

## 295. *The Occurrence of Triterpenes in the Aquifoliaceae and Ericaceae of Hong Kong.*

By H. R. ARTHUR, C. M. LEE, and C. N. MA.

Of the *Ilex* species endemic to the Colony, six contain ursolic acid, but *I. macrocarpa* contains oleanolic acid (the first report of it from this genus) and *I. pubescens* contains both acids; *Rhododendron farrerae* contains cerin, previously isolated only from cork, and *R. westlandii* contains a triterpenoid mixture which includes friedelin, *epifriedelanol*, and a new triterpene.

THE compounds reported below have been isolated from plants of Hong Kong during a search for new triterpenoid compounds.

The Aquifoliaceae of the Colony are confined to the genus *Ilex* of which ten species have been recorded. The leaves of the eight available species are being examined for the presence of triterpenoid compounds, which occur as mixtures in all species except *I. rotunda* from which ursolic acid was isolated without difficulty. Whilst ursolic acid has also been identified in *I. asprella*, *I. cinerea*, *I. hanceana*, *I. memecylifolia*, and *I. triflora*, it has been shown that *I. macrocarpa* contains oleanolic acid, and *I. pubescens* a mixture of both acids. Ursolic acid has been reported before in *Ilex* species <sup>1</sup> but this is the first report of oleanolic acid from this genus.

Regarding previous work carried out on the Ericaceae of the Colony, Arthur and Hui <sup>2</sup> showed that the leaves of all species contained ursolic acid and they <sup>3</sup> showed that the flavanone, matteucinol, was present in the leaves of *Rhododendron simsii*. Arthur <sup>4</sup> found that the leaves of *R. farrerae* contained the new flavanone, farrerol, but other species did not contain flavonoid compounds.

We report here that cerin (previously isolated only from cork <sup>5</sup>) occurs in the leaves of *R. farrerae* and that friedelin, *epifriedelanol*, and a new triterpene occur in a triterpenoid mixture from *R. westlandii*. Since the new triterpene occurs in very low yield and we will not be able to obtain more of it we record in the Experimental section what information we already possess.

### EXPERIMENTAL

M. p.s of Aquifoliaceae compounds are corrected. All compounds have been characterised by mixed m. p. with authentic specimens. Analyses are by Dr. Zimmermann, Melbourne. Alumina used was B.D.H., analysis grade, and the light petroleum had b. p. 60—80°.

*Aquifoliaceae*.—The dried leaves of all *Ilex* species were extracted with ether. Crude triterpene acid was isolated from the ethereal extract as stated by Arthur and Hui <sup>4</sup> for the isolation of ursolic acid from the Ericaceae. Attempted fractional recrystallisation of the crude acid from ethanol or methanol gave for some species crystalline acid of sharp m. p. which was identified by derivatives; from other species crystalline material was not obtained. In those cases the crude triterpenoid mixtures were acetylated. Fractional recrystallisation then

<sup>1</sup> Nooyen, *Pharm. Weekblad*, 1920, **57**, 1128; Mendive, *J. Org. Chem.*, 1940, **5**, 235; Kariyone, Hashimoto, and Tobinaga, *J. Pharm. Soc. Japan*, 1953, **73**, 257; Kariyone, Hashimoto, and Kiguchi, *ibid.*, 1949, **81**, 313.

<sup>2</sup> Arthur and Hui, *J.*, 1954, 4683.

<sup>3</sup> *Idem*, *J.*, 1954, 2782.

<sup>4</sup> Arthur, *J.*, 1955, 3740.

<sup>5</sup> Chevreul, *Ann. Chim.*, 1815, **96**, 141.

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afforded the pure acetates which on hydrolysis gave the acids which were identified as previously stated.

Samples of both acids gave in the Liebermann–Burchardt test a red  $\rightarrow$  violet  $\rightarrow$  blue  $\rightarrow$  green colour. A sample of oleanolic acid was analysed (Found: C, 79.2; H, 10.7. Calc. for  $C_{30}H_{48}O_3$ : C, 78.9; H, 10.6%) [acetate (Found: C, 77.1; H, 10.0; Ac, 7.6. Calc. for  $C_{32}H_{50}O_4$ : C, 77.1; H, 10.1; 1Ac, 8.8%)] and likewise a sample of ursolic acid (Found: C, 79.4; H, 10.7%) [acetate (Found: C, 77.7; H, 10.3%)]. Other experimental results are summarised in the Table.

