AN EXAMINATION OF THE RUBIACEAE OF HONG KONG*

VII.† THE OCCURRENCE OF TRITERPENOIDS, STEROIDS, AND TRITERPENOID SAPONINS

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Earlier papers of this series have reported the work on eleven species of the Hong Kong Rubiaceae, two of which have been shown[1,2] to contain triterpenoids occurring both in the free state and as sapogenins, while another six have been proved to yield free triterpenoids. Either a phytosterol or a phytosterol mixture has been isolated from each of the plants investigated. We report here further work on three more species of the family.

Examination of the leaves of *Ixora chinensis* Lam. revealed the presence of the triterpenoids lupeol and betulin, and the sterols β-sitosterol and stigmasterol.

Investigations on two *Randia* species, *R. canthioides* Champ. ex Benth. and *R. sinensis* (Lour.) Schult., led to the isolation of stigmasterol from the stems of the former and β-sitosterol from the leaves and stems of both species. In the leaves of *R. canthioides*, cincholic acid has been found to occur both in the free state and as sapogenin, the sugar moiety of which is 6-deoxy-D-glucose. The stems of *R. sinensis* have been shown to contain a saponin which on hydrolysis yielded mesembryanthemoidigenic acid and D-glucose.

The occurrence of cincholic acid in sapogenin mixtures has been reported from three plants of the Rubiaceae family,[1-3] but this is the first isolation of it as a free triterpenoid and as a pure sapogenin. The only previous report on mesembryanthemoidigenic acid was made by Tursch *et al.*[4] in 1965. They isolated the new acid as a sapogenin from *Rhipsalis mesembryanthemoides* Steud., and determined its structure to be 3β,29-dihydroxyolean-12-en-28-oic acid.

The mass spectrum of methyl mesembryanthemoidigenate showed prominent ions at *m/e* 486, 7·5% (M^+); 455, 13% (M^+-CH_2OH); 427, 2·5% ($M^+-COOCH_3$); 426, 4% ($M^+-COOCH_3-H$); 278, 33·6% (retro-Diels–Alder fragment *a*); 265, 6·5% (*b*); 247, 100% (*a*$-CH_2OH$ and *b*$-H_2O$); 219, 14·3% (*a*$-COOCH_3$); 218, 14·9% (*a*$-COOCH_3-H$); 207, 24·9% (*c*); 205, 7·5% (*d*); 201, 43·5% (*a*$-COOCH_3-H_2O$).

It is noteworthy that the base peak is the ion *m/e* 247, and not the expected ion *m/e* 219, which is formed by loss of the angular methoxycarbonyl group at C 28

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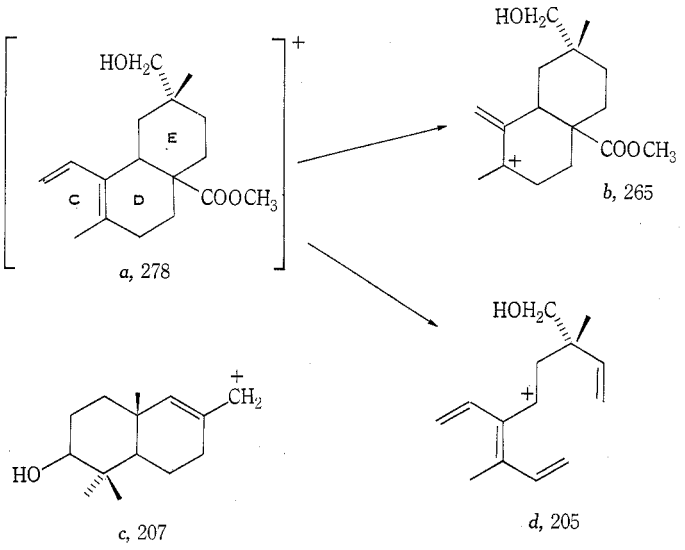
[1] Hui, W. H., and Yee, C. W., *Aust. J. Chem.*, 1968, **21**, 543.

[2] Hui, W. H., and Szeto, S. K., *Phytochemistry*, 1967, **6**, 443.

[3] Tschesche, R., Duphorn, I., and Snatzke, G., *Liebigs Ann.*, 1963, **667**, 151.

[4] Tursch, B., Leclercq, J., and Chiurdoglu, G., *Tetrahedron Lett.*, 1965, 4161.

from the retro-Diels–Alder fragment *a*.[5] This base peak is probably due to the two fragments, *a*—CH_2OH (from C 29) and *b*—H_2O, both having *m/e* 247.



Experimental

Lupenyl acetate, betulin, dimethyl cincholate, mesembryanthemoidigenic acid, stigmasteryl acetate tetrabromide, and β-sitosterol were identified by mixed m.p. and i.r. spectral comparisons with authentic samples and the first four also by elementary analysis (kindly made by Microbial Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd, Japan, and the Microanalytical Laboratory, University of Singapore). Optical rotations are for chloroform solutions unless otherwise stated.

