluted with benzene, giving the *diketone* as plates (from methanol) (70 mg.) m. p. 155—157°, $[\alpha]_D - 62^\circ$ (*c.* 1.03) (Found: C, 77.6; H, 9.45. $C_{32}H_{48}O_4$ requires C, 77.4; H, 9.75. $C_{32}H_{46}O_4$ requires C, 77.7; H, 9.4%). Light absorption: Max., 2360 and 2430 Å; inflexion, 2510 Å; $\epsilon = 5620, 6200, \text{ and } 4120$.

Hydrogenation of "Methyl Polyporene B."—The ester (1.3 g.) in ethanol (125 c.c.) was shaken with hydrogen at 20° in the presence of Adams's catalyst (100 mg.) until the uptake of hydrogen ceased. The product was crystallised from methanol, giving the *dihydro-derivative* as needles (900 mg.), m. p. 173—174°, $[\alpha]_D + 25^\circ$ (*c.* 0.98) (Found: C, 76.55; H, 11.0. $C_{32}H_{54}O_4$ requires C, 76.45; H, 10.85. $C_{32}H_{52}O_4$ requires C, 76.75; H, 10.45%). Light absorption: Max., 2360, 2430, and 2520 Å; $\epsilon = 4850, 6100, \text{ and } 3220$.

Conversion of "Methyl Dihydropolyporene B" into Methyl 3β:16α-Dihydroxyeburico-7:9(11)-dien-21-oate (VI).—The dihydro-ester (400 mg.) in ether (100 c.c.) was added to an ethereal solution of monoporphthalic acid (15 c.c.; 10 mols.), and the mixture kept at 20° for 5 days. The ethereal solution was then washed with sodium hydrogen carbonate solution and with water, dried, and evaporated to dryness. The crude epoxide (300 mg.) in ethanol (50 c.c.) was heated under reflux with concentrated hydrochloric acid (2 c.c.) for 3 hr. Dilution with water and isolation with ether yielded a product which was adsorbed on alumina (15 g.). Elution with benzene-ether (4:1; 200 c.c.) afforded methyl 3β:16α-dihydroxyeburico-7:9(11)-dien-21-oate as needles (150 mg.), m. p. 189—190° (two crystallisations from methanol), undepressed on admixture with an authentic sample {m. p. 188—190°, $[\alpha]_D + 24^\circ$ (Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548)}, $[\alpha]_D + 27^\circ$ (*c.* 0.94) (Found: C, 74.15; H, 10.4. Calc. for $C_{32}H_{52}O_4 \cdot CH_3 \cdot OH$: C, 74.4; H, 10.6%). Light absorption: Max., 2360, 2430, and 2520 Å; $\epsilon = 14,000, 16,500, \text{ and } 10,900$.

Conversion of "Methyl Polyporene B Diacetate" into 3β:16α:21-Trihydroxyeburico-7:9(11):24(28)-triene (IX).—The diacetate (900 mg.) was oxidised in acetic acid (50 c.c.) with chromic acid (450 mg.) at 80—90° for 10 min. After dilution with water, isolation with ether yielded a product which was adsorbed on alumina (50 g.). Elution with benzene (200 c.c.) gave a gum (15 mg.) which was discarded. Further elution with benzene-ether (9:1; 600 c.c.) afforded a gum (350 mg.) which showed maximum light absorption in ethanol at 2540 Å, indicative of the presence of methyl 3β:16α-diacetoxy-7-oxoeburico-8(9):24(28)-dien-21-oate. The gum was dissolved in ether (100 c.c.) and heated under reflux with lithium aluminium hydride (900 mg.) for 3 hr. Decomposition of the complex with dilute sulphuric acid and isolation with

ether yielded a white solid which was heated under reflux with acetic anhydride (50 c.c.) for 1 hr. Dilution with water and extraction with ether afforded a gum which was heated under reflux with methanolic potassium hydroxide (10% ; 50 c.c.) for 30 min. The product (250 mg.) was adsorbed from benzene-ether (9 : 1 ; 50 c.c.) on alumina (10 g.) ; elution with benzene-ether (4 : 1 ; 70 c.c.) gave an impure fraction (50 mg.). Further elution with this solvent (300 c.c.) afforded 3 β : 16 α : 21-trihydroxyeburico-7 : 9(11) : 24(28)-triene as needles (190 mg.), m. p. and mixed m. p. 218—219° (two crystallisations from methanol), $[\alpha]_D + 34^\circ$ (*c*, 0.78 in pyridine). Light absorption : Max., 2360, 2430, and 2520 Å ; $\epsilon = 15,000 ; 17,400, \text{ and } 11,600$.

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