116. *Constituents of the Higher Fungi. Part I. The Triterpene Acids of* Polyporus betulinus *Fr.*

By L. C. Cross, C. G. Eliot, I. M. Heilbron, and E. R. H. Jones.

An examination of the birch tree fungus, *Polyporus betulinus* Fr., has resulted in the isolation, after saponification of the extracts, of *polyporenic acids A, B* and *C*, all of which are probably triterpenoid. Acids A and B appear to be isomeric $C_{30}H_{48}O_4$ acids, and both contain two hydroxyl groups and two ethylenic linkages. Polyporenic acid C may be identical with gypsogenin, $C_{30}H_{46}O_4$, isolated from *Gypsophila* and other species of *Saponaria*.

Chemical studies on the higher fungi have been extensive, the numerous publications of Zellner and his co-workers (*Monatsh.*, 1904, **25**, 537; 1905, **26**, 727; 1908, **29**, 1171; 1909, **30**, 231, 655; 1911, **32**, 133; 1913, **34**, 321; 1915, **36**, 611; 1917, **38**, 319; 1918, **39**, 603; 1920, **41**, 443; 1923, **44**, 9; 1928, **50**, 193, 201; 1929, **53—4**, 146; 1930, **56**, 200; 1933, **62**, 214; 1934, **64**, 6; 1935, **66**, 76) covering a large part of the field in a general survey of the constituents.

In addition to numerous references to the isolation of ergosterol from the higher fungi in the works of Zellner, Ratcliffe (*Biochem. J.*, 1937, **31**, 240) records the isolation of this sterol from *Boletus edulis* along with another sterol closely resembling spinasterol, and King (J., 1922, **121**, 1753) and Rosenthal (*Monatsh.*, 1922, **43**, 237) isolated ergosterol from *Amanita muscaria*. Sumi (*Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1933, **20**, 254) has studied the " ergosterol " content, estimated by digitonin precipitation, of the gills and stipes of various members of the Basidomycetes. There appears to be little doubt that the sterols and other constituents of the non-saponifiable portion of fungal extracts form the usual complicated mixture of phytosterols and while the isolation of pure fungisterol ($C_{25}H_{40}O$?) has been recorded (Zellner and Zikmunda, *Monatsh.*, 1930, **56**, 200; Hartmann and Zellner, *ibid.*, 1928, **50**, 193) it seems probable that this is not a completely homogeneous sterol, but is contaminated with other constituents of the non-saponifiable complex.

As far as we are aware, no well-characterised members of the terpene group, such as the triterpene resinols, have been isolated from fungi; there are, however, a number of substances described in the literature which might possibly fall into this category. Fröschl

and Zellner (*Monatsh.*, 1928, **50**, 201) concluded that "lentinol" ($C_{27}H_{44}O_3$), m. p. 265°, which they isolated from *Lentinus squamosus* Schroet, was a "resinol." The α- and β-pinicolic acids, m. p. 198—208·5° and 265—271° respectively, obtained by the same authors (*Monatsh.*, 1929, **53—4**, 146) from *Polyporus pinicola* Fr. (a fungus parasitic on pine), were suggested to be isomeric $C_{19}H_{30}O_2$ acids; the analytical data are in agreement, however, with a formula $C_{30}H_{50}O_3$, and in the absence of molecular weight determinations their molecular formulæ must remain uncertain. In an earlier paper on the constituents of the same fungus, Hartmann and Zellner (*Monatsh.*, 1928, **50**, 193) describe a number of neutral and acidic substances of high melting point, analyses of which suggest sterol and triterpenoid formulæ, and it is recorded that one of the "resin acids," decomp. about 180°, gives a red-brown colour with greenish fluorescence with the Liebermann-Burchard reagent (cf. polyporenic acid A, *q.v.*). From another member of the Polyporaceæ, *Polyporus sulfureus* L., Zellner and Zikmunda (*loc. cit.*) isolated a crystalline product, m. p. 265° (decomp.), which apparently gave no sterol colour reactions although the analytical data suggested a sterol or triterpenoid formula. A similar crystalline substance, m. p. 250°, exhibiting no sterol colour reactions (Salkowski and Liebermann–Burchard), is recorded by Ellis (*Biochem. J.*, 1918, **12**, 173) as being a constituent of *Polyporus nigricans*, and Sumi (*Chem. Zentr.*, 1931, I, 1773) describes the isolation of an alcohol ($C_{28}H_{48}O$), m. p. 245—255°, from *Cortinellus shiitake*.

