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Translocation of alkaloids in *Datura* species

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The ditigloyl esters of the hydroxytropanes, normally found in the roots of *Datura* spp. occur also in the transpiration stream of *D. innoxia* and *D. cornigera* although they do not accumulate in the aerial parts. An alkaloid previously isolated from the leaves or roots of a number of species, and now obtained from the transpiration stream is probably an optical isomer of norhyoscine. Hyoscine is the principal alkaloid of the sap of both the species investigated and noratropine and hyoscyamine are also present.

HYOSCINE is usually the principal alkaloid of the aerial parts of *Datura innoxia* and *D. cornigera*. In addition, hyoscyamine and meteloidine have been isolated from *D. innoxia* and, noratropine and 3-hydroxy-6-tigloyloxytropane from *D. cornigera*. The roots of both species contain 3,6-ditigloyloxytropane, 7-hydroxy-3,6-ditigloyloxytropane, hyoscine and hyoscyamine or atropine. Noratropine and dihydroxytropane have been obtained from *D. cornigera* roots and tigloidine, tropine and pseudotropine from *D. innoxia* roots (see Evans & Wellendorf, 1959; Evans & Than, 1962; Evans & Griffin, 1963). The roots of both species contain more hyoscyamine or atropine than hyoscine.

As a preliminary step in the study of the metabolism of the ditigloyl esters we have investigated the upward translocation of alkaloids in *D. innoxia* and *D. cornigera* by the analysis of the bleeding sap drawn from the stocks of decapitated plants and by grafts. In previous work on *D. innoxia*, Romeike (1953) has detected hyoscine in the bleeding sap and a hyoscine : hyoscyamine ratio of about 3 :1 in the leaves of *Cyphomandra betacea* (a non-alkaloid producing plant) grafted on to *D. innoxia* stocks.

Methods

ANALYSIS OF SAP

Datura innoxia. 50 flowering plants of *D. innoxia* growing in the field (August, 1963) were decapitated at soil level and the bleeding sap (3·7 kg) from the cut ends of the root stocks absorbed into weighed columns of cellulose (about 100 g). The lower ends of the columns were plugged with absorbent cotton wool and were pressed firmly onto the cut surfaces. After 48 hr the tubes were removed, reweighed and the sap displaced from the columns with acetone and evaporated to dryness under reduced pressure. The residues were treated with chloroform–ammonia† (3 ml), the solvent removed and then, the crude bases in ether, submitted to column chromatography [kieselguhr (15 g) with 0·5 M phosphate buffer solution, pH 6·4 (5 ml)]. Light petroleum (b.p. 40–60°), ether, chloroform and chloroform–ammonia were used successively as developing solvents and the eluted bases were titrated with 0·005 N sulphuric acid where

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† The lower layer produced by shaking chloroform (500 ml) with strong solution of ammonia B.P. (20 ml).

possible. Individual bases were characterised by thin-layer and paper chromatography and where feasible, by the preparation and infra-red examination of the picrates.

Another sample of bleeding sap (340 g) collected in September, 1962, was similarly analysed [kieselguhr (10 g) with 0·25 M phosphate buffer solution, pH 6·1 (3·2 ml)].

For comparison with the above, the dried, coarsely powdered leaves and stems of the 1963 plants were also analysed. A sample (50 g) was mixed with lime (5 g) and moistened with water (15 ml); after standing for 30 min, it was exhausted with ether. The solvent was removed and the residue transferred, in ether, to a chromatographic column [kieselguhr (15 g) with 0·5 M phosphate buffer solution, pH 6·8 (5·0 ml)]. Pigments were removed from the column with light petroleum (b.p. 40–60°) and the alkaloids were fractionally eluted by ether and chloroform.

D. cornigera. Two, four-year old trees of *D. cornigera* growing in a temperate greenhouse, were cut back to within a few feet of the ground and the bleeding sap (481 g) collected and treated as described above [kieselguhr (10 g) with 0·25 M phosphate buffer solution pH 6·1 (3·2 ml)].

ANALYSIS OF GRAFTED PLANTS

Tomato shoots were grafted on to *D. innoxia* stocks when both species were about 8 inches tall. The scions and roots were separately harvested at about 5 months and dried (15 g aerial parts; 3 g stocks). Chromatographic analysis was performed as for *D. cornigera.*

CHARACTERISATION OF NORHYOSCINE

One base, eluted from the chromatographic columns immediately after hyoscine, gave a picrate m.p. 230° and was obtained from the sap of both *D. innoxia* and *D. cornigera.* This base has previously been recorded as a constituent of the roots of *D. innoxia* (Evans & Wellendorf, 1959) and the aerial portions of *D. ferox*, Indian henbane (Evans & Partridge, 1949) and *D. cornigera* (Evans & Than, 1962). For its further study, material obtained from an investigation (Evans & Griffin, 1963) of *D. cornigera* was used. The details of the chemical examination are recorded below.

