274. *The Alkaloids of the Roots of* Datura.

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3α-Tigloyloxytropane, hitherto not recorded as a natural product, has been obtained from the roots of *Datura ferox*. Tigloidine, atropine, tropine, *pseudo*tropine, and an uncharacterised base have been isolated from the roots of *D. innoxia*. The roots of both species contain 7-hydroxy-3 : 6-ditigloyloxytropane, (−)-3α : 6β-ditigloyloxytropane, hyoscine, and meteloidine.

The complex nature of the alkaloid mixture contained in the roots of *Datura* species was shown by application of partition chromatography to the separation of tropane alkaloids.[1] Since then paper chromatography has detected hyoscine, hyoscyamine, and cuscohygrine in the roots of *D. ferox* L.[2,3] and *D. innoxia* Miller and meteloidine [2] in the former. Other bases, the constitutions of which have not been fully elucidated, have been reported in both species.[4,5] 7-Hydroxy-3 : 6-ditigloyloxytropane [6] has been isolated from extracts of the roots of *D. ferox* and (−)-3α : 6β-ditigloyloxytropane [7] from those of *D. innoxia*; the results of the continued investigation of these, and similar extracts, are recorded here.

An ether extract of the roots of *D. ferox* was resolved into six fractions by partition chromatography, light petroleum (b. p. 60—80°), ether, and chloroform being used successively as eluants. Repeated chromatography of the first fraction afforded 3α : 6β-ditigloyloxytropane together with the 7-hydroxy-derivative. The ether eluate contained first hyoscine and then 3α-tigloyloxytropane, which was recovered from the titration liquors as the picrate identical with that of the ester prepared from tropine and tigloyl chloride according to the method of Barger, Martin, and Mitchell.[8] We were unable, however, to obtain 3α-tigloyloxytropane picrate with the m. p. ascribed to it by these workers although the properties of the hydrobromides were identical. The first fractions of the chloroform eluate contained meteloidine and the later ones two other bases which have not been characterised but which, by paper chromatography, appear to be alkamines. No evidence for the presence of hyoscyamine in these roots was obtained.

[1] Evans and Partridge, *Quart. J. Pharm.*, 1948, **21**, 126.
[2] Romeike, *Flora*, 1956, **143**, 67.
[3] Reinouts van Haga, *Nature*, 1954, **174**, 833.
[4] Steinegger and Gessler, *Pharm. Acta Helv.*, 1955, **30**, 279.
[5] Romeike and Zimmermann, *Naturwiss.*, 1958, **45**, 187.
[6] Evans and Partridge, *J.*, 1957, 1102.
[7] Evans and Wellendorf, *J.*, 1958, 1991.
[8] Barger, Martin, and Mitchell, *J.*, 1937, 1820.

From a slightly acid solution of the total alkaloids of *D. innoxia* roots in sulphuric acid, 7-hydroxy-3 : 6-ditigloyloxytropane, together with the small amount of 3α : 6β-ditigloyloxytropane already reported,[7] was collected in chloroform. To portions of the aqueous solutions, aliquot portions of alkali were added, and the fractionally-liberated bases successively removed in chloroform (Fractions A2—A12). Fraction A2 consisted almost entirely of 7-hydroxy-3 : 6-ditigloyloxytropane and fraction A3 afforded tigloidine (3β-tigloyloxytropane) together with hyoscine, meteloidine, atropine, and small quantities of a base previously reported in the leaves of *D. ferox*.[8] The optical rotation of the isolated hyoscine and the m. p. of its salts indicated a mixture of the (−)- and the (±)-form. Fractions A8 to A12 contained a mixture of tropine and *pseudo*tropine and these bases were separated for characterisation by the esterification of the mixture with tigloyl chloride followed by chromatographic fractionation of the mixed esters.

The proportions (%) of the principal alkaloids in samples of these roots are given in the Table.

Root	(I)	(II)	(III)	(IV)	(V)
D. innoxia (Pakistan sample)	0·02	0·11	0·19	0·15	0·02
D. innoxia (English sample)	0·08	0·19	0·22	0·25	0·06
D. innoxia (English, Texas seeds)	0·03	0·12	0·27	0·31	0·06
D. ferox (English, Australian seeds)	0·01	0·03	0·03	0·05 *	0·004

(I), 3α : 6β-Ditigloyloxytropane. (II), 7-Hydroxy-3 : 6-ditigloyloxytropane. (III), Hyoscine. (IV), Atropine and meteloidine (as meteloidine). (V), Tropine and *pseudo*tropine.