Polyporus betulinus Fr., a large white shelf-fungus, parasitic on the birch tree (*Betula alba*) was examined by Zellner (*Monatsh.*, 1913, **34**, 331) during the course of his survey. He found that a light petroleum extract, after saponification, yielded a sterol mixture, m. p. 139—144° (also obtained by Ellis, *loc. cit.*, in an examination of the same fungus), and the fatty acids contained a white "non-acidic" amorphous substance, m. p. about 260° (decomp.), readily separable owing to its sparing solubility in alcohol, ether or chloroform. With the Liebermann test (acetic anhydride–sulphuric acid) a violet coloration was observed, and the Salkowski reagent (chloroform–sulphuric acid) produced a strong red coloration. From the subsequent ethereal extract, after hydrolysis, an amorphous yellow powder, m. p. about 250° (decomp.), was isolated, which Zellner, believing it to be an alcohol, designated "polyporol," and analyses of this substance indicated the formula $(C_6H_{10}O)_x$ or better $C_{31}H_{50}O_5$. Like the high-melting substance isolated from the light petroleum extract, it gave characteristic colour reactions with the Liebermann and Salkowski reagents and it was concluded that they both belonged to the ergosterol or resin alcohol group.

In commencing a systematic examination of these fungi we have undertaken a re-investigation of *Polyporus betulinus* Fr. After many experiments with a variety of solvents we find that the best extraction procedure is to treat the fresh minced fungus, first with alcohol in the cold, and subsequently under reflux with a mixture of acetone and ether. From the alcoholic extract after saponification, in addition to a mixture of sterols containing ergosterol, which is at present being investigated, we have isolated a crystalline acid, m. p. 194°, the analytical data for which suggest a formula $C_{30}H_{48}O_4$ or $C_{31}H_{50}O_4$, and for which we suggest the name *polyporenic acid A*.* Attempts to isolate acetyl and benzoyl derivatives of the acid have been unsuccessful, possibly owing to mixed anhydride formation, but the acid has been characterised by the preparation of a *monomethyl* ester, m. p. 142°, which yields a *methyl ester monoacetate*, m. p. 112°. A Zerewitinoff determination on the methyl ester indicated the presence of two active hydrogen atoms, suggesting that the unidentified oxygen atom may be present either as an unreactive hydroxyl group or as a readily enolised carbonyl group. Titration of the methyl ester with perbenzoic acid and a confirmatory microhydrogenation experiment indicate the presence in the acid of two ethylenic linkages; these are not conjugated, since the acid shows no absorption of appreciable intensity in the ultra-violet region of the spectrum. It is probable that the low melting point of polyporenic acid A is not unconnected with the presence of two ethylenic linkages in the molecule, especially since the melting point of the doubly unsaturated tetracyclic basseol is considerably lower than that of the fully cyclised β-amyrenol

* The name polyporic acid has been given to a hydroxy-quinone pigment present in some of the Polyporaceæ (Kögl, *Annalen*, 1926, **447**, 78) and betulic acid to an oxidation product of betulin which has been isolated from the bark of *Cornus florida* L. (Robertson, Soliman, and Owen, J., 1939, 1267).

(Beynon, Heilbron, and Spring, J., 1937, 989), and the marked green fluorescence observed in the Liebermann–Burchard test (see experimental) is also exhibited by basseol. Preliminary experiments indicate that the acid is unstable towards reagents which are effective in cyclising basseol acetate to β-amyrenyl acetate.