Plant	Acid	M. p. of acid (vac.)	M. p. of acetate	M. p. of Me ester	$[\alpha]_D^{26}$ of acid	$[\alpha]_D^{27}$ of acetate	Yield (%) crude acid (dry leaves)
<i>I. asprella</i> .....	Ursolic	286–288°	291–292°	—	—	+65°	0.03
<i>I. cinevea</i> .....	„	286–288	291	—	+65°	—	1.06
<i>I. hanceana</i> .....	„	285–288	289–290	—	—	+62	1.44
<i>I. macrocarpa</i> ...	Oleanolic	309–311	266–267	198° †	+76	—	1.32
<i>I. memecylifolia</i> ...	Ursolic	285–286	286–287	—	—	+64	1.11
<i>I. pubescens</i> .....	„	287–289	291–292	—	—	+66	0.58
„ .....	Oleanolic	309–310	264–266	—	—	—	0.10
<i>I. rotunda</i> .....	Ursolic	287–290	—	169	+66	—	1.67
<i>I. triflora</i> .....	„	287–289	289–290	—	+66	—	—

\* In MeOH–CHCl<sub>3</sub> (1 : 1).

† In CHCl<sub>3</sub>.

‡ Sinters at 107–112°.

*Ericaceae.*—(a) *Rhododendron farrerae*. Dried leaves (2 kg.) were extracted with ether (10 l.), and the ethereal extract was evaporated to dryness. The dark green residue was boiled repeatedly with methanol to remove ursolic acid and farrerol. The almost colourless methanol-insoluble fraction (8 g.) which remained was recrystallised several times from pyridine and then from benzene. Colourless needles of cerin, m. p. 256–258°,  $[\alpha]_D^{26} -41.1^\circ$  (*c* 0.71 in pyridine) (Found: C, 81.6; H, 11.5. Calc. for  $C_{30}H_{50}O_2$ : C, 81.4; H, 11.4%), were obtained. The infrared spectrum of this sample in the range 900–1350 cm.<sup>-1</sup> was identical with that of an authentic sample. On oxidation with chromic oxide in acetic acid cerin gave friedelindioic acid, m. p. 286–288° (decomp.; vac.) (Found: C, 76.3; H, 10.5. Calc. for  $C_{30}H_{50}O_4$ : C, 75.9; H, 10.6%), characterised as dimethyl ester, m. p. 176–177° (Found: C, 76.5; H, 10.9; OMe, 11.2. Calc. for  $C_{32}H_{54}O_4$ : C, 76.4; H, 10.8; 2OMe, 12.3%). Cerin was further characterised as the 2 : 4-dinitrophenylhydrazone, m. p. 254° (decomp.) (Found: N, 9.2. Calc. for  $C_{36}H_{54}O_5N_4$ : N, 9.0%), and oxime, m. p. 272–276° (Found: C, 78.6; H, 11.3; N, 2.8. Calc. for  $C_{30}H_{51}O_2N$ : C, 78.7; H, 11.2; N, 3.1%).

