

## THE CHEMICAL CONSTITUENTS OF AUSTRALIAN *ZANTHOXYLUM* SPECIES

### VI.\* A FURTHER EXAMINATION OF THE CONSTITUENTS OF THE BARK OF *ZANTHOXYLUM CONSPERSIPUNCTATUM* (RUTACEAE)

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#### Abstract

From the bark of *Zanthoxylum conspersipunctatum* Merr. & Perry the following substances were isolated: chelerythrine and sanguinarine chlorides, 13-acetyldihydrochelerythrine,  $\beta$ -sitosterol, lupeol, lupenone, sesamin, hesperidin, tembamide, *O*-methyltembamide, cyclotrivenatrylene, and psoromic acid. *O*-Methyltembamide is almost certainly an artefact, as is cyclotrivenatrylene, and psoromic acid probably originated from a lichen on the bark.

Johns *et al.*<sup>1</sup> extracted three samples of the bark of *Zanthoxylum conspersipunctatum* Merr. & Perry from different areas of New Guinea, with methanol and worked up the extracts primarily for alkaloids. From each of the extracts the flavanone glycoside, hesperidin, and the amide, tembamide, were isolated but only one yielded alkaloids, which were shown to be  $\alpha$ -allocryptopine ( $\beta$ -homochelidonine), and a new base, very closely related to cryptopine, whose structure was established. The authors noted that these results were consistent with the botanical variability of the species.

Through the kindness of Dr J. A. Lamberton we have been able to examine a fourth sample of the bark which was collected at Okasa near Okapa in New Guinea. This sample also yielded hesperidin and tembamide, but no tertiary bases could be separated. Instead, chelerythrine chloride and sanguinarine chloride were identified and, in addition, 13-acetyldihydrochelerythrine<sup>2</sup> was isolated under circumstances which indicated that it was a genuine natural product rather than an artefact. Also isolated were  $\beta$ -sitosterol, lupeol, lupenone, and the lignan, sesamin, together with *O*-methyltembamide, cyclotrivenatrylene, and psoromic acid. Of the three latter substances, *O*-methyltembamide, which was synthesized from tembamide, was almost certainly an artefact, as was cyclotrivenatrylene, although veratryl alcohol,

\* Part V, *Aust. J. Chem.*, 1970, **23**, 133.

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<sup>1</sup> Johns, S. R., Lamberton, J. A., Tweeddale, H. J., and Willing, R. I., *Aust. J. Chem.*, 1969, **22**, 2233.

<sup>2</sup> Corrie, J. E. T., Green, G. H., Ritchie, E., and Taylor, W. C., *Aust. J. Chem.*, 1970, **23**, 133.

its presumed precursor, was not detected; the psoromic acid was probably derived from lichens growing on the bark. Considerable difficulty was experienced in separating chelerythrine and sanguinarine chlorides which form a mixture notoriously difficult to separate and a completely satisfactory method was not found.

The overall results of the extraction confirm the variability of the species and its relationships to other *Zanthoxylum* species as noted by Johns *et al.*<sup>1</sup>

### Experimental

General directions are as in Corrie *et al.*<sup>2</sup>

(i) The dried milled bark (7.0 kg; NGF 24960) was exhausted at room temperature in turn with light petroleum, ether, and methanol.

The light petroleum extract on concentration to about 200 ml deposited a crystalline mass, PS (20 g), which was collected. The filtrate was diluted with ether and fractionated with 5% aqueous sodium bicarbonate, 5% aqueous sodium carbonate, and 2% aqueous sodium hydroxide to yield fractions P1, P2, P3 respectively, and the neutral fraction P4. Alkaloids were absent.

The ether extract, which also contained no alkaloids, similarly yielded fractions E1, E2, E3, and E4.

The methanol extract on concentration deposited a crystalline solid, MS (10 g). The mother liquor (300 ml) was poured into 2N sulphuric acid (1000 ml); a tar separated. The tar was re-dissolved in methanol and the solution again poured into 2N sulphuric acid to yield a tar, MT, and an aqueous phase. The combined aqueous phases were extracted with ether and the extracts fractionated with alkali as above to yield fractions M1, M2, M3, and M4. The residual aqueous solution was discarded.