Hydrolysis of the acetone–ether extract yields two further acids, *polyporenic acids B* and *C*. The former, m. p. 300—310° (decomp.), gave similar analytical figures to those of acid A and, like the latter, gave uncrystallisable gums on acetylation or benzoylation, but was characterised readily by the preparation of a crystalline *monomethyl* ester, m. p. 160°, on which a Zerewitinoff determination showed the presence of two active hydrogen atoms. Both the acid and its methyl ester give yellow colorations with tetranitromethane in chloroform solution and on microhydrogenation the ester absorbs two moles of hydrogen. *Polyporenic acid C*, m. p. 270—275°, has not so far been available in sufficient quantity for detailed examination, but its *methyl* ester, m. p. 192—193°, is obviously different from those of polyporenic acids A and B, and analysis suggests a formula $C_{31}H_{48}O_4$ (or $C_{31}H_{50}O_4$) for the ester. The melting points of the acid and its ester are practically identical with those (273° and 192° respectively) given for gypsogenin (albsapogenin), $C_{30}H_{46}O_4$ (Karrer, Fioroni, Widmer, and Lier, *Helv. Chim. Acta*, 1924, **7**, 781; Ruzicka and Giacomello, *ibid.*, 1937, **20**, 299; 1936, **19**, 1136), and when further quantities of polyporenic acid C are available a direct comparison will be made.

It is thus apparent that these acids isolated from *Polyporus betulinus* Fr. are comparable with a number of well-defined, naturally occurring triterpene acids ($C_{30}H_{48}O_4$). Among such are hederagenin and sumaresinolic acid (Ruzicka and Furter, *Helv. Chim. Acta*, 1932, **15**, 472), chenopodium sapogenin (Dafert *et al.*, *Sci. Pharmaceutica*, 1934, **5**, 84), gleditschia sapogenin (Kuwada, *J. Pharm. Soc. Japan*, 1935, **55**, 242), and echinocystic acid (Bergsteinsson and Noller, *J. Amer. Chem. Soc.*, 1934, **56**, 1403). Further evidence for the relationship of the polyporenic acids to the above triterpenes must await the results of dehydrogenation experiments, but it is interesting to speculate as to a possible simple inter-relationship between the acids of the parasitic fungus and the dihydric triterpene alcohol betulin, $C_{30}H_{50}O_2$, which forms a major constituent of the bark of the host tree. Such a hypothetical relationship, involving the interchange of a methyl and a carboxyl group, $CO_2H \rightleftarrows CH_3$ is not novel in the triterpene series, where oleanolic acid ($C_{30}H_{48}O_3$) is related in such a way to β-amyrenol ($C_{30}H_{50}O$) and ursolic ($C_{30}H_{48}O_3$) and β-boswellic ($C_{30}H_{48}O_3$) acids are similarly related to α-amyrenol (Ruzicka and Schellenberg, *Helv. Chim. Acta*, 1937, **20**, 1553; Goodson, J., 1938, 999; Ruzicka and Wirz, *Helv. Chim. Acta*, 1939, **22**, 948). The occurrence of betulic acid, an oxidation product of betulin, in which the primary carbinol group has been replaced by a carboxyl group, $CH_2{\cdot}OH \rightleftarrows CO_2H$, in the bark of *Cornus florida* L. has recently been observed (Robertson, Soliman, and Owen, *loc. cit.*) and Ruzicka and Brenner (*Helv. Chim. Acta*, 1939, **22**, 1523) have succeeded in converting betulin into lupeol ($C_{30}H_{50}O$) by replacement of the same primary alcohol group by a methyl group.

Experimental.

