910. *The Alkaloids of* Datura meteloides *D.C.*

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6,7β-Epoxy-3α-atropoyloxytropane (apohyoscine), not previously recorded as a natural alkaloid, has been isolated from the aerial parts of *Datura meteloides* together with hyoscine, norhyoscine, hyoscyamine, meteloidine, and norhyoscyamine. The roots of this species contain (−)-3α,6β-ditigloyloxytropane, 3,6-ditigloyloxytropan-7-ol, hyoscine, norhyoscine, meteloidine, atropine, and probably norhyoscyamine, tropine, pseudotropine, and 3,6-dihydroxytropane.

Hyoscine,[1] meteloidine,[1] and norhyoscyamine [2] have been isolated from *Datura meteloides* D.C., and hyoscyamine and an uncharacterised base indicated [3] by paper chromatography. No systematic investigation of the root alkaloids appears to have been reported. The results of the investigation of the alkaloidal mixture obtained from a number of samples of aerial parts and roots of the authentic plant are recorded here.

An ether extract of the leaves in light petroleum (b. p. 40—80°) was submitted to chromatography at pH 5·6 and, after elution with more solvent, yielded a new natural base in about 0·01% yield. The alkaloid was identical with a small quantity of base isolated from *D. innoxia* leaves, and on investigation proved to be apohyoscine, previously synthesised from hyoscine by Willstätter and Hug.[4] On the evidence for the stereochemical structure

[1] F. L. Pyman and W. C. Reynolds, *J.*, 1908, **93**, 2077.
[2] F. H. Carr and W. C. Reynolds, *J.*, 1912, **101**, 946.
[3] G. Verzárné Petri and S. Sárkány, *Acta Pharm. Hung.*, 1961, **31**, 22.
[4] R. Willstätter and E. Hug, *Z. physiol. Chem.*, 1912, **79**, 146.

of hyoscine,[5] the alkaloid is 6,7β-epoxy-3α-atropoyloxytropane. The alkaloids remaining on the column were recovered in ammoniacal chloroform and refractionated at pH 6·5. By successive elution with ether, chloroform, and ammoniacal chloroform, hyoscine, norhyoscine, meteloidine, hyoscyamine, and norhyoscyamine were obtained.

The ether extracts of various root samples were dissolved in ether and passed through kieselguhr loaded with dilute acid. Impurities were washed from the column with ether, and the ditigloyl esters eluted with chloroform and refractionated again at pH 5·6. Those bases remaining on the column were collected in ammoniacal chloroform and resubmitted to chromatography at pH 6·8. Although there was some variation in the relative amounts of the different alkaloids in different samples, the overall alkaloidal pattern for all of them was similar. The following alkaloids were characterised, the figures denoting the percentage of alkaloid in the various dry roots: 3α,6β-ditigloyloxytropane (0·01—0·03), 3,6-ditigloyloxytropan-7-ol (0·02—0·16), hyoscine (0·01—0·1), norhyoscine (0·1), meteloidine (0·1), hyoscyamine (0·03—0·1), and tropine (0·01). By the use of thin-layer chromatography, bases with R_F values corresponding to those of 3α-tigloyloxytropane, norhyoscyamine, pseudotropine, and 3,6-dihydroxytropane were detected.

The range of alkaloids found in *D. meteloides* resembles that of some other *Datura* species.[6] We consider the apohyoscine to be a normal constituent of the plant, and not formed from hyoscine during the drying process, because it has occurred in all samples analysed but has never been detected in similarly dried samples of *D. ferox* or *D. cornigera*, two other hyoscine-containing species.

On the basis of paper chromatography [3,7] Verzárné Petri has suggested that an unknown alkaloid of the leaves of *D. meteloides* and *D. innoxia* is 3,6-ditigloyloxytropan-7-ol. Although the ditigloyl esters are transported in the transpiration stream from the roots to the leaves of *Datura* species,[8] in all the Nottingham-grown samples examined, they do not appear to accumulate there.[9]

Experimental

Plant Material.—All the material analysed was grown in Nottingham from seed samples received from Florida, U.S.A., the Blakeslee collection, and from Gatersleben, Germany. The flowering plants possessed the characteristics of *D. meteloides* Dun in D.C.[10]

