776. *The Chemistry of Western Australian Plants. Part VI.* The Occurrence of Betulic, Oleanolic, and Ursolic Acids.*

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Ursolic and oleanolic acids have been identified in *Anthocercis littorea*, *Anthocercis odgersii*, and the coastal form of *Alyxia buxifolia*. Oleanolic acid has also been obtained from *Petalostigma sericea*. Betulic acid has been obtained from the inland form of *Alyxia buxifolia*, from *Nuytsia floribunda* and from six *Melaleuca* species. " Melaleucin " is probably betulic acid.

Although fairly widely distributed in the plant kingdom ursolic acid has been reported only from the Solanaceæ in the Australian genera *Duboisia* (Trautner and Neufeld, *Aust. Chem. Inst. J. and Proc.*, 1947, **14**, 17; Bottomley and White, *Australian J. Sci. Res.*, 1951, *A*, **4**, 107) and *Anthotroche* (Bottomley and White, *Australian J. Sci. Res.*, 1950, *A*, **3**, 516). It has now been found, together with oleanolic acid, in two further solanaceous plants, *Anthocercis littorea* Labill. and *Anthocercis odgersii* F. Muell. A third member of the same genus, *Anthocercis intricata* F. Muell., also contains a mixture of triterpene acids which is probably similar, although the acids have not been separated.

In the Apocynaceæ, *Petalogstigma sericea* (R.Br.) C. A. Gardn. and *Alyxia buxifolia* R.Br. have been examined. The former contains oleanolic acid, while the coastal form of the latter, like the *Anthocercis* spp., contains both oleanolic and ursolic acids. However, *Alyxia buxifolia* specimens collected from low-rainfall areas away from the coast contain betulic acid.

Betulic acid has also been isolated from *Nuytsia floribunda* (Labill.) R.Br. (Loranthaceæ), the Western Australian Christmas tree, where it is accompanied by a small amount of betulin, and from the barks of six Melaleuca species, *M. rhaphiophylla* Schau., *M. cuticularis* Labill., *M. viminea* Lindl., *M. leucadendron* L., *M. parviflora* Lindl., and *M. pubescens* Schau. The first five of these belong to the group popularly known as " paper-barks " and crystalline triterpene acid can be seen in places between the thin papery layers of the bark.

The substance isolated from the bark of *Melaleuca leucadendron* and named " melaleucin " by Isii and Osima (*J. Agric. Chem. Soc., Japan*, 1939, **15**, 839) is probably impure betulic acid. The formula proposed by them, $C_{28}H_{45}O_3$, contravenes valency requirements and requires carbon and hydrogen values (C, 78·2; H, 10·6%) reasonably close to those for $C_{30}H_{48}O_3$ (C, 78·9; H, 10·6%), while the m. p.s reported for the compound (304°) and its acetate (280°) are not far removed from those of betulic acid and its acetate, especially if they were uncorrected.

Experimental

M. p.s are corrected. Unless otherwise indicated they were determined in open tubes. Analyses are by Drs. Weiler and Strauss, Oxford, and Dr. Zimmermann, Melbourne. The identity of all the compounds prepared was checked by mixed m. p. with authentic specimens. No depressions were observed. Rotations are in chloroform solution unless otherwise indicated.

Alyxia buxifolia.—(*a*) Leaves (4·5 kg.) collected at Kalgoorlie in October, 1943, and near Morawa in August, 1945, were powdered and extracted with alcohol, and the extract (350 g.) extracted with ether. Sodium salts (43·5 g.) were precipitated by treatment of the ethereal solution with N-sodium hydroxide, and were purified by treatment of a solution in 50% alcohol with charcoal, followed by acidification. The precipitated acid was dissolved in a 5% solution of potassium hydroxide in 50% alcohol, the solution treated with charcoal, and then with acid, and the solid (35 g.) crystallised from methanol (7 times) and then from ethanol (3 times); it had m. p. 308—309°. When it was acetylated, betulic acid acetate, m. p. 289—290°, was obtained, and this was hydrolysed to betulic acid, m. p. 310—311° [methyl betulate, m. p. 222—223° (Found: C, 79·05; H, 10·75. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7%)].

(*b*) Leaves (1 kg.), collected at Point Peron in February, 1951, were extracted with ether,

* Part V, Bottomley and White, *Australian J. Sci. Res.*, 1951, *A*, **4**, 112.

and the insoluble sodium salts (45 g.) precipitated from this extract, dissolved in methanol (charcoal), and acidified. The crude acid was dissolved in boiling methanol; on cooling, solid (*A*) (8 g.) separated. A solid (*B*) (22 g.) was obtained on concentration of the filtrate.

On crystallisation of (*A*) from ethanol (charcoal), ursolic acid (0·8 g.), m. p. 286—288°, $[\alpha]_D^{16}$ +69° (*c*, 0·68 in 1 : 1-methanol–chloroform), was obtained (acetate, m. p. 289—290°; methyl ester, m. p. 170—171°; and methyl ester acetate, m. p. 245—246°).

Crystallisation of (*B*) from ethanol and removal of a low-melting first crop gave oleanolic acid (0·8 g.), m. p. 309—312°, $[\alpha]_D^{15}$ +80° (*c*, 0·93), after six crystallisations from ethanol (acetate, m. p. 267—268°; methyl ester, m. p. 201°; and methyl ester acetate, m. p. 222°).