The minced fungus (3·4 kg.) was kept under alcohol at room temperature for 3 days; the filtered solution on dilution with water then yielded a white flocculent precipitate, which was taken up in ether. The aqueous solution was saturated with salt and further extracted, and the combined extracts washed and dried. The residual fungus was now heated under reflux with a mixture of ether (1500 c.c.) and acetone (1500 c.c.) for 6 hours, and the solution filtered, washed with water, and dried. After removal of solvent from the combined ethereal extracts the residue, which formed a greasy yellow solid (70 g.), was hydrolysed by heating under reflux for 5 hours with potassium hydroxide (75 g.) in methyl alcohol (1500 c.c.); the orange-red solution was diluted with water and extracted with ether. This extraction was rendered difficult by the presence of large amounts of insoluble potassium salts. Concentration of the dried ethereal solution of the non-saponifiable portion gave a residue, from which, by crystallisation, some impure ergosterol, m. p. 151—153°, was isolated, giving with antimony trichloride the characteristic red coloration changing to blue. *Light absorption in alcohol:* Maxima, 2815, 2720 A.; $E^{1\%}_{1\,cm.}$ 210 (for pure ergosterol, $E^{1\%}_{1\,cm.}$ about 310). The filtered potassium salts were dissolved in hot acetic acid; on cooling, crude *polyporenic acid B* (1 g.) separated in an amorphous

form, m. p. 295—300°. After cautious addition of water to the hot filtrate a gelatinous precipitate of *polyporenic acid C* separated on cooling, which when dry formed a horny mass (2 g.), m. p. about 240°. On further dilution of the mother-liquors with water, crude *polyporenic acid A* separated as a pale yellow solid (15 g.), m. p. 180°. In subsequent experiments the alcoholic and ether–acetone extracts were saponified separately, the former yielding mainly acid A and the latter acids B and C.

Polyporenic Acid A.—The acid after several crystallisations from dilute acetic acid, aqueous methyl alcohol or acetone, separated in needles, m. p. 194°, readily soluble in cold pyridine, moderately easily soluble in alcohol, acetone, ethyl acetate and acetic acid, but sparingly soluble in ether, chloroform and benzene. With tetranitromethane in chloroform solution the acid gives a yellow coloration, with chloroform and sulphuric acid a red colour with slight green fluorescence and with the Liebermann–Burchard reagent it gives an immediate red coloration with brilliant green fluorescence, slowly changing to brown with the fluorescence unimpaired; it gives no colour with antimony trichloride in chloroform solution. $[\alpha]_D^{20°}$ +69° (l = 1, c = 1·0 in pyridine). For analysis the acid was dried in a high vacuum for 3 hours at 80° [Found: C, 76·4, 76·6; H, 10·7, 10·6; *M* (by titration), 485, 484, 486, 485, 486. $C_{30}H_{48}O_4$ requires C, 76·2; H, 10·2%; *M*, 472. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%; *M*, 486]. *Methyl ester.* The acid in dry acetone was treated with an excess of diazomethane in ether. After several hours the ethereal solution was washed with water and sodium carbonate solution, dried, and evaporated, and the crystalline mass taken up in methyl alcohol. Cautious addition of water and cooling gave the *ester* in needles, which after two further crystallisations had the constant m. p. 142°. It is readily soluble in most organic solvents, and gives with the Liebermann–Burchard test a pinkish-violet coloration. $[\alpha]_D^{20°}$ +77° (l = 1, c = 3·0 in chloroform). For analysis the ester was dried in a high vacuum for 3 hours at 80° (Found: C, 76·6; H, 10·7. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%. $C_{32}H_{52}O_4$ requires C, 76·7; H, 10·5%). *Active hydrogen determination* (Zerewitinoff): The ester (6·291 mg.) evolved 0·65 c.c. of methane at 763 mm. and 18°, equivalent to 2·1 active hydrogen atoms per mole. *Quantitative microhydrogenation:* 6·621 mg. of the ester required 0·62 c.c. of hydrogen at 766 mm. and 19° (after heating to 95°), corresponding to 1·9 double bonds.

The methyl ester (150 mg.) was set aside at 0° with a solution of perbenzoic acid in chloroform (20 c.c.; 0·2N). At intervals samples of the solution were titrated against 0·1N-sodium thiosulphate, all measurements being standardised against a blank consisting of the same volume of perbenzoic acid in chloroform. After 48 and again after 90 hours it was found that 1 mole of methyl ester had absorbed 2·04 atoms of oxygen.