(ii) Fractions P1, E1, and M1 were combined and chromatographed on silica gel. Elution with benzene-ether (9 : 1) afforded psoromic acid (0.09 g), m.p. 265°, after recrystallization from methanol. It was identified by comparison (mixed m.p., and i.r. spectrum) with an authentic specimen.

(iii) Fractions P2, E2, and M2 were combined and chromatographed on silica gel but the only crystalline fraction obtained (0.4 g), m.p. 75–77°, was evidently (i.r. spectrum) a fatty acid mixture which was not examined further.

(iv) The fractions P3, E3, and M3 were intractable and were discarded.

(v) Fraction PS was fractionally crystallized from methanol to yield lupeol (4 g), m.p. 216°, identified by comparison with an authentic specimen. The material in the mother liquors was dissolved in benzene (300 ml) and light petroleum (300 ml) and the solution extracted several times with methanol-water (9 : 1). The unextracted material in the hydrophobic phase was crude lupeol (6 g), which was best purified as its acetate, m.p. 217°, from methanol. The methanol-water extracts contained sesamin (8 g) which crystallized from methanol in colourless plates, m.p. 125–126°,  $[\alpha]_D^{25} + 50^\circ$  (c, 1% in  $\text{CHCl}_3$ ) [lit.<sup>3</sup> m.p. 123°,  $[\alpha]_D^{20} + 68^\circ$  (c, 1.6% in  $\text{CHCl}_3$ )]. The natural occurrence of partly racemic sesamin has been previously reported<sup>3</sup> and the m.p. stated to lie in the range 122–130°. In the present instance the substance was identified by its n.m.r. spectrum (cf.<sup>4</sup>).

(vi) Fractions P4 and E4 similarly yielded lupeol (3 g) and sesamin (2 g). The combined mother liquors were evaporated and the residue chromatographed on alumina. Elution with benzene-light petroleum (1 : 1) gave lupenone (1 g), m.p. 168° after crystallization from aqueous ethanol; it was identified by comparison with an authentic specimen. Sesamin (11 g) was eluted by benzene, lupeol (5 g) by benzene-ether (10 : 1), and  $\beta$ -sitosterol (0.4 g) by benzene-ether (5 : 1). The latter, m.p. 138–140° after crystallization from methanol, was identical with an authentic specimen.

(vii) The solid, MS, on recrystallization from methanol afforded hesperidin, m.p. 257–260° (dec.), identified by comparison with an authentic specimen.

(viii) Fraction M4 was chromatographed on silica gel. Elution with benzene-ether (10 : 1) afforded sesamin (2 g), and with benzene-ether (1 : 1) cyclotrimeratrylene<sup>5</sup> (0.15 g), m.p. 233° after

<sup>3</sup> Carnalm, B., Erdtman, H., and Pelchowicz, Z., *Acta chem. scand.*, 1955, 9, 1111.

<sup>4</sup> Ludwig, C. H., Nist, B. J., and McCarthy, J. L., *J. Am. chem. Soc.*, 1964, 86, 1186.

<sup>5</sup> Lindsey, A. S., *Chemistry Ind.*, 1963, 823.

recrystallization from benzene; it was identical with a synthetic specimen. Tembamide (0.3 g), m.p. 155°,  $[\alpha]_D^{25}$  0° (c, 2% in EtOH) (lit.<sup>1</sup> m.p. 156°), after crystallization from chloroform–light petroleum, was eluted by ether–methanol (99:1) (Found: C, 70.7; H, 6.6; N, 5.1. Calc. for  $C_{16}H_{17}NO_3$ : C, 70.8; H, 6.2; N, 5.2%). The acetyl derivative, colourless needles from benzene–light petroleum, had m.p. 140° (lit.<sup>6</sup> 141°).

(ix) The tar, MT, was dissolved in methanol and the solution was shaken with dilute hydrochloric acid. The interfacial precipitate, MTP, was collected but no substance could be isolated from the aqueous extract. The organic layer was evaporated and the residue triturated with methanol to yield a further amount of MTP and a filtrate MTF.

