787. *The Occurrence of Nicotine, Anabasine, and* iso*Pelletierine in* Duboisia myoporoides.

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Nicotine, anabasine, and *iso*pelletierine have been isolated from *Duboisia myoporoides*.

Two species of the genus *Duboisia* (family Solanaceae), namely, *D. myoporoides* R.Br. and *D. Leichhardtii* F. Muell, are known to contain tropane alkaloids, principally hyoscine and hyoscyamine, and *D. Hopwoodii* (F. Muell) F. Muell contains nicotine and nornicotine.[1] Recently, alkaloids of both groups have been found in individual plants of *D. myoporoides* of New Caledonian origin.[2]

In 1954, *D. myoporoides*, growing on the Acacia Plateau, near Killarney, South Queensland, Australia, was for the first time used as a commercial source of leaf for export. In late 1954 the exporters informed one of us (P. I. M.) that the leaf was reported to contain nicotine and investigation was begun in Canberra. In the meantime a consignment which had been sent to England was examined at the Wellcome Research Laboratories, Beckenham, in 1955 and although it was found to contain a high percentage of total alkaloids, it had comparatively little hyoscine, and hyoscyamine could not be detected. The work carried out independently at Canberra and Beckenham indicated that the leaf contained the alkaloids hyoscine, (−)-nicotine, anabasine, and (±)-*iso*pelletierine. The occurrence of *iso*pelletierine is remarkable since its only other natural source is the pomegranate, *Punica granatum* L.

Two independent procedures were adopted for isolation of the alkaloids and are described in the Experimental section.

The samples examined at Canberra and Beckenham were not identical and showed considerable variation in their alkaloid contents, *viz.*:

	Nicotine (%)	Anabasine (%)	*iso*Pelletierine (%)	Hyoscine (%)
Beckenham	0·3	1·4	0·25	0·08
Canberra	0·06	0·39	0·09	0·09

Because of its unusual occurrence the identification of the (±)-*iso*pelletierine was pursued to considerable length and confirmed by comparison of a number of its derivatives with those of a synthetic sample of (±)-2-piperidylacetone kindly provided by Professor J. P. Wibaut.

Experimental

Extractions.—Method 1. Ground leaf (3 kg.; 60 mesh) was intimately mixed with sodium hydrogen carbonate (450 g.) and water (1·8 l.) and percolated with trichloroethylene. The percolate (20 l.) was extracted with 2% sulphuric acid (2 l., 1 l., 1 l.). The acid extracts were washed with ether (1 l.), then strongly basified with 20% aqueous sodium hydroxide, saturated

[1] Henry, " The Plant Alkaloids," 4th Edn., Churchill, London, 1949.
[2] Hills, Bottomley, and Mortimer, *Nature*, 1953, **171**, 435.

with sodium chloride, and extracted with ether (3 × 1·5 l.). The ethereal solution was dried (Na_2SO_4) and concentrated under slightly reduced pressure to give the total base (bases 1) (65 g., 2·16%).

Method 2. Ground leaf (48·2 kg.) was extracted with ethanol containing 1% v/v of sulphuric acid until little further material was extracted. The alcohol was removed under reduced pressure and the residue taken up in water and filtered. The filtrate (7 l.) was washed with chloroform (1 l.), made strongly alkaline with sodium hydroxide, and then extracted with chloroform (1 l., 0·5 l., 0·5 l.). The bases were recovered from the chloroform extracts by 5N-sulphuric acid (900 ml.) (bases 2).

Bases 1 and 2 were found by paper chromatography, on Whatman No. 1 paper with butan-1-ol–concentrated hydrochloric acid (9 : 1) saturated with water [3] to contain nicotine, anabasine, hyoscine, and an unidentified base (R_F 0·5).

Fractionation of Bases.—Bases 1 were distilled through a 20 cm. Fenske column to give: (*A*) b. p. 65—70°/3 mm., base R_F 0·5 + a little nicotine; (*B*) b. p. 70—90°/3 mm., trace of base R_F 0·5 + nicotine + a little anabasine; (*C*) b. p. 90—105°/3 mm., anabasine + a little nicotine; and (*D*) residue, containing hyoscine.

Fractions (*A*) and (*B*) were combined and redistilled in nitrogen to give: (*AA*) b. p. 45—50°/0·15 mm., n_D^{20} 1·4771 (7·5 g.), base R_F 0·5, and (*AB*) b. p. 56—72°/0·15 mm., n_D^{20} 1·5162 (8·0 g.), nicotine + trace of anabasine.

