THE OCCURRENCE OF (+)-HYGROLINE IN *GYNOTROCHES AXILLARIS* BL. (RHIZOPHORACEAE)*

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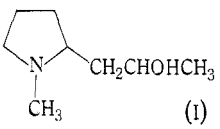

(I)

Gynotroches axillaris Bl., a New Guinea tree belonging to the family Rhizophoraceae, gives a poor and variable yield of alkaloids, and (+)-hygroline (I), isolated from the bark and detected in the leaves, is the only alkaloidal constituent to be identified. (+)-Hygroline has previously been found[1] as the major alkaloid of the leaves of the related species *Carallia brachiata* (Lour.) Merr., which belongs to the subfamily Rhizophoroidae and tribe Gynotroche of the family Rhizophoraceae.

All leaf specimens of *G. axillaris* gave negative field tests and only trace amounts of alkaloids in laboratory assays. Bark samples sometimes gave negative field tests that were confirmed by laboratory assay, but bark from one tree (80 ft by 2·5 ft) from rain forest near Mt Shungol in the Morobe District, Territory of New Guinea, gave a positive field test and yielded alkaloids on extraction. Very little of this material was available for chemical work, and a subsequent collection gave a much poorer yield. (+)-Hygroline was detected in the crude alkaloid fraction from this low-yielding material, and was isolated in sufficient quantity for positive identification. The yield of alkaloid from leaves was too small for separation of the alkaloids, but thin-layer chromatography and gas chromatography indicated the presence of hygroline in the crude alkaloidal extracts.

Experimental

Milled dry bark of *G. axillaris* (102 kg) was extracted by percolation with warm ethanol, and the crude alkaloids (2·2 g) were isolated according to the method already described.[2] The crude alkaloids still contained much non-basic material. Comparison by thin-layer chromatography with a sample of alkaloids from *Carallia brachiata* and with authentic hygroline indicated that hygroline was a component of the mixture. A benzene extract of the crude alkaloids, most of which remained undissolved, gave on concentration an oily residue that contained a major component having the same retention time as hygroline in gas chromatograms. Small samples collected by preparative gas chromatography had the same mass spectrum as hygroline.

As attempts to separate the hygroline directly from the crude alkaloids were not successful, a 1-g sample of the crude alkaloids was dissolved in acetic anhydride and allowed to stand at room temperature. After removal of excess acetic anhydride under vacuum the residue was separated into basic and non-basic fractions by partition between chloroform and 2N sulphuric acid. Approximately 60–70% of the total was recovered from the chloroform, and as this fraction

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[1] Fitzgerald, J. S., *Aust. J. Chem.*, 1965, **18**, 589.

[2] Johns, S. R., Lamberton, J. A., and Occolowitz, J. L., *Aust. J. Chem.*, 1966, **19**, 1951.

showed *O*-acetate but not amide absorption in its i.r. spectrum it must have been non-alkaloidal. The basic fraction (*c.* 30%) was recovered from the acid solution by basification with NH_3 and extraction into $CHCl_3$. The major portion passed rapidly through a column of neutral alumina in benzene solution, and its n.m.r. spectrum and i.r. spectrum indicated that it was largely hygroline acetate. A small portion yielded a picrate, m.p. 128–129°, which showed no depression of m.p. when mixed with a sample of (+)-hygroline acetate picrate. Hydrolysis of the remainder with 5% KOH in ethanol at room temperature yielded hygroline which was finally purified by sublimation under vacuum. This gave (+)-hygroline as colourless crystals, m.p. 27–29°, $[\alpha]_D$ +82° (*c*, 0·17 in H_2O). (Lit.[1] m.p. 29–31°, $[\alpha]_D$ +87° in water.)

A crude alkaloid fraction from *G. axillaris* leaves was obtained in too low yield for fractionation. However, the portion extracted by hot benzene appeared to contain hygroline from comparative thin-layer chromatograms and gas chromatograms.

Leaf and bark of *G. axillaris* collected from a tree (40 ft by 1 ft) in rain forest near the Busu River, 12 miles north of Lae, gave negative field tests for both leaf and bark, and assays of 0·003% leaf and 0·007% bark alkaloids. Of several collections the only sample to give a positive field test was bark from a tree at Mt Shungol. Extraction of this bark (8·5 kg) afforded 1·2 g alkaloids.