ISOLATION OF (−)-HYOSCYAMINE FROM *ANTHOTROCHE PANNOSA* ENDL.*

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An earlier investigation[1] of *Anthotroche pannosa* Endl. and *A. blackii* F. Muell. resulted in the isolation of ursolic acid from the dried leaves and stems of both species, but surprisingly, alkaloids could not be detected in either of these solanaceous plants. Later, Webb[2] found that a 29-year-old herbarium specimen of a third member of the genus, *A. truncata*, also failed to give a test for alkaloids.

A. pannosa has now been re-examined and a low yield of basic material has been obtained from methanolic extracts of both fresh and shade-dried leaves and stems. Thin-layer chromatography of the crude base revealed the presence of at least seven alkaloids. The major component of this mixture has now been isolated by partition chromatography[3] and identified as (−)-hyoscyamine, an alkaloid which occurs frequently in other genera belonging to the Solanaceae.[4]

Experimental

Melting points are uncorrected. Thin-layer chromatography (t.l.c.) was carried out on Merck silica gel G using chloroform–diethylamine (9 : 1) as the mobile phase. Alkaloids were revealed by spraying the air-dried chromatogram with an iodoplatinate reagent.[5]

Examination of Shade-dried Anthotroche pannosa

Leaves and stems, collected from flowering plants growing near Wyola, W.A., in September 1962, were shade-dried. The milled material (2·4 kg) was extracted exhaustively with methanol at room temperature and the combined extracts evaporated under reduced pressure. From time to time the evaporation was interrupted in order to remove the crude ursolic acid (77 g) which had precipitated.

The viscous residue was then partitioned between ether (2 l.) and water (1·3 l.). The ethereal layer contained more ursolic acid (the insoluble sodium salt (13 g) was isolated) but only traces of alkaloids. The aqueous layer was basified with aq. NH_3 (d 0·88) and extracted with chloroform. The alkaloids were extracted with 1% HCl, the acid solution basified, and re-extracted with chloroform; the crude base was then obtained as a gum (0·7 g) which gave a positive Vitali reaction.[6] T.l.c. revealed the presence of at least seven alkaloids, R_F 0·22, 0·35,

* Manuscript received September 25, 1967.

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[1] Bottomley, W., and White, D. E., *Aust. J. scient. Res.* A, 1950, **3**, 516.

[2] Webb, L. J., CSIRO Aust. Bull. No. 268, p. 93, 1952.

[3] Evans, W. C., and Pe Than, M., *J. Pharm. Pharmac.*, 1962, **14**, 147.

[4] Willaman, J. J., and Schubert, B. G. Tech. Bull., U.S. Dep. Agric. No. 1234, 1961.

[5] Smith, I., "Chromatographic and Electrophoretic Techniques." 2nd Edn, Vol. 1, p. 396. (Heinemann: London 1960.)

[6] Carr, F. H., in "Allen's Commercial Organic Analysis." 5th Edn, Vol. 7, p. 823. (J. A. Churchill: London 1929.)

0·50, 0·65, 0·75, 0·86, and 0·92. The spot at R_F 0·50 was the most intense; authentic (−)-hyoscyamine and (−)-hyoscine had R_F 0·50 and 0·66, respectively, under these conditions.

Examination of fresh Anthotroche pannosa

Leaves and stems of *A. pannosa* were collected from the same locality in September 1963. The fresh plant material (32 kg) was chopped and allowed to stand in methanol for 4 days. The marc was dried in air overnight, then milled and extracted exhaustively with methanol at room temperature. The combined extracts were worked up as described above when the crude base was obtained as a reddish resin (2·44 g). T.l.c. revealed the same seven alkaloids to be present.

A solution of the crude base (1·77 g) in ether (90 ml) and chloroform (10 ml) was placed on a column of kieselguhr (30 g) supporting 5N H_2SO_4 (20 ml). Some coloured impurities (0·33 g) were eluted from the column with ether (1 l.), then the alkaloids were recovered with ammoniacal chloroform (750 ml). Evaporation of the solvent yielded a reddish resin (1·15 g), a portion (0·72 g) of which was redissolved in chloroform (3 ml), and the solution applied to a column of kieselguhr (40 g) loaded with 0·5M phosphate buffer, pH 6·2 (20 ml). The chromatogram was developed with light petroleum (b.p. 56–60°; 325 ml) and then ether (675 ml). The eluate was collected in 5-ml fractions; t.l.c. revealed that the light petroleum eluate and earlier fractions of the ether eluate contained mixtures, and that later fractions of the ether eluate contained the virtually pure, major alkaloid having the same R_F value as (−)-hysocyamine. The base (17 mg) obtained by evaporation of these particular fractions was converted into the picrate which crystallized from ethanol, as yellow needles, m.p. 165–166°, undepressed on admixture with an authentic sample of (−)-hyoscyamine picrate.

The identity of the picrates was confirmed by comparison of the infrared spectra (KBr) of the two samples.

Acknowledgments

The authors thank Mr T. E. H. Aplin for identifying the plant material investigated. The award of a CSIRO Junior Postgraduate Studentship (to J.B.B.) is gratefully acknowledged.