Results

CONSTITUENTS OF THE SAP

D. innoxia. From the titration liquids of the light petroleum eluate a colourless base (2·5 mg) was obtained which, neutralised with sulphuric acid (0·02 N) and treated with sodium picrate solution gave 3,6-ditigloyloxytropane picrate, needles from aqueous ethanol, m.p. and mixed m.p. with authentic material 151°. The infra-red spectrum and Rf value by paper chromatography were identical with those of the reference compound. The first portion of the ether eluate (25 ml) contained a base (2·2 mg) affording an alkaloid picrate, plates from aqueous ethanol, m.p. and mixed m.p. with authentic 7-hydroxy-3,6-ditigloyloxytropane picrate,

183°. Comparison of the infra-red spectrum of the picrate and Rf value of the base with those of the authentic compound further substantiated the identification. From the second portion of the ether eluate (150 ml) another base (95 mg) was obtained; it was shown to be hyoscine by the characters of the picrate, needles from aqueous ethanol, m.p. and mixed m.p. with authentic hyoscine picrate, 188°. Found: C, 51·6; H, 4·5. Calc. for $C_{17}H_{21}NO_4$, $C_6H_3N_3O_7$: C, 51·9; H, 4·5%. Two peaks were evident on the titration curve of the chloroform eluate. The first (6·8 ml) gave a base, picrate m.p. 230° on one recrystallisation and the further investigation of this is recorded below; the second peak corresponded to hyoscyamine (30 mg), picrate m.p. and mixed m.p. with authentic hyoscyamine picrate 163°. Found: C, 53·1; H, 4·9. Calc. for $C_{17}H_{23}NO_3$,$C_6H_3N_3O_7$: C, 53·3; H, 5·0%. The chloroform–ammonia eluate contained a mixture of bases which was resolved by chromatography on alumina (50 g). Ether: ethanol (75:25) eluted hyoscyamine (3 mg), characterised as the picrate and ethanol eluted noratropine (7 mg), picrate, needles from ethanol m.p. and mixed m.p. with authentic material, 227°. The isolated noratropine base and the reference compound had the same Rf values on thin-layer chromatograms (alumina with chloroform–ethanol 1:9 as developing solvent).

From the *D. innoxia* bleeding sap collected in 1962, hyoscine (4·5 mg) and hyoscyamine (5·4 mg) were isolated as the corresponding picrates; 3,6-ditigloyloxytropane (0·5 mg) was indicated by paper chromatography.

The partition chromatographic separation of the basic mixture derived from the decapitated shoots yielded hyoscine (145 mg) and hyoscyamine (56 mg); both were identified by the preparation of picrates and by thin-layer chromatography (alumina with chloroform–ethanol 1 : 1 as developing solvent). No alkaloids were detected in the coloured light petroleum eluate.

D. cornigera. The light petroleum eluate contained a base (1·8 mg) having the same Rf value as 3,6-ditigloyloxytropane (alumina with ether as developing solvent) and which, on neutralisation and treatment with sodium picrate solution, gave 3,6-ditigloyloxytropane picrate, m.p. 150° undepressed on admixture with the authentic compound. The regenerated base, in dilute hydrochloric acid, gave with chloroplatinic acid solution 3,6-ditigloyloxytropane chloroplatinate, feathery needles, m.p. 228° undepressed by admixture with authentic material, m.p. 230°. Two bases were evident in the ether fraction. The first (initial 35 ml eluate) was equivalent to 0·5 ml 0·005 N acid and was tentatively identified by thin-layer chromatography (alumina with ether as developing solvent) as 7-hydroxy-3,6-ditigloyloxytropane although a derivative could not be prepared. The remainder of the ether eluate contained hyoscine (10·5 mg), which yielded hyoscine picrate m.p. 188°, undepressed by admixture with authentic hyoscine picrate. The titration curve obtained from the chloroform eluate showed a sharp, small peak followed by a larger one. The base (1·5 mg) corresponding to the first peak, on neutralisation and treatment with aqueous sodium picrate gave a picrate, serrated needles from aqueous ethanol m.p. 232°, the characterisation of which is described

below. The oily base (5 mg) from the second peak was shown by thin-layer chromatography (alumina, developing solvent chloroform : ethanol 1 : 1) to consist largely of hyoscyamine with traces of noratropine. Noratropine (1·5 mg) was eluted from the column by chloroform–ammonia and on neutralisation and treatment with aqueous sodium picrate afforded noratropine picrate, long needles from aqueous ethanol m.p. 226°, undepressed by admixture with authentic noratropine picrate.