* Meteloidine only.

The two ditigloyl esters appear to be constant constituents of the roots of *Datura* species, both alkaloids having also been isolated from *D. stramonium*.[6,7] The occurrence of tigloidine, not previously reported in this genus, forms another phytochemical link with the genus *Duboisia*. Racemised hyoscine and hyoscyamine, together with alkamines of the tropane esters, appear to be chiefly confined to the roots of *Datura* spp.; with the aerial organs of the plants they are usually found only in poorly dried and stored material.

Experimental

The Alkaloids of the Roots of D. ferox.—The chromatographic column from which light petroleum (b. p. 60—80°) had removed 7-hydroxy-3 : 6-ditigloyloxytropane [6] was developed with ether and aliquot portions of the eluate were titrated with 0·02N-sulphuric acid; this gave three more bands. From the first, equivalent to 0·04 g. of hyoscine, crystalline products could not be obtained; the second afforded hyoscine (0·15 g.) [aurichloride, m. p. and mixed m. p. 209° (Found: C, 31·9; H, 3·7; Au, 30·9. Calc. for $C_{17}H_{21}O_4N,HAuCl_4$: C, 31·7; H, 3·4; Au, 30·6%)]. The third ether fraction was eluted as a wide homogeneous band and the aqueous titration liquors, made alkaline with ammonia solution, were shaken with chloroform; separation and removal of solvent left a gum (0·05 g.) which, neutralised with dilute sulphuric acid and treated with sodium picrate, gave *3α-tigloyloxytropane picrate* (from aqueous alcohol), m. p. 180° (Found: C, 50·7; H, 5·5; N, 12·5. $C_{13}H_{21}O_2N,C_6H_3O_7N_3$ requires C, 50·4; H, 5·3; N, 12·4%). Chloroform was then used to elute two more bands from the column. The base isolated from the first was transferred to a column of kieselguhr (40 g.) loaded with 0·5M-phosphate buffer (40 ml.; pH 7·2), and eluted with ether. The recovered base (0·52 g.), in neutral solution, with sodium picrate afforded meteloidine picrate, m. p. and mixed m. p. 178° (Found: C, 47·4; H, 5·0; N, 11·9. Calc. for $C_{13}H_{21}O_4N,C_6H_3O_7N_3$: C, 47·1; H, 4·9; N, 11·6%). Paper chromatography of the second chloroform fraction indicated two alkamines having approximately the same R_F value as tropine and giving a similar purple colour when chromatograms of it were sprayed with modified Dragendorff's reagent.

For the isolation of 3α : 6β-ditigloyloxytropane, the powdered root (225 g.) was moistened with water (60 ml.) and kept for 2 hr. Calcium hydroxide (20 g.) was mixed in and the powder exhaustively extracted with ether. The percolate was evaporated to dryness and the green residue transferred to kieselguhr (20 g.) loaded with 0·5M-phosphate buffer (12 ml.; pH 6·6). The first fraction eluted with light petroleum (b. p. 60—80°), which previous observations had indicated as homogeneous,[6] was resubmitted to partition chromatography on kieselguhr (20 g.)

and 0·5M-phosphate buffer (12·0 ml.; pH 6·0). Elution of the chromatogram with light petroleum afforded two fractions. The base (0·014 g.) recovered from the first was neutralised with dilute sulphuric acid and on treatment with sodium picrate furnished 3α : 6β-ditigloyloxytropane picrate, m. p. and mixed m. p. 150° (Found: C, 52·2; H, 5·7. Calc. for $C_{18}H_{27}O_4N,C_6H_3O_7N_3$: C, 52·4; H, 5·5%). The alkaloid (0·077 g.) from the second fraction was neutralised with alcoholic hydrobromic acid; addition of ether to the solution precipitated 7-hydroxy-3 : 6-ditigloyloxytropane hydrobromide, m. p. and mixed m. p. 215° (Found: C, 51·5; H, 6·6. Calc. for $C_{18}H_{27}O_5N,HBr$: C, 51·7; H, 6·7%).