The Alkaloids of the Aerial Parts.—Powdered herb (730 g.) was mixed with calcium hydroxide (80 g.), moistened with water (300 ml.), set aside for 2 hr., and then exhaustively extracted with ether. The green residue from the extract was dissolved as completely as possible in light petroleum (b. p. 40—60°), and transferred to a column of kieselguhr (80 g.) supporting 0·5M-phosphate buffer solution (60 ml.), pH 5·8. A green tar, insoluble in the light petroleum, was softened by warming, and shaken several times with warm solvent; the total transference of the alkaloids involved about 100 ml. of solvent. The pigmented light petroleum eluate (400 ml.) from the column was agitated with dilute Bromocresol Green solution (50 ml.), and the system neutralised with 0·1N-sulphuric acid. The aqueous solution was collected, rendered alkaline with ammonia solution, and shaken with chloroform (4 × 20 ml.). Removal of the chloroform afforded a gum (0·07 g.) which was dissolved in ethanol (2 ml.) and neutralised with 1·0N-sulphuric acid (0·3 ml.). Water (2 ml.) was added, and the suspension filtered after standing for 2 days at 3°. Treatment of the solution with sodium picrate solution furnished *apohyoscine picrate* (0·05 g.), needles from aqueous ethanol, m. p. 216° (decomp.) (Found: C, 53·8; H, 4·9. $C_{17}H_{19}NO_3,C_6H_3N_3O_7$ requires C, 53·7; H, 4·2%). Apohyoscine picrate prepared from hyoscine by the method of Willstätter and Hug [4] gave no m. p. depression with the picrate of the natural base and had an identical infrared (i.r.) spectrum. On thin-layer chromatography on alumina (ether as solvent) the two bases had the same R_F value, and both

[5] P. Dobó, G. Fodor, G. Janzsó, I. Koczor, J. Tóth, and I. Vincze, *J.*, 1959, **3461**.
[6] W. C. Evans and M. Wellendorf, *J.*, 1959, 1406; W. C. Evans and M. Pe Than, *J. Pharm. Pharmacol.*, 1962, **14**, 147; W. C. Evans and N. A. Stevenson, *ibid.*, p. 664.
[7] G. Verzárné Petri, *Gyógyszerészet*, 1961, **5**, 401.
[8] W. C. Evans and W. J. Griffin, *J. Pharm. Pharmacol.*, 1964, **16**, 337.
[9] W. C. Evans and W. J. Griffin, *Phytochemistry*, 1964, **3**, 503.
[10] S. Danert, *Pharmazie*, 1954, **11**, 349.

gave an insignificant Vitali–Morin reaction. The natural base, regenerated from the picrate and neutralised with dilute hydrochloric acid, furnished, with aurichloric acid, an aurichloride, m. p. 184—185°, undepressed on admixture with authentic apohyoscine aurichloride m. p. 186°. By hydrolysis [11] with barium hydroxide solution the alkaloid gave atropic acid, m. p. and i.r. spectrum consistent with authentic atropic acid. The alkamine, also produced by the hydrolysis, on treatment with tigloyl chloride afforded 3,6α-epoxy-7β-tigloyloxytropane (tigloyloscine), picrate as nodular masses, m. p. and mixed m. p. 185°. The i.r. spectra of the picrate and of the authentic picrate were identical.

The remaining bases were recovered from the original chromatographic column in ammoniacal chloroform and, after removal of the solvent, were transferred to a kieselguhr column (50 g.) supporting 0·5M-phosphate buffer solution (35 ml.), pH 6·5. Hyoscine (0·29 g.), picrate m. p. and mixed m. p. 187°, was eluted with ether. It was followed by *dl*-norhyoscine (0·10 g.), picrate as serrated needles from aqueous ethanol, m. p. and mixed m. p. 245° (Found: C, 50·9; H, 4·15. Calc. for $C_{16}H_{19}NO_4,C_6H_3N_3O_7$: C, 51·0; H, 4·3%). The i.r. spectra of the picrate and of the authentic picrate, and the R_F value (0·45) of the base on thin-layer alumina chromatograms (ether–ethanol; 1 : 1) further confirmed the identity of the alkaloid. Chloroform eluted meteloidine (0·10 g.), picrate m. p. and mixed m. p. 175°, and hyoscyamine (0·06 g.), picrate as needles from aqueous ethanol, m. p. and mixed m. p. 164—165° (Found: C, 53·3; H, 5·2. Calc. for $C_{17}H_{23}NO_3,C_6H_3N_3O_7$: C, 53·3; H, 5·0%). A mixture of bases removed from the chromatographic column with ammoniacal chloroform afforded norhyoscyamine (0·1 g.) as the main constituent. It was identified by comparison of its R_F value on thin-layer chromatography on alumina (chloroform–ethanol; 1 : 1) and of the m. p. and i.r. spectrum of the picrate with those of the authentic alkaloid.

An Alkaloid of Datura innoxia *Miller.*—During the routine analysis [6] of the aerial parts of *D. innoxia*, small quantities of an unknown base were detected in the light petroleum eluate. This base, isolated from the combined fractions of many analytical samples, was shown by its picrate (m. p., mixed m. p., and i.r. spectrum) and aurichloride (m. p. and mixed m. p.) to be identical with the apohyoscine isolated from *D. meteloides*.

