Occurrence of Nicotine together with Hyoscine in *Duboisia myoporoides* R. Br.

THE genus *Duboisia* comprises three species: *D. hopwoodii* F. Muell. is confined to the interior of the Australian continent, *D. leichhardtii* F. Muell. has a restricted occurrence in the highlands of south-eastern Queensland, and *D. myoporoides* R. Br. occurs along the eastern coast of Australia and in New Caledonia. The leaves of all three species contain major quantities of alkaloids; but the genus is unique in that one species, *D. hopwoodii*, contains the pyridine alkaloids nicotine and *nor*-nicotine, whereas the other two contain various alkaloids of the tropane group. Efforts to detect tropane alkaloids among the minor alkaloids of *D. hopwoodii* have failed. Similarly, although the other two Australian species contain volatile bases, the occurrence of nicotine or *nor*-nicotine has not been demonstrated.

A sample of seed of *D. myoporoides* from New Caledonia was recently obtained for us by the South Pacific Commission. Seedlings were established at Canberra both in the field and in a heated glasshouse. Although the plants in the field were stunted, those in the glasshouse grew well and were very similar to adjacent plants of *D. myoporoides* grown from Australian seed.

Alkaloid assays were carried out by a modification of the partition chromatographic method described by Loftus Hills and Rodwell[1]. Working with *D. hopwoodii*, it had been found that nicotine and *nor*-nicotine are well separated by the procedure in routine use for separation of tropane bases. In all chromatograms of material of New Caledonian origin, peaks were obtained in the positions for nicotine, hyoscine and *nor*-nicotine. The identity of hyoscine in each chromatogram was established by preparation of the picrate or reineckate, the melting points of which were not depressed on admixture with the compound prepared from authentic hyoscine. It gave a positive Vitali–Morin reaction, and when run on a paper chromatogram, using butanol – acetic acid – water to form the phase pair, a single spot was obtained with the same R_F value as that obtained with hyoscine. To identify nicotine, the dipicrate was prepared and checked by mixed melting point with nicotine dipicrate prepared from tobacco. The *nor*-nicotine was similarly identified, using *nor*-nicotine from *D. hopwoodii* for comparison. Also, for both these fractions, paper chromatograms showed single spots in the expected positions.

The partition chromatograms also showed small amounts of other bases. Material eluted in the position where hyoscyamine would be expected gave a positive Vitali reaction, and a paper chromatogram showed a spot with the same R_F value as hyoscyamine. The leaf samples contained approximately 0·1 per cent of this material.

The method of assay, although giving reliable quantitative data for hyoscine, only gives an indication of the amounts of nicotine and *nor*-nicotine present, as losses on drying are uncertain[2]. Of the seven samples of *D. myoporoides* from New Caledonian seed examined, the first was a composite bulk from eight field trees, and the remainder were from individual plants in the glasshouse. The amounts of hyoscine, nicotine and *nor*-nicotine, expressed as a percentage of the dry weight of the leaf, are shown in the accompanying table.

D. myoporoides (*ex* NEW CALEDONIAN SEED)

Origin of sample	As percentage of dry weight of leaf		
	Nicotine	*nor*-Nicotine	Hyoscine
Field, Canberra A.C.T.	0·9	0·2	0·4
Single plants, glass-house, Canberra, A.C.T.	0·7	0·2	0·25
	0·6	0·3	0·4
	0·9	0·2	0·4
	0·5	0·25	0·35
	0·7	0·3	0·5
	0·7	0·2	0·55

This appears to be the first occasion upon which alkaloids of the tropane and pyridine groups have been observed to occur in the same plant, or even in the same species. In the genus *Duboisia* in Australia, these two phytosynthetic mechanisms are sharply isolated by the species boundaries; but they exist together in this New Caledonian form, the origin of which is a matter of conjecture.

The geographical remoteness of New Caledonia from the area in which *D. hopwoodii* occurs and the morphological similarity of this material with *D. myoporoides* do not support a theory of hybrid origin. Possibly internal changes involving the pattern or nature of the genes were responsible. On the other hand, the New Caledonian material may be a relict of a primitive form from which the genotypes of the Australian mainland originated.

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[1] Loftus Hills, K., and Rodwell, C. N., *Aust. J. Sci. Res.*, B, **4**, 486 (1951).
[2] Bottomley, W., and White, D. E., *Aust. J. Sci. Res.*, A, **4**, 107 (1951).

Uptake of Dyes into Cut Leaves

THE limitation of petiolar uptake of solutes by detached leaves shown in the following experiments does not seem to have been previously recorded.

Leaves of *Atropa belladonna* L. and of cherry laurel (*Prunus laurocerasus* L.) were placed with their petioles in dilute solutions of the basic dyes methylene blue and safranin, and of the acid dye acid fuchsin. In two to three hours approximately 0·25 ml. of the acid fuchsin had been taken up and could be seen by the naked eye, and by the microscope, to have reached the finest ramifications of the leaf vascular system.

Uptake of the basic dyes was very much slower; but the safranin experiment was prolonged until approximately 0·5 ml. had been taken in. No colouring of the leaf was noticeable, and lengthwise cutting showed that all the dye had been retained by the first half-inch of the petiole; methylene blue, which is not so basic, had stained approximately the first inch. Such an immovable accumulation of dye molecules in the xylem vessel lumen would naturally impede fluid intake, and the leaves did, in fact, wilt.

The experiment was repeated for cherry laurel leaves using penicillin and streptomycin, which are respectively acidic and basic antibiotics. The concentration in both cases was 1,000 units/ml., and the solutions were at *p*H 5. Uptake of penicillin solution was rapid, but of streptomycin solution very slow. At varying times disks were removed by means of a cork-borer from parts of the leaf distal and proximal