STUDIES ON *DATURA LEICHHARDTII* MUELL. EX BENTH.

PART II. ALKALOIDAL CONSTITUENTS

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The variation in the proportion of hyoscyamine and hyoscine in the aerial parts of *Datura leichhardtii* has been investigated. From the roots, the alkaloids 7-hydroxy-3,6-ditigloyloxytropane, 3α-tigloyloxytropane and meteloidine have been isolated.

BRIEF reports are available on the poisonous nature of *Datura leichhardtii* Muell. ex Benth. towards sheep and other animals (Hurst, 1942; Gardner and Bennetts, 1953). The presence of alkaloids in the plant (Sievers, 1921) has been noted, but no investigation into the composition of the alkaloidal mixture has been made. An investigation on this point, together with an ontogenetical study of alkaloid production, was therefore undertaken.

EXPERIMENTAL

The source of plant material is as indicated in Part I (Evans and Stevenson, 1962).

Plants were raised from seed in a temperate greenhouse, and to prevent early ageing were exflorated until a height of about 45 cm. was attained. Flower buds were then allowed to develop until the corollas protruded through the calyces, when the aerial parts and roots were separately harvested and dried at 65°.

Alkaloids of the Aerial Parts

The powdered, dried leaves and stems gave a positive Vitali reaction and when analysed for tropic acid esters by the method of Colby and Beal (1952) gave an alkaloidal content of approximately 0·1 per cent. The powder (200 g.) was moistened with water (120 ml.), allowed to stand overnight and then triturated with calcium hydroxide (20 g.). The mixture was shaken (1 hr.) with solvent ether (1·5 litres), the supernatant liquid decanted and the marc further percolated with ether (2·5 litres). Ether extracts were concentrated and passed through a column of purified kieselguhr (30 g.) loaded with N sulphuric acid (20 ml.). Pigments were removed by elution with ether and the alkaloids were recovered with chloroform/ammonia.†

The basic residue thus obtained was chromatographed on kieselguhr (30 g.) loaded with phosphate buffer (pH 6) (20 ml.) as described by Evans and Partridge (1952). Ether (500 ml.) yielded (−)-hyoscine

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† The lower layer produced by shaking chloroform (500 ml.) with Strong Solution of Ammonia B.P. (20 ml.).

(98 mg.), picrate m.p. 187° giving no depression on admixture with an authentic specimen. Chloroform (28 ml.) gave a mixture of bases (57 mg.) which could not further be separated: the picrate had m.p. 158–160° after extensive recrystallisation. Chloroform (a further 175 ml.) yielded hyoscyamine (144 mg.), picrate m.p. 164–165° giving no depression with an authentic sample. Finally, treatment of the column with chloroform/ammonia yielded an unidentified base (11 mg.) whose picrate had m.p. 228° after extensive recrystallisation.

Paper chromatography of extracts from other samples of *D. leichhardtii* showed that hyoscyamine and hyoscine were the principal alkaloids. Quantitative analyses (Evans and Partridge, 1952; Evans and Than, 1962) are shown in Table I.

TABLE I

ALKALOIDS OF AERIAL PARTS OF *DATURA LEICHHARDTII* MUELL. EX BENTH.

Sample*	Total alkaloids in dried sample per cent (as hyoscyamine)	Hyoscine as per cent total alkaloid where applicable
Seedlings, 2–3 days after cotyledons appeared	—	Principal alkaloid had R_F value = hyoscyamine; some hyoscine and traces of two bases of low R_F value.
Seedings, 1 week after emergence of cotyledons	—	Hyoscine and hyoscyamine spots of about equal magnitude. Traces of other bases of lower R_F value.
Seedlings, 2 weeks old‡	0·13†	55
Seedings, 4 weeks old	0·10	53
Young plants, 6 weeks old	0·16	47
Plants, 8 weeks old	0·09	46
Plants, 10 weeks old, average height 30 cm., 1 capsule	0·14	29
Plants, 12 weeks old, average height 35 cm., 2–3 young capsules	0·13	31
Plants, 14 weeks old, average height 35 cm., 2–3 mature capsules	0·08	29
Plants, analysed at end of season after removal of ripe fruits	0·02	7
Plants with young capsules. Raised in 1959	0·04	20
Dried, Australian grown sample with fruits, 1952	0·03	20
Ripe seeds	0·16	28

* Raised in 1960, except where indicated.
† Method of Evans and Partridge (1952); Evans and Than (1962).
‡ Composite sample from different batches.

