

THE OCCURRENCE OF TRITERPENOIDS AND STEROIDS IN SOME PLANTS OF THE RUBIACEAE FAMILY OF HONG KONG*

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Fifty-eight species of Rubiaceae from 28 genera have been identified in Hong Kong.¹ Of these only a few are used medicinally. The compounds reported here have been isolated from three of these medicinal plants during a search for the active principles.

Oldenlandia corymbosa L. and *Oldenlandia diffusa* Roxb. are very similar plants known under the common name of *pae hua she shê ts'ao* (the snake-tongued grass with white flower). The former has square branches and small flowers in bundles; the latter has round branches and single flowers.² Decoctions of both plants have long been prescribed by herbalists for intestinal disorders, and they are also used for treating cancer. The third plant *Paederia scandens* (Lour.) Merr. is used as a "blood purifier".

Triterpene acids and sterols have previously been isolated from a few *Oldenlandia* species. *O. biflora* and *O. herbacea* have been reported to contain ursolic acid,³ and the former has also been shown to contain β -sitosterol and γ -sitosterol.⁴ The occurrence of ursolic acid, oleanolic acid, and γ -sitosterol has been reported in *O. corymbosa*.⁵ Neither *O. diffusa* nor *Paederia scandens* appear to have previously been investigated chemically.

We record here from *O. corymbosa* the isolation of stigmasterol, in addition to γ -sitosterol, ursolic acid, and oleanolic acid which have formerly been reported.⁵ The petroleum extract of the plant on concentration gave a precipitate consisting mainly of triterpene acid mixture, which gave a red→violet→blue→green colour in the Liebermann-Burchardt test. This mixture after purification through the sodium salts was fractionally recrystallized to yield ursolic acid as the chief product. Oleanolic acid was obtained in very small yield in the mother liquors. The petroleum solution, after removal of triterpene acids, on chromatography yielded the steroid mixture, from which stigmasterol and γ -sitosterol were separated from each other as acetates by the method⁶ of Windaus and Hauth. Further extraction of the plant material with methanol gave a second yield of γ -sitosterol,

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¹ "Check List of Hong Kong Plants." p. 12. (Colonial Herbarium, Hong Kong Govt.: Hong Kong 1962.)

² Chuang, S. C., *Present-day Chin. Med. J.*, 1961, **105**, 4.

³ Govindachari, T. R., Nagarajan, K., Pai, B. R., and Rajappa, S., *J. Sci. Ind. Res., India*, 1958, **17B**, 73.

⁴ Bhakuni, D. S., *J. Sci. Ind. Res., India*, 1959, **18B**, 445.

⁵ Khastgir, H. N., Sengupta, S. K., and Sengupta, P., *J. Amer. Pharm. Ass. (Sci. Ed.)*, 1960, **49**, 562.

⁶ Windaus, A., and Hauth, A., *Ber. dtsch. chem. Ges.* 1906, **39**, 4378; *Ber. dtsch. chem. Ges.*, 1907, **40**, 3681; Nabenhauer, F. P., and Anderson, R. J., *J. Amer. Chem. Soc.* 1926, **48**, 2972.

ursolic acid, and oleanolic acid. *Oldenlandia diffusa* was investigated similarly: it yielded stigmasterol, γ -sitosterol, and ursolic acid, but did not appear to contain oleanolic acid.

The petroleum extract of *Paederia scandens* on concentration precipitated a low-melting substance which is under further investigation. The mother liquor after removal of this low-melting material, when chromatographed on alumina, yielded γ -sitosterol, a second quantity of which was obtained on further extraction of the plant with methanol. No triterpene acid was isolated.

Experimental

Analyses were by the Microanalytical Laboratory, University of Singapore. Melting points of triterpene acids and derivatives were determined in vacuum tubes and corrected, and those of sterols and derivatives on a Kofler block. Mixed melting points with authentic samples were determined with ursolic acid, oleanolic acid, and γ -sitosterol, and some of their derivatives. No depressions were observed. The alumina used for chromatography was B.D.H. analysis grade. Light petroleum refers to the fraction of b.p. 60–80°. Infrared spectra were taken on a Perkin-Elmer Infracord (Model 137) spectrophotometer.

Oldenlandia corymbosa.—Air-dried plant (800 g) was extracted with light petroleum (10 l.) in a Soxhlet apparatus for 30 hr. The extract was concentrated to 300 ml, allowed to stand overnight, and filtered. The solids (5 g) were collected, dissolved in ether, and shaken with 4% sodium hydroxide solution. The greyish precipitate of crude sodium salts which separated at the interface was filtered, washed, and dissolved in hot ethanol (charcoal). The solution was acidified and cooled; the almost colourless acid mixture which separated yielded, after repeated crystallizations from ethanol, colourless needles of ursolic acid (3 g), m.p. 289–290°, $[\alpha]_D +68.8^\circ$ (c, 0.229 in 1 : 1 MeOH-CHCl₃) (Found: C, 79.0; H, 10.7%. Calc. for C₃₀H₄₈O₃: C, 78.9; H, 10.6%). It formed methyl ursolate, m.p. 171–172°, $[\alpha]_D +62.7^\circ$ (c, 0.57 in CHCl₃) (Found: C, 79.2; H, 10.7%. Calc. for C₃₁H₅₀O₃: C, 79.1; H, 10.7%) and ursolic acid acetate, m.p. 293–295° (Found: C, 77.1; H, 10.0%. Calc. for C₃₂H₅₀O₄: C, 77.1; H, 10.1%).

