93. *Constituents of the Bark of* Balanops australiana *F. Muell.*

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Friedelin, friedelan-3β- and -3α-ol, cerin, and betulic acid have been obtained from the bark of *Balanops australiana* F. Muell. The sternutatory constituent of the bark could not be isolated.

Balanops australiana F. Muell., popularly known as the " pimply ash," is found mainly in the rain-forests of North Queensland[1] (only two *Balanops* species are native to Australia[2]). Field tests for alkaloids in the bark have proved negative.[3] An examination of the bark was undertaken because of its reputation as a powerful sternutator, and an attempt was made by Dr. J. R. Price, C.S.I.R.O., Melbourne, to isolate the physiologically active constituent(s). By extraction with a series of boiling solvents, Price and McKenzie isolated the triterpene ketone friedelin (0·6% of bark) and the derived

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[1] Francis, " The Australian Rain-Forest Trees," Forestry and Timber Bureau, Sydney, 1951, p. 385.

[2] Bailey, " The Queensland Flora," Diddams, Brisbane, 1902, Part V, p. 1457.

[3] Webb, " An Australian Phytochemical Survey," Part I, C.S.I.R.O. Bulletin No. 241, 1949, p. 13; Part II, No. 268, 1952, p. 28.

axial alcohol friedelan-3β-ol ("*epi*friedelanol"; 0·07% of bark).[4,5] They were unable to isolate any pure physiologically active substance from the extracts.

By systematic extraction of the bark with a series of solvents at room temperature we have also isolated friedelin (0·65% of bark), friedelan-3β-ol (0·047% of bark) together with the equatorial epimer friedelan-3α-ol (0·107% of bark), the related α-hydroxy-ketone cerin (0·022% of bark), and the unsaturated hydroxy-acid betulic acid (0·009% of bark). Friedelin and friedelan-3β-ol occur in the bark and wood of several Australian trees[6] including *Siphonodon australe* Benth.,[7] from which authentic specimens were obtained. Friedelan-3α-ol has not previously been isolated from a natural source. Cerin accompanies friedelin in the bark of the cork oak, *Quercus suber*.[8,9] Betulic acid is a common constituent of the bark, wood, and leaves of many trees and shrubs;[6] an authentic specimen was obtained from *Syncarpia laurifolia* Tenn.[10]

The work of Price and his co-workers indicated that the physiologically active constituents might be expected in the more polar extracts of the bark. The investigation of these extracts, particularly the methanol extract, was complicated by the large amount of tannin extracted from the bark and the very stable foam produced in aqueous solutions of the extracts, and no pure physiologically active substance was isolated.

EXPERIMENTAL

M. p.s were determined on a Kofler block and are corrected. $[\alpha]_D$ refer to chloroform solutions at ~20°. Infrared absorption spectra were measured by use of a Perkin-Elmer model 21B spectrophotometer. Unless otherwise stated, aluminium oxide used was Spence's Type H (activity II). Light petroleum refers to the fraction of b. p. 60—90°. The substances isolated were identified by direct comparison (mixed m. p.s and infrared spectra) with authentic specimens.

Extraction.—The dried, finely milled bark (13·44 kg.), collected at Ravenshoe, Queensland, was exhausted by percolation at room temperature in turn with light petroleum, ether, acetone, and methanol. Each extract was concentrated to small volume under reduced pressure and refrigerated for several days before being worked up.

Colourless crystals (m. p. 240—259°; 81·5 g., 0·61% of bark) were slowly deposited from the yellow light petroleum concentrate. This crude triterpenoid mixture (10 g.) was chromatographed on aluminium oxide (300 g.) in light petroleum. Elution with light petroleum (4 l.) and benzene–light petroleum (1 : 1, 3 l.) gave friedelin (8·02 g., 0·49% of bark), m. p. and mixed m. p. 258—262°, $[\alpha]_D$ −24° (*c* 1·8), after crystallisation from ethyl acetate. Elution with benzene (1 l.) and crystallisation from benzene furnished friedelan-3β-ol (424 mg., 0·026% of bark), m. p. and mixed m. p. 283—285°, $[\alpha]_D$ +24° (*c* 1·0). Elution with ether (4 l.) and crystallisation from benzene afforded friedelan-3α-ol (1·41 g., 0·086% of bark), m. p. and mixed m. p. 292—301°, $[\alpha]_D$ +18° (*c* 1·0). Authentic specimens of friedelin and friedelan-3β-ol were obtained from *Siphonodon australe* Benth.[7] Friedelan-3α-ol was obtained by reduction of friedelin with sodium in pentyl alcohol.[11,12] Courtney and Gascoigne[7] found m. p. 261—264°, $[\alpha]_D$ −21°, for friedelin and m. p. 280—282°, $[\alpha]_D$ +21° for friedelan-3β-ol. Spring *et al.*[11] give m. p. 299—302°, $[\alpha]_D$ +18°, for friedelan-3α-ol.

The pale yellow ether concentrate deposited, on storage, a mixture of friedelin (3·2 g.), friedelan-3β-ol (0·24 g.), and friedelan-3α-ol (0·48 g.). The material deposited from the deep red acetone concentrate contained, in addition to friedelin (2·6 g.), friedelan-3β-ol (0·5 g.), and friedelan-3α-ol (0·41 g.), the hydroxy-ketone cerin (0·32 g.), m. p. 249—252°, $[\alpha]_D$ −44° (*c* 0·62),

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[4] Dr. J. R. Price, personal communication.

[5] Jefferies, *J.*, 1954, 473.

[6] White, *Rev. Pure and Appl. Chem. (Australia)*, 1956, **78**, 5041.

[7] Courtney and Gascoigne, *J.*, 1956, 2115.

[8] Istrati and Ostrogovich, *Compt. rend.*, 1899, **128**, 1581.