GRAFTS OF TOMATO ON *D. innoxia*

The light petroleum eluate from the chromatographic analysis of the scions was shaken three times with 0·02 N acid (5 ml each) and the basic material (0·7 mg) recovered from the acid solution in chloroform. Paper chromatography of the base showed it to have an Rf value similar to that of 3,6-ditigloyloxytropane but insufficient material precluded the preparation of a derivative. Hyoscine (10·0 mg), picrate needles from ethanol m.p. 188° was obtained from the ether eluate and hyoscyamine (5·0 mg), picrate prisms from aqueous ethanol m.p. 160°, from the main chloroform eluate. The initial portion of the chloroform eluate contained a small quantity of base (about 0·4 mg) having an Rf value on paper chromatography intermediate between that of hyoscyamine and 7-hydroxy-3,6-ditigloyloxytropane and similar to that of tigloyloxytropane. Insufficient material prevented the preparation of a derivative.

The *D. innoxia* stocks of the graft combination contained 3,6-ditigloyloxytropane (1·5 mg), picrate m.p. and mixed m.p. 150°; 7-hydroxy-3,6-ditigloyloxytropane (1·5 mg), picrate m.p. and mixed m.p. 182°; hyoscine (0·6 mg), picrate m.p. 187° and a hyoscyamine fraction (3·0 mg) from which it was not possible to prepare a crystalline derivative.

CHARACTERISATION OF NORHYOSCINE

Analysis of the picrate m.p. 232° gave: C, 51·1, 50·7; H, 4·2, 4·2; N, 11·4, 10·6. Calc. for $C_{16}H_{19}NO_4$, $C_6H_3N_3O_7$: C, 51·0; H, 4·3; N, 10·8%; no *N*-methyl groups were present and the base gave a positive Vitali-Morin reaction. By many recrystallisations of the picrate it was possible to raise its m.p. to 238° and admixture with ±-norhyoscine picrate m.p. 245° resulted in no depression of the m.p. below 238°. The infra-red spectra of the two compounds were virtually identical. The base (25 mg), regenerated from the picrate, in methanol (0·5 ml) was treated with methyl iodide (0·02 ml). After standing at room temperature for 2 hr, the mixture was gently warmed to remove the solvent. Thin-layer chromatography (alumina with ether : ethanol 1 : 1 as developing solvent) of the residue indicated it to contain a mixture of unchanged alkaloid, hyoscine and hyoscine methiodide. The mixture was transferred to alumina (30 g) and with ether–ethanol (90 : 10) as eluant, a base (5 mg) was obtained which afforded a picrate, short needles from aqueous ethanol, m.p. 175–179°, undepressed by admixture with authentic (−)-hyoscine picrate. The infra-red spectra of the two compounds showed them to be the same. Unchanged alkaloid (9·0 mg), picrate m.p. 237°

was eluted by ether–ethanol (75 : 25). Insufficient base was obtained for the measurement of its optical rotation.

Discussion

The occurrence of 3,6-ditigloyloxytropane and 7-hydroxy-3,6-ditigloyloxytropane in the rising sap and in the roots of *D. innoxia* and *D. cornigera* suggests that further metabolism of these alkaloids takes place in the aerial parts of the plant since they do not accumulate in the leaves. This is further supported by the observation that 3,6-ditigloyloxytropane also appears in tomato scions grafted on to *D. innoxia* stocks. The hyoscine : hyoscyamine ratios found in the aerial portions of grafted plants and in the bleeding saps, more closely resemble those found in the normal leaves than in the roots of these species. This is in general agreement with previous studies on *D. innoxia* (Romeike 1953), although hyoscyamine was not reported as a component of the sap.

The alkaloid, picrate m.p. 230–232°, obtained from a number of *Datura* spp. appears to be norhyoscine. Norhyoscine was discovered as the ±-form in mother liquors remaining from the manufacture of hyoscine by Gmelin (1941). The infra-red spectra of the picrates of authentic material and the isolated alkaloid and of hyoscine and the *N*-methyl derivative afford good evidence for identification. The low m.p. of the picrate compared with ±-norhyoscine picrate (245°) and its gradual rise on recrystallisation without change in composition suggest that racemisation of the optically active salt may be taking place as also occurs with some samples of hyoscyamine and norhyoscyamine. In accordance with this is the somewhat low m.p. of the chemically pure *N*-methyl derivative (hyoscine). A larger quantity of the alkaloid is now required for an optical rotation measurement.

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