After removal of seven crops of ursolic acid from the ethanolic mother liquors, the eighth crop on recrystallization from ethanol yielded colourless needles of oleanolic acid (0.06 g), m.p. 309–310°, $[\alpha]_D +78.3^\circ$ (c, 0.209 in CHCl₃) (Found: C, 78.4; H, 11.0%. Calc. for C₃₀H₄₈O₃: C, 78.9; H, 10.6%). It formed methyl oleanolate, m.p. 200–202° (Found: C, 79.2; H, 10.5%. Calc. for C₃₁H₅₀O₃: C, 79.1; H, 10.7%) and oleanolic acid acetate, m.p. 256–258°.

The original petroleum mother liquor was further concentrated and chromatographed on alumina (600 g). The sterol mixture (0.6 g) was obtained by elution with light petroleum–benzene mixture (1 : 4). It had m.p. 150–152°. The mixture was acetylated, and to the product dissolved in ether was added a solution of bromine in acetic acid. The highly insoluble crystalline precipitate formed, on recrystallization from chloroform–ethanol, gave plates of stigmasteryl acetate tetrabromide (0.25 g), m.p. 200–201° (decomp.) (Found: C, 48.2; H, 6.61%. Calc. for C₃₁H₅₀O₂Br₄: C, 48.1; H, 6.52%). The tetrabromide was debrominated by refluxing with zinc powder in acetic acid and the product, on recrystallization from ethanol, yielded plates of stigmasteryl acetate, m.p. 143–144° (Found: C, 81.9; H, 11.2%. Calc. for C₃₁H₅₀O₂: C, 81.9; H, 11.1%). The acetate was hydrolysed by ethanolic sodium hydroxide, and the product was recrystallized from ethanol to yield stigmasterol, m.p. 168–170°, which gave an identical i.r. spectrum (in Nujol) with that recorded for stigmasterol by Johnson, Grostic, and Jensen.⁷ The solution after removal of stigmasteryl acetate tetrabromide was debrominated similarly to give γ -sitosteryl acetate (0.03 g), m.p. 141–142°, $[\alpha]_D -43^\circ$ (c, 0.34 in CHCl₃) (Found: C, 82.2; H, 11.3%. Calc. for C₃₁H₅₂O₂: C, 81.5; H, 11.5%) which was hydrolysed to γ -sitosterol, m.p. 143–146°, $[\alpha]_D -41.0^\circ$ (c, 0.32 in CHCl₃) (Found: C, 83.5; H, 12.1%. Calc. for C₂₉H₅₀O: C, 84.0; H, 12.2%). The i.r. spectrum (in CCl₄) was identical with that of an authentic sample of γ -sitosterol.

⁷ Johnson, J. L., Grostic, M. F., and Jensen, A. O., *Analyt. Chem.* 1957, **29**, 468.

The plant material, after extraction with light petroleum, was further extracted with boiling methanol for 30 hr. The solution was concentrated to 100 ml and allowed to stand overnight. The green precipitate (10 g) was collected, dissolved in ether, and shaken with 4% sodium hydroxide solution. The precipitate (10 g) at the interface was worked up as before to give ursolic acid (5 g), m.p. 288–290°, and oleanolic acid (0.1 g), m.p. 308–309°. The methanol mother liquor was evaporated to dryness, dissolved in petroleum, and chromatographed on alumina. Elution with light petroleum–benzene (1 : 4) gave needles (0.15 g), m.p. 135–140°, which after several recrystallizations from ethanol yielded γ -sitosterol as plates (0.10 g), m.p. 143–147°.

Oldenlandia diffusa.—Dried plant material (900 g) was extracted with light petroleum and then with methanol, and the extracts were worked up by methods similar to those for *O. corymbosa*. The crude acid mixture (5 g) on repeated recrystallization yielded ursolic acid (3.2 g), m.p. 289–290°, $[\alpha]_D^{25} + 67.0^\circ$. (It formed methyl ursolate, m.p. 170–171°; and ursolic acid acetate, m.p. 292–294°.) No mother-liquor crops had a high m.p.; this indicated the absence of oleanolic acid. The steroid fraction (0.64 g) gave stigmasteryl acetate tetrabromide (0.20 g), m.p. 200–202° (decomp.), debrominated to stigmasteryl acetate, m.p. 143°, which was hydrolysed to stigmasterol, m.p. 168–170°. The mother liquor after removal of stigmasteryl acetate tetrabromide on debromination yielded γ -sitosteryl acetate, m.p. 140–141°, hydrolysed to γ -sitosterol, m.p. 143–146°.

Paederia scandens.—The dried plant (900 g) was extracted with light petroleum at room temperature for 7 days, the solution was concentrated to 50 ml, and allowed to stand overnight. A semicrystalline substance (m.p. 75–80°) was filtered off. The filtrate was chromatographed on alumina (400 g). On elution with light petroleum–benzene (1 : 3), needle-shaped crystals (1.5 g), m.p. 135–138°, were obtained. These, after ten recrystallizations from ethanol, gave plates of γ -sitosterol, m.p. 143–146°, characterized by forming the acetate, m.p. 135–137°.

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