Fraction *C*, redistilled, gave a small forerun and a main fraction, b. p. 94—98°/1·5 mm. (47·6 g.), $[\alpha]_D^{22}$ −5·87° (*c* 6 in EtOH), $[\alpha]_{5461}^{21}$ +1·55° (*c* 7·5 in N-HCl), consisting of anabasine and a trace of nicotine. Fraction (*AA*) was converted into the picrate and recrystallised from ethanol and aqueous ethanol, to give yellow prisms, m. p. 149—150°, identified as (±)-*iso*-pelletierine picrate by mixed m. p. with a specimen, m. p. 149°. (−)-Nicotine, $[\alpha]_{5461}^{22}$ −198·4° (no solvent), was isolated from fraction *AB* by steam-distillation and identified by conversion into the dihydrochloride.

The oil (47·6 g.) from redistillation of fraction *C* was dissolved in light petroleum (100 ml.; b. p. 60—80°) and cooled to −20°, colourless crystals separating which were filtered off on a Buchner funnel fitted with a freezing jacket and washed with cold light petroleum. The crystals were redistilled (b. p. 96°/12 mm.), to give (±)-anabasine (25 g.), n_D^{20} 1·5440, $[\alpha]_D^{21}$ −0·44° (no solvent), $[\alpha]_{5461}^{21}$ −0·22° (*c* 18 in H_2O), m. p. (from f. p. curve) 9·5°. The light petroleum filtrate was concentrated and re-cooled, to give a further crop of anabasine (10 g.), $[\alpha]_{5461}^{20}$ −2·08° (*c* 1·0 in H_2O). The filtrate and washings were concentrated *in vacuo*, giving an oil (10 g.), $[\alpha]_{5461}^{20}$ −32·44° (*c* 3 in H_2O). Steam-distillation gave, in the distillate, (−)-nicotine (1 g.) and, in the residue, anabasine (8 g.), $[\alpha]_{5461}^{20}$ −13·53° (*c* 2·5 in H_2O).

Hyoscine was isolated in 0·082% yield from a further batch of bases 1 by distribution between chloroform and 0·1M-citric acid–sodium phosphate buffer (pH 5·0) in a countercurrent separator after 50 transfers (*K* 0·5). It was identified by mixed m. p. of its picrate (m. p. 187°) with that of authentic hyoscine picrate.

Fractionation of bases 2. Preliminary partition chromatographic analysis [4] showed the presence of four bases. The individual bases were separated by distribution between phosphate buffers and organic solvents according to the scheme on p. 3969. A distribution system of five vessels was used in the diamond-shaped arrangement of Bush and Densen.[5]

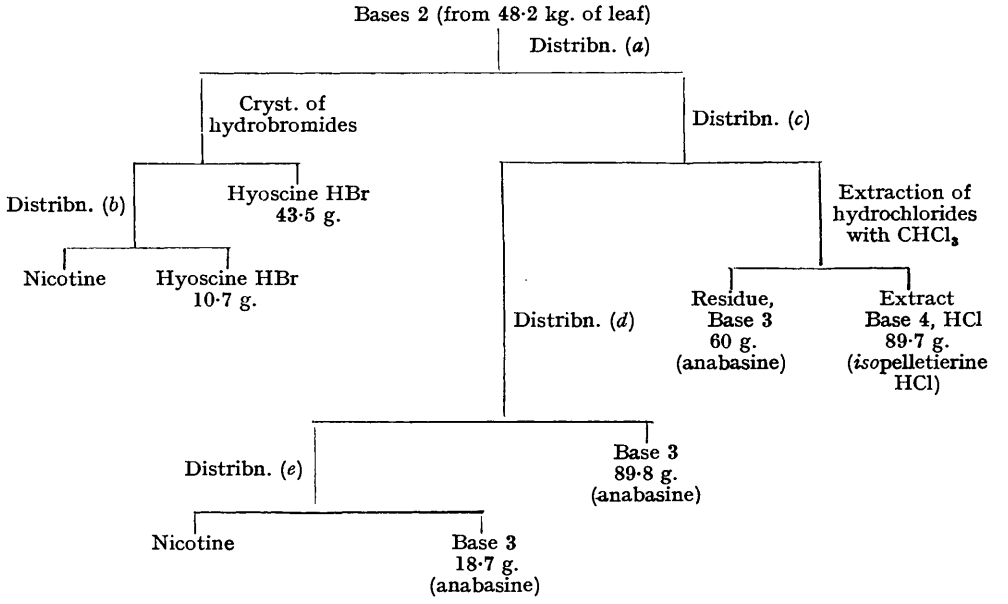
" Distribution " refers to separation using potassium phosphate buffers (*d* 1·15) and an organic phase (see below) to give organic-phase material (left) and buffer-phase material (right):

(*a*) buffer pH 6·0; $CHCl_3$ (*d*) buffer pH 7·8; light petroleum–ether (1 : 1)
(*b*) ,, 7·9; light petroleum–ether (3 : 1) (*e*) ,, 6·6; $CHCl_3$
(*c*) ,, 7·0; $CHCl_3$

The portion from distribution (*c*) containing base 4 was dissolved in a 40% excess of concentrated hydrochloric acid and continuously extracted for 80 hr. with chloroform. The extracted material crystallised on concentration and cooling (89·7 g. in three crops) and recrystallised from acetone containing a little ethanol, to give (±)-*iso*pelletierine hydrochloride, m. p. 142—143°. The nicotine and base 3 (anabasine) fractions when analysed by partition chromatography were each found to contain less than 1% of other bases.

