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ALKALOIDS OF *DUBOISIA HOPWOODII*

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Key Word Index—*Duboisia hopwoodii*; Solanaceae; leaf; root; pyridine alkaloids; tropane alkaloids; identification.

Abstract—Leaf and root collections of *Duboisia hopwoodii* were made from Alice Springs in central and Western Australia. From *D. hopwoodii* collected at Alice Springs were isolated nornicotine, nicotine, myosmine and *N*-formylnornicotine; cotinine, *N*-acetylnornicotine, anabasine, anatabine, anatalline and bipyridyl were detected by GC/MS. Root material contained hyoscyamine, scopolamine, nicotine and nornicotine; *N*-formylnornicotine was detected by GC/MS. *D. hopwoodii* from Western Australia yielded nicotine, nornicotine, hyoscyamine and metanicotine. Root material contained nornicotine, hyoscyamine, myosmine and *N*-formylnornicotine, GC/MS detected cotinine and *N*-acetylnornicotine.

INTRODUCTION

Duboisia hopwoodii is one of the few drug-yielding plants used by the Australian aborigine. Known as 'pituri' by central Australian tribes, after drying and preparation the leaves were chewed as a narcotic [1]. Unlike other species, *D. myoporoides* and *D. leichhardtii*, the alkaloids are derived mainly from pyridine rather than tropane. Nornicotine and nicotine were shown to be major alkaloids by Hicks and LeMessurier [2]. Bottomley and White examined 67 leaf samples from Western Australia and showed that in most instances both nicotine and nornicotine could be detected [3]. More recently, the tropane base, hyoscyamine, was isolated from the roots of *D. hopwoodii* [4].

As part of a study of medicinal plants used by the Australian aborigine, leaf and root collections of *D. hopwoodii* were made from central Australia (Alice Springs) and Western Australia. Previous investigations of other *Duboisia* species [5, 6] had demonstrated a complex mixture of alkaloids. Thus the total alkaloid spectrum of *D. hopwoodii* was investigated.

RESULTS AND DISCUSSION

A preliminary TLC investigation of extracts of the samples obtained from the Northern Territory and Western Australia did not demonstrate diversity in the nature of alkaloid type. The major alkaloid of the leaves of the Northern Territory sample was nornicotine; hyoscyamine was the major root alkaloid. The three samples collected from Western Australia showed variation in leaf shape and total alkaloid content. However, all three contained nicotine as the major alkaloids of the leaves along with traces of hyoscyamine. The root samples had been mixed at the time of collection. The major root alkaloids were nornicotine and hyoscyamine.

Further investigation of the crude extracts by various chromatographic methods resulted in the isolation of the pyridine bases nornicotine, nicotine, myosmine and *N*-formylnornicotine which were characterized by full physical identification and microanalysis. Metanicotine was identified by IR and GC/MS. The tropane alkaloids, hyoscyamine and hyoscine were isolated from the roots. Characterization was effected by full physical identification and microanalysis. Minor alkaloids were identified by the characteristic fragmentation patterns by GC/MS. These were cotinine, *N*-acetylnornicotine, 2,2′-bipyridyl, anatabine and anatalline (Table 1).

All identified pyridine alkaloids have been detected previously in *Nicotiana* species. Due to the presence of the nicotine metabolites, nornicotine [7], myosmine [8], cotinine [9] and *N*-formylnornicotine [10] and nicotine alkaloids, it is likely that the microsomal enzyme systems that catalyse the metabolism of pyridine alkaloids in *D. hopwoodii* and *Nicotiana* are the same.

The results of this investigation suggest chemical variation within *D. hopwoodii*. Nicotine was the major alkaloid in the Western Australian collection while nornicotine was predominant in the Northern Territory sample. Whether this variation results from environmental factors or is due to existence of chemical races remains to be determined. Tropane alkaloids are found in the roots of *D. hopwoodii* with traces occurring in the leaves. A possibility of an interesting relationship between the metabolism of tropane and pyridine alkaloids supports further metabolic studies.

EXPERIMENTAL

Plant material. Roots and leaves of *D. hopwoodii* F. Muell. were collected 170 km SW of Alice Springs (24.1.78) and identified by Mr. P. K. Latz, Department of Northern Territory; a sample was lodged with the Queensland Herbarium and annotated BRI 261969.

Roots and leaves of *D. hopwoodii* were collected between Pinda and Wurarga, Western Australia (25.2.78). One collection was a single bush, another from a group of trees; leaves from the group

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Table 1. Distribution of alkaloids in samples of *D. hopwoodii* from two locations

Alkaloid	% Total alkaloid			
	Northern Territory		Western Australia	
	Roots	Leaves	Roots	Leaves
Nornicotine	17.7	86.2	47.8	20.2
Nicotine	19.1	8.8	tr	77.1
N-Formylnornicotine	tr	1.1	3.8	tr
Myosmine	—	1.4	7.8	tr
Hyoscyamine	55.6	—	40.7	1.9
Hyoscine	6.0	—	—	—
Cotinine	—	tr	tr	tr
N-Acetylnornicotine	—	tr	tr	tr
Metanicotine	—	—	—	tr
2,2′-Bipyridyl	—	tr	—	—
Anatabine	—	tr	—	—
Anatalline	—	tr	—	—
Anabasine	—	tr	—	—

were divided into broad and narrow leaf types but the roots were supplied as a mixed sample. Identification was by Dr. J. Dodd, University of West Australia and a voucher specimen lodged with the Queensland Herbarium and annotated BRI 261968.

Extraction and isolation of alkaloids

Northern Territory sample. Leaves—first extraction. Leaves (1 kg) were milled and extracted by percolation with 6 l. of EtOH. The extract was concd to 300 ml and the pH adjusted to between 1 and 2 with H_2SO_4. The ppt was filtered off and the filtrate was shaken with Et_2O to extract pigments. An excess of NH_3 soln was then added and the bases extrd with $CHCl_3$. After evapn a crude extract of 28.8 g was obtained.