(b) *Rhododendron westlandii*. Dried leaves (11.5 kg.) were extracted with light petroleum at room temperature and the extract was concentrated in stages until coarse colourless crystals no longer separated. These crops were combined and recrystallised from chloroform, giving hexagonal plates (15 g.) of *epifriedelanol*, m. p. 277–278° (vac.)  $[\alpha]_D^{23} -18.1^\circ$  (*c* 0.99 in CHCl<sub>3</sub>) (Found: C, 84.6; H, 12.3%. *M*, 425. Calc. for  $C_{30}H_{52}O$ : C, 84.0; H, 12.2%; *M*, 429). *epiFriedelanol*, which gave a pink  $\rightarrow$  purple  $\rightarrow$  brown colour in the Liebermann–Burchardt reaction, was characterised by conversion into the acetate (with pyridine and acetic anhydride), which separated from benzene in plates, m. p. 291–292° (vac.)  $[\alpha]_D^{27} -45.0^\circ$  (*c* 0.32 in CHCl<sub>3</sub>) (Found: C, 82.0; H, 11.7. Calc. for  $C_{32}H_{54}O_2$ : C, 81.6; H, 11.6%). The acetate resisted hydrolysis in boiling solutions of potassium hydroxide in ethanol and in ethylene glycol, and also in sulphuric–chromic acid, although the acetate group was shown to be present by infrared analysis. The benzoate (prepared with benzoyl chloride and pyridine) separated from benzene–methanol in plates, m. p. 251–252° (decomp.) (vac.)  $[\alpha]_D^{22} -37.6^\circ$  (*c*, 1.32 in CHCl<sub>3</sub>) (Found: C, 83.4; H, 10.6. Calc. for  $C_{37}H_{56}O_2$ : C, 83.4; H, 10.6%). *epiFriedelanol* was converted into friedelindioic acid, m. p. 286° (decomp.), by chromic oxide in acetic acid, and in stabilised acetone (Bowers *et al.*<sup>6</sup>) into friedelin which separated from benzene in needles, m. p. 259–260° (vac.)  $[\alpha]_D^{23} -27.3^\circ$  (*c* 0.39 in CHCl<sub>3</sub>) (Found: C, 84.6; H, 11.9. Calc. for  $C_{30}H_{50}O$ : C, 84.4; H, 11.8%). Friedelin was characterised by conversion into friedelanol, m. p. 297–298°  $[\alpha]_D^{23} -11.1^\circ$  (*c* 0.07 in CHCl<sub>3</sub>), by the method of Drake and Campbell<sup>7</sup> and into the 2 : 4-dinitrophenylhydrazone, m. p. 291° (decomp.; vac.) (Found: C, 71.4; H, 9.1; N, 9.3. Calc. for  $C_{36}H_{54}O_2N_4$ : C, 71.2; H, 9.0; N, 9.2%). *epiFriedelanol methyl ether* was prepared by boiling *epifriedelanol* (3.0 g.) with methyl iodide (500 ml.) and silver oxide for 35 hr. Recrystallisation from chloroform of the residue obtained after removal of methyl iodide yielded *epifriedelanol*. The chloroform mother-liquor was evaporated to dryness and the

<sup>6</sup> Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2555.

residue was dissolved in light petroleum and chromatographed over alumina, with benzene as eluant. Early fractions contained *epifriedelanol* but later fractions on recrystallisation from ethyl acetate deposited needles of the methyl ether, m. p. 219—220°,  $[\alpha]_D^{21} - 21.4^\circ$  (*c* 0.49 in  $\text{CHCl}_3$ ) (Found: C, 84.8; H, 12.0.  $\text{C}_{31}\text{H}_{54}\text{O}$  requires C, 84.2; H, 12.3%).

The mother-liquor of the original petroleum extract from the plant after removal of *epifriedelanol* was concentrated and the colourless crystalline mixture (27 g.) which separated was collected and its light petroleum solution was chromatographed on alumina, light petroleum being used as eluant. The first fraction (0.1 g.) on recrystallisation from light petroleum deposited needles of a *substance*, m. p. 201—203°,  $[\alpha]_D^{28} - 80^\circ$  (*c* 0.11 in  $\text{CHCl}_3$ ) (Found: C, 85.1; H, 11.7.  $\text{C}_{30}\text{H}_{48}\text{O}$  requires C, 84.8; H, 11.4.  $\text{C}_{30}\text{H}_{50}\text{O}$  requires C, 84.4; H, 11.8%). Like  $\beta$ -amyrin, this compound gave a deep red colour in the Liebermann-Burchardt test but it depressed the m. p. of the former by 32°, and was recovered unchanged after 4 hours' boiling in acetic anhydride and pyridine. It was isomerised by boiling ethanolic hydrochloric acid.

The second fraction (1.0 g.) from the column, obtained by elution with light petroleum-benzene (3 : 2), gave on recrystallisation from benzene needles of *friedelin*, m. p. 248—252°.  $[\alpha]_D^{29} - 27.5^\circ$  (*c* 0.34 in  $\text{CHCl}_3$ ) (Found: C, 84.6; H, 11.8. Calc. for  $\text{C}_{30}\text{H}_{50}\text{O}$ : C, 84.4; H, 11.8%) [2 : 4-dinitrophenylhydrazone, m. p. 288° (decomp.; vac.) (Found: C, 71.6; H, 8.9; N, 9.7. Calc. for  $\text{C}_{36}\text{H}_{54}\text{O}_4\text{N}_4$ : C, 71.2; H, 9.0; N, 9.2%); oxime, m. p. 302—303° (decomp.; vac.) (Found: C, 81.4; H, 11.7; N, 3.4. Calc. for  $\text{C}_{30}\text{H}_{51}\text{ON}$ : C, 81.6; H, 11.7; N, 3.2%)].

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UNIVERSITY OF HONG KONG.

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