Anthocercis littorea.—Powdered leaves and stems, collected at City Beach, near Perth, were extracted with alcohol, the residue, obtained by removal of the alcohol, was washed with 1% hydrochloric acid, and the insoluble material (42 g.) dissolved in a 2% solution of sodium hydroxide in 70% aqueous methanol and warmed (charcoal) for 30 minutes. Acidification of the filtered solution, gave the crude acid (23·5 g.), from which some impurities were removed by light petroleum. A solution of the acid in methanol (670 ml.) was treated with charcoal and then evaporated (about 100 ml. at a time), yielding six fractions, which were fractionated from methanol by a triangular scheme. The less soluble fractions and the most soluble fractions, after repeated crystallisation from ethanol, yielded ursolic acid (1·5 g.), m. p. 286—287° (vac.), $[\alpha]_D^{15}$ +70° (*c*, 1·02 in 1 : 1-methanol–chloroform) [methyl ester, m. p. 171°; acetate, m. p. 292°; and methyl ester acetate, m. p. 246—247° (vac.)]. From the fourth fraction oleanolic acid (0·5 g.), m. p. 311—312° (vac.), $[\alpha]_D^{15}$ +79° (*c*, 0·63), was obtained after two crystallisations from methanol and three from ethanol [methyl ester, m. p. 201°; acetate m. p. 268° (vac.); and methyl ester acetate, m. p. 222°].

Anthocercis odgersii.—Leaves and stems (420 g.), collected at Chandler, near Merredin, were powdered and extracted with alcohol, and the extract (42 g.) was worked up as described for *A. littorea*. Ursolic acid (10 g.), m. p. 286—287°, was obtained [methyl ester, m. p. 169—170°; acetate, m. p. 287—289°; and methyl ester acetate, m. p. 242—243°] together with a small amount of oleanolic acid, m. p. 311—312° (vac.) [methyl ester, m. p. 201°].

Anthocercis intricata.—This plant, collected in the Geraldton district, yielded a similar mixture of triterpene acids, but pure components have not been separated.

Melaleuca *spp.*—The bark (400 g.) of *Melaleuca rhaphiophylla*, collected at South Perth, was powdered and extracted with ether. The extract was treated with sodium hydroxide solution, and the precipitated sodium salts collected, dried, and dissolved in methanol (charcoal), and the solution acidified with hydrogen chloride. The crude acid, crystallised from methanol and then from ethanol, had m. p. 314—315°, $[\alpha]_D^{25}$ +8·4° (*c*, 1·36 in pyridine) (Found : C, 78·9; H, 10·8%; equiv., 446. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·6%; equiv., 456·7). It formed betulic acid acetate, m. p. 293—294° (Found : C, 77·3; H, 10·0. Calc. for $C_{32}H_{50}O_4$: C, 77·1; H, 10·1%), and methyl betulate, m. p. 223—224° (Found : C, 79·0; H, 10·5. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7%). *M. cuticularis*, *M. leucadendron*, *M. parviflora*, *M. pubescens*, and *M. viminea* barks also yielded betulic acid (identified as the acetate and methyl ester) when extracted as described above.

Nuytsia floribunda.—Leaves and stems (2 kg.), collected from the University grounds, were powdered and extracted with alcohol. The alcohol was removed, the residue was extracted with ether, and the sodium salts (55 g.) were precipitated. A solution in 50% ethanol (charcoal) was acidified, and the crude acid (34 g.) purified by crystallisation from ethanol and methanol, whereby needles of betulic acid, m. p. 320—321°, $[\alpha]_D^{15}$ +12° (*c*, 0·94 in pyridine), were obtained [acetate, m. p. 295°, $[\alpha]_D^{15}$ +24° (*c*, 1·56) (Found : C, 77·3; H, 10·0%); methyl ester, m. p. 222°, $[\alpha]_D^{15}$ +7·2° (*c*, 1·11) (Found : C, 78·9; H, 10·8%)].

From the ethereal extracts, betulin, m. p. 256° (diacetate, m. p. 215°), separated.

Petalostigma sericea.—Husks of the fruit (720 g.) were extracted with ether, the crude sodium salts were precipitated and dissolved in methanol, and the solution was acidified with hydrogen chloride. Oleanolic acid, m. p. 309—311°, $[\alpha]_D^{16}$ +82° (*c*, 0·84), was obtained on crystallisation of the precipitate from methanol (Found : C, 78·9; H, 10·4. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·6%). It was identified by conversion into the acetate, m. p. 267—268° (Found : C, 77·1; H, 10·0; Ac, 8·5. Calc. for $C_{32}H_{50}O_4$: C, 77·1; H, 10·1; Ac, 8·6%), methyl ester, m. p. 201° (Found : C, 79·4; H, 10·5. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7%), and methyl ester acetate, m. p. 222°, $[\alpha]_D^{16}$ +71° (*c*, 0·98).

Thanks are due to the Commonwealth Research Grant to Australian Universities for financial support, to Mr. C. A. Gardner, Dr. N. H. Brittan, and Mr. G. G. Smith for the collection and

identification of the plants, to Mr. W. J. Dunstan for authentic specimens of oleanolic acid and its derivatives, and to Mr. D. T. Downing and Mr. L. S. Zampatti for assistance with some of the experimental work.

UNIVERSITY OF WESTERN AUSTRALIA, NEDLANDS. [*Received, June 3rd,* 1952.]