Hydrolysis of the ester (200 mg.) with methyl-alcoholic potassium hydroxide (25 c.c.; 5%) by heating on the steam-bath for 3 hours yielded, after extraction of the diluted alkaline solution with ether, unchanged ester (110 mg.). Acidification of the aqueous portion with acetic acid, isolation by means of ether, and crystallisation from aqueous acetone gave polyporenic acid A (75 mg.), m. p. 190—191°, alone and mixed with authentic material.

Methyl ester acetate. The ester (100 mg.) was heated on the steam-bath for 4 hours with pyridine (1 c.c.) and acetic anhydride (1 c.c.); the mixture was then poured into water, and the acetate isolated by means of ether. Three crystallisations from aqueous methyl alcohol gave the *methyl ester acetate* in needles, m. p. 112°, $[\alpha]_D^{20°}$ +88° (l = 1, c = 1·2 in chloroform). For analysis it was dried in a high vacuum at 60° for 3 hours (Found: C, 75·0; H, 9·85. $C_{33}H_{52}O_5$ requires C, 74·9; H, 9·9%. $C_{34}H_{54}O_5$ requires C, 75·2; H, 10·0%).

Polyporenic Acid B.—This acid usually separates from dilute alcohol in a gelatinous form, but it can be crystallised from either acetone or acetic acid, separating from the latter solvent in an asbestos-like mass, m. p. 300—310° (decomp.). After drying in a high vacuum at 100° for 12 hours the acid melts at 275—280°, but recovers the original m. p. after solution in alcoholic alkali and precipitation with acid. The acid is very soluble in cold pyridine, sparingly soluble in alcohol, acetone, and ethyl acetate, and practically insoluble in ether, benzene, and chloroform. It gives a yellow coloration with tetranitromethane and an orange coloration with chloroform and sulphuric acid; with the Liebermann–Burchard reagent a violet colour is produced. For analysis the acid was dried in a high vacuum at 100° for 12 hours (Found: C, 76·3; H, 10·2; *M*, 470, 474, 488. $C_{30}H_{48}O_4$ requires C, 76·2; H, 10·2%; *M*, 472. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%; *M*, 486). *Methyl ester.* This was prepared with diazomethane in dry acetone solution, and crystallised first from acetone and finally from aqueous methyl alcohol, separating in needles, m. p. 160° after softening at 155°. For analysis the *ester* was dried in a high vacuum at 80° for 3 hours (Found: C, 76·7; H, 10·4. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%. $C_{32}H_{52}O_4$ requires C, 76·7; H, 10·5%). *Active hydrogen determination:* The ester (4·587 mg.) evolved

0·46 c.c. of methane at 17° and 752 mm., equivalent to 2·03 active hydrogen atoms per mole. *Quantitative microhydrogenation:* 3·50 Mg. of the ester required 0·34 c.c. of hydrogen at 767 mm. and 17° (after heating to 95°), corresponding to 2·0 double bonds.

Polyporenic Acid C.—This acid, which has not yet been obtained analytically pure, separates from aqueous alcohol or dilute acetic acid in microscopic crystals, m. p. about 263—266°; this can be raised to 270—275° by solution of the acid in hot alcoholic potassium hydroxide and precipitation with dilute hydrochloric acid. With chloroform and sulphuric acid, the acid gives an orange lower layer, with acetic anhydride and sulphuric acid a blue-violet coloration is produced, and with the Liebermann–Burchard reagent a reddish-violet colour, which rapidly fades, is observed. *Methyl ester.* The *ester*, prepared in the usual manner, crystallises from aqueous methyl alcohol in needles or thick prisms, m. p. 192—193° after softening at 189°. For analysis the ester was dried in a high vacuum at 80° for 3 hours (Found: C, 76·6, 76·5; H, 9·8, 9·7. $C_{31}H_{48}O_4$ requires C, 76·8; H, 10·0%. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%).

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