(*a*) Ixora chinensis *Lam.*

Air-dried leaves (3·5 kg) were milled and extracted twice at room temperature with light petroleum. The concentrated combined extracts were chromatographed on alumina (1·5 kg). Elution with light petroleum (b.p. 60–80°) gave low-melting waxy material. Elution with light petroleum–benzene (1 : 1) yielded crude lupeol (0·26 g), m.p. 194–215°, purified through its acetate, m.p. 218–219°, $[\alpha]_D$ +47·0°. Elution with light petroleum–benzene (1 : 2) gave a sterol mixture (0·85 g), m.p. 140–149°, the acetates of which on bromination deposited crystals of stigmasteryl acetate tetrabromide (0·2 g), m.p. 203°. The filtrate after debromination and deacetylation yielded β-sitosterol, m.p. 138–140°, $[\alpha]_D$ −35°. Elution with chloroform gave a substance (0·9 g), m.p. 230–240°, which on rechromatography gave betulin, m.p. 256–258°, $[\alpha]_D$ +20·0°. This yielded the diacetate, m.p. 224–226°, $[\alpha]_D$ +21·2°, and the dibenzoate, m.p. 176–179°.

Air-dried stems were investigated in the same manner, but neither sterols nor triterpenoids could be isolated. Nor did the ethanol extracts of the stems or leaves yield any triterpene acids or saponins.

(*b*) Randia canthioides *Champ. ex Benth.*

Milled dried leaves (1·4 kg) were extracted with light petroleum and the extract chromatographed on alumina (700 g) as *I. chinensis*. Elution with light petroleum–benzene (1 : 4) yielded

[5] Budzikiewicz, H., Wilson, J. M., and Djerassi, C., *J. Am. chem. Soc.*, 1963, 85, 3688.

β-sitosterol (0·3 g), m.p. 140–141°. Two further extractions with ethanol at room temperature gave a solution which on evaporation under vacuum left a solid mass. After repeated extraction with ether the combined ethereal solutions were thoroughly extracted with 8% sodium hydroxide solution. The aqueous layer was acidified, and the precipitate (1·0 g) formed was methylated with diazomethane in ether, and the dried product was dissolved in light petroleum–benzene (2 : 3) and chromatographed on alumina (60 g). Elution with light petroleum–benzene (1 : 4) gave dimethyl cincholate, m.p. 217–220°, $[\alpha]_D$ +104°. It formed acetyl dimethyl cincholate, m.p. 249–250°. The ether-insoluble part of the ethanol extract gave a strong frothing test in water. It was extracted with 25% aqueous ethanol, the solution was made 4N with respect to hydrochloric acid, and then boiled under reflux for 4 hr. The crude sapogenin which precipitated was collected and extracted with ether. The crude triterpene acid in the ethereal solution was purified through the sodium salt, methylated, and chromatographed as described above to yield dimethyl cincholate, m.p. 218–220°, $[\alpha]_D$ +104°. Paper chromatography of the neutralized concentrated filtrate from the hydrolysis of the crude sapogenin (after removal of other organic matter by extractions with chloroform and ether) using the developer n-butanol–ethanol (4 : 1) with boric acid–borax buffer[6] revealed one single spot with an R_F value corresponding to that of 6-deoxy-D-glucose. The ozasone prepared from the same solution had m.p. 197–198° (lit.[2] 193–194°).

The light petroleum extract of the stems (1·9 kg) on chromatography in the usual way yielded a sterol mixture (0·1 g), which was separated into stigmasterol and β-sitosterol as described for *I. chinensis* leaves. No triterpene acid or saponin could be obtained from the ethanol extract of the stems.

(*c*) Randia sinensis (*Lour.*) *Schult.*

Chromatography of the light petroleum extract of both the leaves (1·2 kg) and stems (9 kg) gave only β-sitosterol (0·3 g and 0·4 g respectively). The ethanol extract of the leaves again did not yield any triterpenoids, but that of the stems, which was almost insoluble in ether, gave a strong frothing test in aqueous solution. It was hydrolysed with hydrochloric acid in aqueous ethanol as in (*b*). The crude sapogenin (6·5 g) purified through the sodium salt was methylated and chromatographed in benzene on alumina (250 g). Elution with benzene–chloroform (3 : 2) gave a solid which crystallized from ethanol to give methyl mesembryanthemoidigenate, $C_{31}H_{50}O_4$, m.p. 216–218°, $[\alpha]_D$ +66·0°, as needle-shaped crystals, which were acetylated to give the diacetyl methyl ester, $C_{35}H_{54}O_6$, m.p. 245–247°, $[\alpha]_D$ +59·7°, and hydrolysed with potassium hydroxide in ethylene glycol to yield the acid $C_{30}H_{48}O_4$, m.p. 320–323°, $[\alpha]_D$ +69·2° ($CHCl_3$–EtOH). Paper chromatography of the filtrate (after the usual treatment) from the crude sapogenin, using collidine saturated with water as developer,[7] showed one single spot with R_F value identical with that of D-glucose. The solution forms an osazone, m.p. 212–213°.

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[6] Krauss, M. T., Jäger, H., Schindler, O., and Reichstein, T., *J. Chromat.*, 1960, **3**, 63.
[7] Sandberg, F., Ahlenius, B., and Thorsen, R., *Svensk farm. Tidskr.*, 1958, **62**, 541.