3,6α-*Epoxy*-7β-*tigloyloxytropane Picrate.*—Oscine (0·15 g.) was treated with tigloyl chloride (0·15 g.), and the ester prepared according to the method of Barger *et al.*[12] for tigloyloxytropane. The viscous base (0·17 g., 74%), on neutralisation with dilute sulphuric acid and treatment with sodium picrate solution, furnished 3,6α-*epoxy*-7β-*tigloyloxytropane picrate* (tigloyloscine picrate), as nodular masses from aqueous ethanol, m. p. 186° (Found: C, 48·9; H, 5·0. $C_{13}H_{19}NO_3$, $C_6H_3N_3O_7$ requires C, 48·9; H, 4·7%).

The Alkaloids of the Roots.—In a typical extraction, the alkaloids of the finely powdered root (94 g.) were extracted in a manner similar to that used for the aerial parts. The evaporated ether extract was transferred, in ether, to a kieselguhr column (20 g.) loaded with 1·0N-sulphuric acid (10 ml.). Pigments and oily material were washed from the column in ether and the chloroform-soluble alkaloid sulphates collected in chloroform (1·5 l.). The chloroform solution was evaporated to 50 ml., shaken with 5N-ammonia solution (25 ml.), washed with water (2 × 10 ml.), and the solvent removed (Fraction A). Those bases remaining on the column were recovered in chloroform from the extruded kieselguhr made alkaline with 15N-ammonia solution (5 ml.); the chloroform was removed (Fraction B).

In the following fractionations all the identified alkaloids were checked against authentic compounds by thin-layer chromatography on alumina with ether, ether–ethanol (1 : 1) or chloroform–ethanol (1 : 1) as solvents, and a saturated solution of iodine in carbon tetrachloride as locating reagent. Fraction A in light petroleum (b. p. 40—60°) was submitted to chromatography on kieselguhr (20 g.) loaded with 0·5M-phosphate buffer solution, pH 5·6. The light petroleum eluate was collected in two fractions, the first of which furnished 3α,6β-ditigloyloxytropane (0·03 g.), picrate as long needles from aqueous ethanol, m. p. and mixed m. p. 149—150°, i.r. spectrum identical with that of an authentic specimen. The second fraction gave 3,6-ditigloyloxytropan-7-ol (0·07 g.), picrate m. p. and mixed m. p. 184° (Found: C, 51·3, 51·5; H, 5·3, 5·2. Calc. for $C_{18}H_{27}NO_5,C_6H_3N_3O_7$: C, 50·9; H, 5·3%). From the regenerated base, neutralised by dilute hydrochloric acid, the chloroplatinate, m. p. and mixed m. p. 253°, and reineckate, m. p. and mixed m. p. 193°, were prepared. Small quantities of unidentified bases were also present in the subsequent ether and chloroform eluates of the column. Fraction B,

[11] W. C. Evans and M. W. Partridge, *J.*, 1957, 1102.
[12] G. Barger, W. F. Martin, and W. Mitchell, *J.*, 1937, 1820.

in ether, was resolved on kieselguhr (20 g.) containing 0·5M-phosphate buffer solution (10 ml.), pH 6·8. Four bases were eluted with ether; (i) (*ca.* 0·002 g.) not identified; (ii) hyoscine (0·01 g.), picrate as needles from aqueous ethanol, m. p. and mixed m. p. 186° (Found: C, 51·7; H, 4·6. Calc. for $C_{17}H_{21}NO_4,C_6H_3N_3O_7$: C, 51·8; H, 4·5%); (iii) norhyoscine (0·01 g.), picrate as serrated needles, m. p. 234—236°, i.r. spectrum identical with that of the authentic picrate; (iv) material with the same R_F value on thin-layer chromatography as 3α-tigloyloxytropane; there was insufficient for complete examination. With chloroform, meteloidine (about 0·07 g.) was eluted, picrate as nodules of needles, m. p. and mixed m. p. 177° (Found: C, 46·7; H, 4·9. Calc. for $C_{13}H_{21}NO_4,C_6H_3N_3O_7$: C, 47·1; H, 5·0%). Incompletely separated from the meteloidine was atropine (*ca.* 0·032 g.), picrate m. p. and mixed m. p. 175° (Found: C, 53·3; H, 5·0%). Continued elution with chloroform afforded tropine, characterised as the picrate, m. p. 290°, and norhyoscyamine. Lack of material prevented the complete characterisation of the latter, and also of a number of bases which were extracted in small yield in ethanol from the ether-exhausted marc of the roots; these had R_F values on thin-layer chromatograms corresponding to pseudotropine and 3,6-dihydroxytropane. The identity of the alkamines was supported by converting them into the corresponding tigloyl esters, the R_F values of which on thin-layer chromatography corresponded to tigloyloxytropane, tigloidine, and 3,6-ditigloyloxytropane.

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