[9] Drake and Jacobsen, *J. Amer. Chem. Soc.*, 1935, **57**, 1570; Drake and Shrader, *ibid.*, p. 1854.

[10] Hodgson, Ritchie, and Taylor, *Austral. J. Chem.*, 1960, **13**, 385.

[11] Brownlie, Spring, Stevenson, and Strachan, *J.*, 1956, 2419.

[12] Drake and Campbell, *J. Amer. Chem. Soc.*, 1936, **58**, 1681.

$\nu_{\max}$ (in Nujol) 3450, 1705, and 1030 cm.^{-1} (after crystallisation from benzene). White [8,9] gives m. p. 250—254°, $[\alpha]_D$ $-41°$, for cerin. Treatment with acetic anhydride–pyridine overnight yielded cerin acetate, m. p. 263—265°, $[\alpha]_D$ $-36°$ (*c* 1·31), $\nu_{\max}$ (in Nujol) 1700, 1220, and 1005 cm.^{-1}, after sublimation at 220° (bath temp.)/1 mm. and crystallisation from methanol. Brownlie *et al.*[11] give m. p. 262—263°, $[\alpha]_D$ $-36°$, for cerin acetate. Cerin acetate was converted into friedelin by treatment with zinc dust in refluxing acetic acid according to their directions. No product appeared on storage of the dark brown methanol concentrate.

After removal of the deposits formed on further storage, the extracts were further concentrated under reduced pressure to thick oils, which were extracted with ether and separated into basic (extracted with 5% hydrochloric acid), acidic (extracted with 5% sodium hydrogen carbonate or 5% sodium carbonate solution), phenolic (extracted with 2% sodium hydroxide solution), and neutral fractions. No basic fractions were obtained.

Acidic and phenolic fractions. The acidic fraction (2 g.) obtained from the light petroleum fraction by extraction with 5% sodium hydrogen carbonate solution was dissolved in ether and treated with an excess of ethereal diazomethane. Chromatography of the product on aluminium oxide (60 g.) in light petroleum and elution with benzene gave methyl betulate (0·26 g.), m. p. and mixed m. p. 221—224°, $[\alpha]_D$ $+7°$ (*c* 2·6), after crystallisation from methylene chloride–methanol. Hydrolysis of this ester by refluxing with 5% ethanolic potassium hydroxide for 1 hr. gave betulic acid, m. p. and mixed m. p. 310—314°, $[\alpha]_D$ $+9°$ (*c* 2·0), after crystallisation from methanol. Authentic specimens of the acid and its methyl ester were obtained from *Syncarpia laurifolia* Tenn.[10] Anstee *et al.*[13] give m. p. 320—321°, $[\alpha]_D$ $+12°$, for betulic acid and m. p. 223—224°, $[\alpha]_D$ $+7°$, for methyl betulate.

Extraction of the ethereal solution of the light petroleum extract, after removal of the acids, with 2% sodium hydroxide solution produced an interfacial precipitate. The free acid was regenerated from this insoluble sodium salt by solution in aqueous ethanol (100 c.c.) at room temperature and acidification with 3N-hydrochloric acid. Ether-extraction and crystallisation of the product from methanol afforded betulic acid (1·0 g.), m. p. and mixed m. p. 310—314°.

Attempts to isolate individual substances from the very dark acidic and phenolic fractions of the ether, acetone, and methanol extracts by chromatography on acid-washed aluminium oxide or on silica gel, or by chromatography or distillation after methylation with diazomethane, were unsuccessful.

Neutral fractions. The neutral fraction from the light petroleum extract, a yellow-green oil (41·3 g.), afforded by chromatography on aluminium oxide (1200 g.), in order of elution, amorphous wax (13·48 g.), friedelin (7·91 g.), friedelan-3β-ol (1·43 g.), friedelan-3α-ol (1·45 g.), and a red tar (17 g.). The last was treated with acetic anhydride–pyridine at 20° overnight, and rechromatographed, to give cerin acetate (1·2 g.).

The neutral fraction (11·55 g.) from the ether extract similarly afforded amorphous wax (0·3 g.), friedelin (7·85 g.), friedelan-3β-ol (0·5 g.), and friedelan-3α-ol (0·3 g.), together with some amorphous material which gave cerin acetate (0·4 g.) by acetylation and rechromatography. The acetone neutral fraction (4·18 g.) yielded friedelin (0·5 g.), friedelan-3β-ol (0·2 g.), friedelan-3α-ol (0·2 g.), and cerin (0·19 g.), together with a thick oil (3·0 g.) which was acetylated and rechromatographed to give cerin acetate (0·5 g.). The methanol neutral fraction (6·2 g.) gave friedelin (0·2 g.), friedelan-3β-ol (0·12 g.), friedelan-3α-ol (0·09 g.), and a red oil (5·7 g.), which afforded cerin acetate (0·45 g.) by acetylation and rechromatography.

Ether-insoluble residues. Investigation of the dark red ether-insoluble residues from the acetone and methanol extracts was made difficult by the large amount of amorphous tannin present and the very stable foam produced in aqueous solution. Attempts to isolate pure substances from these fractions by adsorption chromatography on aluminium oxide or silica gel, partition chromatography on cellulose, or ion-exchange chromatography were unsuccessful.

Total analysis. The total analysis of the crystalline constituents isolated from the bark (13·44 kg.) was friedelin (87·66 g., 0·65% of bark), friedelan-3β-ol (6·44 g., 0·047%), friedelan-3α-ol (14·43 g., 0·107%), cerin (2·91 g., 0·022%), and betulic acid (1·22 g., 0·009%).

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[13] Anstee, Arthur, Beckwith, Dougall, Jefferies, Michael, Watkins, and White, *J.*, 1952, 4065.

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