[3] Munier and Macheboeuf, *Bull. Soc. Chim. biol.*, 1951, **33**, 846.
[4] Bottomley and Mortimer, *Austral. J. Appl. Sci.*, 1954, **5**, 255.
[5] Bush and Densen, *Analyt. Chem.*, 1948, **20**, 121.

The anabasine fractions were distilled separately, to give the amounts shown in the diagram, finally combined and distilled (b. p. 121°/3·5 mm.) to give anabasine, $[\alpha]_D^{19}$ −9·06° (no solvent), n_D^{19} 1·5435, $\lambda_{max.}$ in 95% EtOH containing HCl [6] 2600 Å (ε 5010), $\lambda_{min.}$ 2330 Å (ε 860).



The nicotine fractions were combined and distilled as a colourless oil (27·2 g.), b. p. 109°/7·8 mm., $[\alpha]_D^{25}$ −163·2° (no solvent).

Hyoscine hydrobromide fractions were recrystallised from 70% ethanol to give colourless crystals, m. p. 193°, undepressed on admixture with authentic hyoscine hydrobromide, and having $[\alpha]_D^{25}$ −21·4° (*c* 5·0 in H_2O, for anhydrous salt). The picrate recrystallised from ethanol had m. p. 187°, not depressed on admixture with authentic hyoscine picrate.

The following derivatives were prepared during the course of the identification of nicotine, anabasine, and *iso*pelletierine.

(−)-*Nicotine*: $[\alpha]_D^{22}$ +15·69° (1·0199 g. in 12·5 ml. of 1·0076N-HCl diluted to 25 ml. with water).

Dihydrochloride, m. p. 160—162° (from acetone–ethanol), $[\alpha]_D^{22}$ +10·94° (*c* 1·1 in H_2O) (Found: C, 50·7; H, 6·9; Cl, 30·3. Calc. for $C_{10}H_{16}N_2Cl_2$: C, 51·0; H, 6·85; Cl, 30·2%).

The rotation of the (−)-nicotine dihydrochloride was at variance with the figure $[\alpha]_D$ +102·2° quoted for (−)-nicotine monohydrochloride.[7,8] A number of rotation measurements were therefore carried out on an authentic sample of (−)-nicotine, *viz.*:

Nicotine (g.)	Solvent	$[\alpha]_D^{22}$
1·3284	25 ml. conc. HCl	+15·92°
1·4192	25 ml. 1·0076N-HCl	+16·75°
1·3453	8·14 ml. 1·0076N-HCl to 25 ml. with water	+13·74°
1·9936	16·49 ml. 1·0076N-HCl to 25 ml. with water	+16·54°
1·1161	6·84 ml. 1·0076N-HCl to 10 ml. with water	+19·36°

Dipicrate, m. p. 218—220° (prisms from ethanol) (Found: C, 43·0; H, 3·1; N, 17·7; picric acid spectrophotometrically, 74·1. Calc. for $C_{10}H_{14}N_2,2C_6H_3O_7N_3$: C, 42·6; H, 3·25; N, 18·1; picric acid, 73·8%).

(±)-iso*Pelletierine*: The base was an oil, b. p. 75°/3 mm., which became brown within an hour.

[6] Swain, Eisner, Woodward, and Brice, *J. Amer. Chem. Soc.*, 1949, **71**, 1341.
[7] Ref. 1, p. 36.
[8] Manske and Holmes, " The Alkaloids," New York, 1949, Vol. 1, p. 235.

Hydrochloride, m. p. 144—145° (needles from acetone), $[\alpha]_{5461}^{25}$ 0·00° (*c* 4·64 in H_2O) (Found: C, 54·4; H, 9·0; N, 8·1; Cl, 20·3; O, 9·4. Calc. for $C_8H_{16}ONCl$: C, 54·05; H, 9·05; N, 7·9; Cl, 20·0; O, 9·05%).

Hydrobromide, prepared by addition of hydrobromic acid to freshly distilled base, dehydration over P_2O_5, and recrystallisation from acetone, m. p. 138°.

Picrate, m. p. 149—150° (prisms from ethanol) (Found: C, 45·2; H, 4·8; N, 15·2; picric acid, 61·7. Calc. for $C_8H_{15}ON,C_6H_3O_7N_3$: C, 45·4; H, 4·9; N, 15·1; picric acid, 61·9%).