Fraction MTP was recrystallized from dilute hydrochloric acid to yield orange-yellow needles (0.8 g), m.p. 168–170°. This material, which from its n.m.r. spectrum (ratio of signal intensities from methoxyl to methylenedioxy protons) consisted of chelerythrine chloride (lit.<sup>7</sup> m.p. 202–203°) and sanguinarine chloride (lit.<sup>8</sup> m.p. 278°) in the approximate ratio 3:1, was very difficult to separate. Eventually the following method was adopted. A sample (0.5 g) was heated in a sublimation apparatus at 200°/0.5 mm. The sublimate (0.3 g) was digested with hot ethanol to leave an insoluble fraction which on crystallization from pyridine afforded crude norsanguinarine (0.07 g), m.p. 263–266° (lit.<sup>8</sup> 278–280°) (Found: mol. wt, 317. Calc. for  $C_{19}H_{11}NO_4$ : mol. wt, 317). The ethanol-soluble material after recrystallization from butanol–2-methoxyethanol afforded norchelerythrine (0.2 g), m.p. 214° (lit.<sup>7</sup> 212–214°), which on methylation afforded chelerythrine chloride, m.p. 202–203°, identical with an authentic specimen.

(x) The filtrate, MTF, was evaporated and a solution of the residue in chloroform filtered through a column of silica gel. Several of the fractions on slow evaporation deposited small amounts of crystalline material which on recrystallization from methanol–chloroform afforded 13-acetyl-dihydrochelerythrine (0.04 g), m.p. 190–192° (lit.<sup>2</sup> 194–195°), identical with an authentic specimen.

The remaining fractions were combined and evaporated. The residue (35 g) was extracted with hot benzene (300 ml) and the soluble portion chromatographed on silica gel. Elution with benzene–ether (10:1) yielded sesamin (2.0 g), and a further amount of cyclotrivatrylene (0.05 g) was eluted by benzene–ether (1:1). Ether eluted *O*-methyltembamide (0.3 g), m.p. 98–99° after recrystallization from benzene–light petroleum (Found: C, 71.6; H, 6.8; N, 4.7;  $OCH_3$ , 20.8.  $C_{17}H_{19}NO_3$  requires C, 71.6; H, 6.7; N, 4.9;  $2 \times OCH_3$ , 21.7%).  $\nu_{max}$  3400, 1640, 1620, 1580  $cm^{-1}$ ;  $\lambda_{max}$  228, 269, 279 nm,  $\epsilon$  21000, 2300, 1700; n.m.r. spectrum ( $CD_3OD$ ): m, 9, 7.8–6.8, ArH; m, 1, 4.62, CH; s, 3, 3.80,  $ArOCH_3$ ; m, 2, 3.62–3.50,  $CH_2$ ; s, 3, 3.23,  $CHOCH_3$ .

A solution of tembamide (0.1 g) in methanol (50 ml) containing boron trifluoride etherate (1 ml) was kept at room temperature for 48 hr, and then diluted with ether. The solution was washed thoroughly with aqueous sodium bicarbonate and water, then dried and evaporated. The residue on crystallization from benzene–light petroleum yielded *O*-methyltembamide (0.07 g), m.p. 99°, identical with the above product.

Final total yields were: psoromic acid, 0.9 g; lupeol, 18 g; lupenone, 1 g;  $\beta$ -sitosterol, 0.4 g; sesamin, 25 g; hesperidin, 10 g; cyclotrivatrylene, 0.2 g; tembamide, 0.3 g; *O*-methyltembamide, 0.3 g; chelerythrine and sanguinarine chlorides, 0.8 g; 13-acetyldihydrochelerythrine, 0.04 g.

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<sup>6</sup> Albonico, S. M., Kuck, A. M., and Deulofeu, V., *J. chem. Soc.*, 1967, 1327.

<sup>7</sup> Bailey, A. S., and Worthing, C. R., *J. chem. Soc.*, 1956, 4535.

<sup>8</sup> Beke, D., Barczai, M. B., and Toke, L., *Chem. Abstr.*, 1960, 54, 17437.