The Alkaloids of the Roots of D. innoxia.—An extract of Pakistan root (32 kg.) containing an equivalent of 128 g. of hyoscyamine was obtained in a faintly acid [sulphuric acid] solution and repeatedly shaken with chloroform.[7] Eleven other chloroform solutions of alkaloids of increasing basicity were similarly obtained by the successive addition of aliquot portions (usually 30 ml.) of N-sodium hydroxide to a portion of the solution. Removal of the solvent gave fractions A1—A12. The interaction of sodium picrate and a neutral solution of fraction A1 (0·5 g.) afforded, after repeated recrystallisation from aqueous alcohol, a picrate as prisms, m. p. 173—173·5° (Found: C, 52·2; H, 5·5%). From the original mother liquor of this picrate, the isolation of 3α : 6β-ditigloyloxytropane picrate has already been recorded.[7] The infrared spectrum of the picrate, m. p. 173—173·5°, compared with that of 7-hydroxy-3 : 6-ditigloyloxytropane picrate showed reduced bands at 1695, 1557, 1107, 1020, and 745 cm.$^{-1}$ together with a partial disappearance of the doublet at 1073 and 1183 cm.$^{-1}$ and a corresponding tendency towards the single band of 3α : 6β-ditigloyloxytropane picrate at 1077 cm.$^{-1}$. The picrate (0·28 g.) in chloroform (40 ml.) was washed with successive quantities (40 ml.) of aqueous ammonia until the chloroform solution was colourless. Removal of the solvent left a gum, $[\alpha]_D^{20}$ −3·6° (*c* 7·9 in ethanol, *l* 5 cm.), which was transferred to kieselguhr (20 g.) loaded with 0·5M-phosphate buffer (12·4 ml.; pH 6·0). The petroleum eluate was collected in two fractions the first of which furnished 3α : 6β-ditigloyloxytropane (0·024 g.), $[\alpha]_D^{20}$ −18·7° (*c* 7·9 in ethanol, *l* 5 cm.), which in neutral solution with dilute sodium picrate afforded the picrate, m. p. and mixed m. p. 150—151° (Found: C, 52·4; H, 5·4%). The second fraction gave 7-hydroxy-3 : 6-ditigloyloxytropane [α = 0°; picrate, m. p. and mixed m. p. 180·5° (Found: C, 51·3; H, 5·4. Calc. for $C_{18}H_{27}O_5N,C_6H_3O_7N_3$: C, 50·9; H, 5·3; N, 9·9%)].

A neutral solution of fraction A2 in dilute hydrobromic acid deposited 7-hydroxy-3 : 6-ditigloyloxytropane hydrobromide, m. p. and mixed m. p. 214—215°, from which the picrate, m. p. and mixed m. p. 181° (Found: C, 50·9; H, 5·3; N, 9·9%), was prepared.

Paper chromatograms showed fraction A3 (4·5 g.) to contain a mixture of bases, principally of intermediate R_F values. The fraction was obtained in neutral solution with dilute sulphuric acid, and the alkaloids were fractionally liberated by the addition of ten aliquot parts (20 ml.) of 0·1N-sodium hydroxide and successively collected in chloroform. Removal of the solvent gave fractions B1—B10 of which B5 to B8 were further fractionated by chromatography on kieselguhr (20 g.) loaded with 0·5M-phosphate buffer (10 ml.; pH 6·6). The petroleum fraction, which paper chromatography indicated to be homogeneous, afforded a base (0·04 g.) that with dilute hydrobromic acid deposited tigloidine hydrobromide, m. p. and mixed m. p. 232·5° (Found: C, 51·3; H, 7·1. Calc. for $C_{13}H_{21}O_2N,HBr$: C, 51·3; H, 7·2%). From the hydrobromide, tigloidine picrate was prepared, m. p. and mixed m. p. 240° (Found: C, 50·7; H, 5·5%). Continued elution of the column with ether afforded two bases: hyoscine (1·2 g.), $[\alpha]_D^{20}$ −7·4° (*c* 3·8 in water, *l* 5 cm.), characterised as the hydrobromide, m. p. 182° (softening 172°) (Found: C, 52·8; H, 5·5. Calc. for $C_{17}H_{21}O_4N,HBr$: C, 53·1; H, 5·7%), and the picrate m. p. finally 192° (softened 178°). A minor component (0·015 g.) of the ether eluate afforded a picrate, prisms from aqueous alcohol, m. p. 230° undepressed on admixture with the picrate of the alkaloid of identical R_F value obtained from the leaves of *D. ferox*.[9] Finally chloroform was used to elute meteloidine (0·173 g.) from which the picrate, m. p. and mixed m. p. 176—177° (Found: C, 47·1; H, 4·9; N, 11·4%), was prepared. Paper chromatograms showed fractions B9 and B10 to contain bases with R_F values identical with those of meteloidine and atropine. The basic mixture (0·3 g.) in neutral solution (with dilute sulphuric acid) on treatment with sodium picrate gave atropine picrate (0·2 g.), m. p. and mixed m. p. 173—174° (Found: C, 53·4; H, 5·2. Calc. for $C_{17}H_{23}O_3N,C_6H_3O_7N_3$: C, 53·3; H, 5·0%).