Picrolonate, m. p. 178—179° (needles from aqueous alcohol).

2 : 4-*Dinitrophenylhydrazone hydrochloride*, m. p. 242° (prisms from ethanol) (Found: C, 46·7; H, 5·7; N, 19·7; Cl, 10·1. $C_{14}H_{20}O_4N_5Cl$ requires C, 47·0; H, 5·6; N, 19·6; Cl, 9·9%), $\lambda_{max.}$ in EtOH, (synthetic) 2270 (ε 14,500), 2530 (21,000), (natural) 2270 (ε 14,800), 2530 (ε 21,000).

Thiosemicarbazone hydrochloride, m. p. 212° (needles from ethanol) (Found: C, 43·3; H, 7·7; N, 22·6; S, 13·0. $C_9H_{19}N_4SCl$ requires C, 43·1; H, 7·6; N, 22·4; S, 12·8%).

Semicarbazone hydrochloride, m. p. 169—171° (decomp.) (needles from aqueous alcohol) [anhydrous, m. p. 184° (decomp.)] (Found: C, 42·9; H, 8·3; N, 22·1; Cl, 14·1; O, 12·1; loss at 120°; 7·1. $C_9H_{19}ON_4Cl,H_2O$ requires C, 42·8; H, 8·3; N, 22·2; Cl; 14·1; O, 12·7; H_2O, 8·5%).

Oxime, prepared in acetate-buffered solution,[9] b. p. 134°/2·5 mm., 146°/5·6 mm., two forms, m. p. 105·5° and 90° (from ether) (Found: C, 61·9; H, 10·3; N, 18·4. Calc. for $C_8H_{10}ON_2$: C, 61·5; H, 10·3; N, 17·9%) [the oxime, m. p. 105·5°, gave a picrate, m. p. 176° (from ethanol)].

N-Acetyl derivative, b. p. 138°/4·2 mm.

N-*Benzoyl derivative*, b. p. 165°/0·5 mm., m. p. 74° (from ether) (Found: C, 73·9; H, 7·9; N, 5·8. $C_{15}H_{19}O_2N$ requires C, 73·4; H, 7·3; N, 5·7%).

Anabasine: *Monopicrate*, m. p. 163° (addition of aqueous picric acid to the base in water) (Found: C, 49·1; H, 4·1; N, 17·7; picric acid, 58·8. $C_{10}H_{14}N_2,C_6H_3O_7N_3$ requires C, 49·1; H, 4·35; N, 17·9; picric acid, 58·6%).

Dipicrate, m. p. 213° (addition of excess of picric acid to base in ethanol, or hot aqueous picric acid to solution of base in water adjusted to pH 3·0 with N-HCl) (Found: C, 42·5; H, 3·3; N, 17·6; picric acid, 73·8. Calc. for $C_{10}H_{14}N_2,2C_6H_3O_7N_3$: C, 42·6; H, 3·25; N, 18·1; picric acid, 73·8%).

Dipicrolonate, m. p. 257—259° (needles from aqueous alcohol) (Found: C, 52·25; H, 4·5; N, 20·05; O, 23·3. Calc. for $C_{10}H_{14}N_2,2C_{10}H_8O_5N_4$: C, 52·2; H, 4·3; N, 20·3; O, 23·2%).

N-Nitroso-derivative, b. p. 145—146°/1 mm., $[\alpha]_{5461}^{22}$ 0·00° (no solvent) (Found: C, 62·8; H, 6·8; N, 21·9. Calc. for $C_{10}H_{13}ON_3$: C, 62·8; H, 6·3; N, 22·0%).

N-Benzoyl derivative, prepared by the method of Orechoff and Norkina,[10] b. p. 180°/0·2 mm., m. p. 82—83° (from light petroleum–ether), $[\alpha]_D^{25}$ 0·00 (*c* 5 in EtOH) (Found: C, 76·8; H, 6·9; N, 10·8. Calc. for $C_{17}H_{18}ON_2$: C, 76·7; H, 6·8; N, 10·5%).

2 : 3-Dipyridyl monopicrate: dehydrogenation of anabasine with zinc[11] gave 2 : 3-dipyridyl (19%), b. p. 137°/4·0 mm., identified as the monopicrate, m. p. 151° (Found: C, 50·0; H, 2·95; N, 17·7. Calc for $C_{10}H_8N_2,C_6H_3O_7N_3$: C, 49·9; H, 2·9; N, 18·2%), and the dipicrate, m. p. 167°.

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[9] Hückel and Sachs, *Annalen*, 1932, **498**, 166.
[10] Orechoff and Norkina, *Ber.*, 1932, **65**, 1126.
[11] Orechoff and Menschikoff, *Ber.*, 1931, **64**, 273.