Ontogenetic Production of Alkaloids

Seedlings and plants of varying ages were examined for alkaloidal content at regular intervals. Analyses are recorded in Table I. The separated alkaloids of the 4, 6 and 8 week-old plants were combined and identified by the preparation of their picrates and measurement of their R_F values by paper chromatography. The alkaloids were similarly identified in each subsequent sample.

Alkaloids of the Roots

Roots (100 g.) were extracted as described above for the aerial parts and then transferred with light petroleum to a column of purified kieselguhr (25 g.) loaded with 0·5M phosphate buffer solution (15 ml.), pH 6·6.

Light petroleum (b.p. 40–60°) (25 ml.) afforded an oil (33 mg.) which, after chromatography a second time gave 7-hydroxy-3,6-ditigloyloxytropane, picrate m.p. 177° undepressed on admixture with an authentic specimen. Ether (148 ml.) yielded a material (5·5 mg.) with an R_F value equivalent to that of tigloidine; the picrate m.p. 210° raised to 224–230° (decomp.) after four recrystallisations had m.p. 238° when admixed with a sample of tigloidine picrate, m.p. 240°. Ether (288 ml.) gave 3α-tigloxytropane (13 mg.), picrate m.p. 178–179° giving no depression on admixture with an authentic sample. Chloroform (38 ml.) yielded meteloidine (64 mg.), picrate m.p. 178–179° undepressed on admixture with an authentic sample. Chloroform (a further 185 ml.) gave unidentified bases (11 mg.).

Final elution of the column with chloroform/ammonia gave a small quantity of basic material with a low R_F value.

Discussion

The composition of the alkaloids from the aerial shoots of *D. leichhardtii* is similar to that in *D. stramonium*. In the flowering and young fruiting plants, hyoscyamine and hyoscine, the principal alkaloids, occur

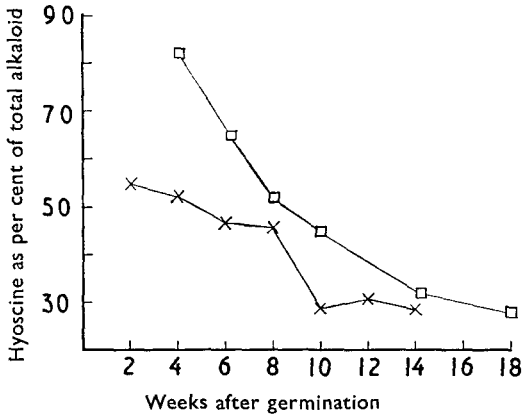

FIG. 2. Variation of the proportion of hyoscine in the total alkaloids of *Datura* spp. with age of plants. ×——×, *D. leichhardtii*; □——□, *D. stramonium* var. *godronii* (from Evans and Partridge, 1953).

in the ratio of about 2:1, whereas in most *Datura* species examined to date, hyoscine is the main alkaloid of the aerial parts. In old plants the ratio of hyoscyamine to hyoscine may rise to 9:1. This increase during development (Table I) is similar to that observed in *D. stramonium* (Hegnauer, 1951; Evans and Partridge, 1953) (see Fig. 1) and also agrees with the conclusions reached for *D. stramonium* on the basis of isotopic feeding experiments (Romeike and Fodor, 1960 and references there cited). Surprisingly, however, we have been able to find neither hyoscyamine nor hyoscine in the roots of mature *D. leichhardtii* plants. Instead the principal alkaloids here are 7-hydroxy-3,6-ditigloyloxytropane, 3α-tigloyloxytropane, meteloidine and possibly tigloidine, although the presence of

tropyl esters in small quantity cannot be excluded. Further experiments are planned to extend our observations on root-alkaloids to all ages of the plant and over a range of environmental conditions.

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DISCUSSION

The paper was presented by MR. STEVENSON. The following points were made in the discussion.

The total alkaloid content was relatively low in this species, which therefore did not have commercial value. Up to 0·04 per cent of tropyl esters had been found in the roots. The third base from the aerial parts gave a picrate of m.p. 228° and was thought to be noratropine. Many factors were involved in the chromatography of the bases and the conditions had to be adjusted empirically. The first hybrids produced a high yield of alkaloids. Diurnal variation was not studied: the plants were collected in the morning.