Paper chromatograms of samples of fractions A8—A11, sprayed with modified Dragendorff's reagent, indicated the presence of two bases of low R_F value; one gave a purplish, and the

[9] Evans and Partridge, *J. Pharm. Pharmacol.*, 1949, **1**, 593.

other an orange stain with the reagent. A neutral solution of a portion (0·06 g.) of these fractions (with sulphuric acid) gave with sodium picrate, tropine picrate (0·07 g.), m. p. and mixed m. p. 274° (decomp.) (Found: C, 45·8; H, 5·3; N, 15·3. Calc. for $C_8H_{15}ON,C_6H_3O_7N_3$: C, 45·4; H, 4·9; N, 15·1%). Another portion (0·38 g.) and tigloyl chloride (0·4 g.) were kept at 105° for 3 hr.; the unchanged acid chloride was then decomposed by warming the mixture with water, and tiglic acid removed with ether. The esterified bases were collected in chloroform. Removal of the solvent gave a gum (0·41 g.) which was transferred in ether on to kieselguhr (20 g.) loaded with 0·5M-phosphate buffer (12·8 ml.; pH 6·0). Two alkaloids were obtained by elution of the chromatogram with ether. The weaker base (0·07 g.) in neutral solution yielded tigloidine picrate, m. p. and mixed m. p. 237° (Found: C, 50·3; H 5·2%). The stronger base was obtained as a gum (0·28 g.) which in neutral solution in alcoholic hydrobromic acid deposited, after concentration of the solution, 3α-tigloyloxytropane hydrobromide, m. p. and mixed m. p. 206·5° (Found: C, 51·1; H, 7·0%). The addition of sodium picrate to the aqueous hydrobromide afforded the picrate, m. p. and mixed m. p. 180° (Found: C, 50·4; H, 5·4; N, 12·5%).

3α-Tigloyloxytropane Hydrobromide and Picrate.—These compounds were prepared by the method of Barger *et al.*[8] from tropine (0·03 g.) obtained by hydrolysis of atropine. The ester was purified by chromatography, kieselguhr (15 g.) and 0·25M-phosphate buffer (10 ml.; pH, 6·0) being employed. The chromatogram was washed with ether, and then the tigloyloxytropane (0·05 g., 95%) removed in ether–chloroform (1 : 1 v/v). The properties of the hydrobromide were those ascribed to it by Barger *et al.* but the picrate, m. p. 180° (Found: C, 50·1; H, 5·3; N, 11·9%), prepared from it could not be obtained with m. p. 200°.[8]

Quantitative Determination of the Principal Alkaloids of Datura *Roots.*—The powdered roots (3 or 5 g.) were assayed by Evans and Partridge's method [10] except that carbon tetrachloride was replaced by light petroleum which was collected in aliquot parts (7·5 ml.) and each titrated to give an estimate of the 3α : 6β-ditigloyloxytropane and 7-hydroxy-3 : 6-ditigloyloxytropane content. The ether fraction corresponded to hyoscine, the chloroform eluate to the total meteloidine and atropine and, the final ammoniacal chloroform eluate contained the tropine–*pseudo*tropine mixture. The homogeneity of the fractions was checked by paper chromatography.

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[10] Evans and Partridge, *J. Pharm. Pharmacol.*, 1952, **4**, 769.