Nornicotine. Fractional liberation of the crude extract by adding subequivalent amounts of M NaOH and subsequent extraction with $CHCl_3$ yielded fractions 1–9 with increasing basicity. The last fraction was obtained from $CHCl_3$ extraction after an excess of NH_3 was added. Fractions 5–10 constituted 91% of the extracted base and contained mostly a strong base which formed a crystalline picrate with aq. picric acid, identified as nornicotine dipicrate mp 191–192°. (Found: C, 41.59; H, 2.87. Calc. for $C_{21}H_{18}O_{14}N_8$: C, 41.59; H, 2.99%.) The purified base was optically active; $[\alpha]_D^{20}$ +26.38° (EtOH; *c* 5.8). GC/MS *m/z* (rel. int.): 148 [M^+] (28), 119 (100), 70 (83), 118 (32), 147 (29). IR (liq.): 3300 (*s*) NH, 716 (*s*) 3-pyridyl. ^{1}H NMR (100 MHz, $CDCl_3$): 2.0 and 2.4 (*m*, 5 H, H-3 and 4 of pyrrolidine ring and –NH), 3.3 (*m*, 2 H, H-5), 4.3 (*t*, 1 H, H-2), 7.3–8.8 ppm (*m*, 4 H, pyridine protons). *Leaves—second extraction.* Leaves (2 kg) were extracted by the same method. The early fractions obtained by fractional liberation of the crude extract were examined for minor alkaloids. The first fraction obtained by extraction of the neutral soln with *n*-hexane was referred to as fraction N. Thus the second, third, etc. were referred to as fraction 1, 2, etc.

Nicotine. Fraction 1 was further subfractionated into nine fractions. The fifth fraction (71 mg) was chromatographed on a kieselguhr partition column (10 g) containing 3 ml of 0.5 M Pi buffer, pH 6.6. Nicotine (36 mg) was eluted with Et_2O. It yielded a dipicrate, mp 220°. (Found: C, 42.40; H, 3.57. Calc. for $C_{22}H_{20}O_{14}N_8$: C, 42.58; H, 3.20%.) GC/MS *m/z* (rel. int.): 162 [M^+] (20), 84 (100), 133 (38), 161 (18). The IR spectrum prepared from the picrate was identical to that of an authentic sample.

Myosmine. Subfractions from the first extraction were combined with 0.26 g of fraction N and chromatographed on an Al_2O_3 column developed with mixtures of Et_2O and EtOH. Myosmine (52 mg) was eluted with Et_2O–EtOH (19:1) and gave myosmine picrate, mp 182–183°. (Found: C, 40.68; H, 2.98. Calc. for $C_{21}H_{16}O_{14}N_8$: C, 41.72; H, 2.64%.) Myosmine is optically inactive [12]. GC/MS *m/z* (rel. int.): 146 [M^+] (71), 118 (100), 145 (155). IR (liq. in Nujol): 1620 (m) $\diagdown$C=N– conjugated with an aromatic ring, 1560 (w) and 1585 (w) characteristic absorptions of myosmine [13, 14]. ^{1}H NMR (100 MHz, $CDCl_3$): 2.42 (*m*, 2 H, H-4 of pyrrolidine ring), 3.55 (*t*, 2 H, H-5), 4.29 (*t*, 2 H, H-3), 7.5–9.4 ppm (*m*, 4 H, pyridine protons).

N-Formylnornicotine. Fraction N (0.2 g) was chromatographed on a Si gel adsorption column, using THF as solvent. The early fractions (20–45) were combined and chromatographed on Al_2O_3, developed with Et_2O–EtOH (9:1). The eluted alkaloid which was later identified as *N*-formylnornicotine, did not form a crystalline picrate with either an aq. or ethereal soln of picric acid. GC/MS *m/z* (rel. int.): 176 [M^+] (50), 147 (100), 119 (65), 70 (32), 148 (30), 175 (15). IR (liq. in Nujol): 1660 (s)—CONH. ^{1}H NMR (100 MHz, $CDCl_3$): 2.0 (*m*, 4 H, H-3 and 4 of pyrrolidine ring), 3.7 (*m*, 2 H, 5 H), 4.9 (*m*, 1 H, H-2), 7.0–8.5 ppm (*m*, 5 H, pyridine protons and an aldehyde proton appeared at 8.1 and 8.3 ppm as two singlets at 28°, upon raising the temp. to 58° the two singlets were closer by 2 Hz.

Other minor alkaloids. Numerous minor alkaloids were identified by interpretation of their GC/MS fragmentation patterns and where possible comparison with lit. values. Fraction 20–45 referred to above contained cotinine [15]. GC/MS *m/z* (rel. int.): 176 [M^+] (22), 98 [M^+ –78; 100], and *N*-acetylnornicotine, GC/MS *m/z* (rel. int.): 190 [M^+] (18), 147 [M^+ –43; 100], 175 [M^+ –15; 22], the MS in the region below 147 resembled that of nornicotine. Some strongly basic fractions were combined and by GC/MS the following alkaloids were identified; anabasine [15], GC/MS *m/z* (rel. int.): 162 [M^+] (21), 84 [M^+ –78; 100], 105 [M^+ –57; 81], 106 [M^+ –56; 58], 119 [M^+ –43; 50], 133 [M^+ –29; 42], 120 [M^+ –42; 18]. Anatabine, GC/MS *m/z* (rel. int.): 160 [M^+] (86), 105 [M^+ –55; 100], 54 [M^+ –106; 85], 131 [M^+ –29; 63], 106 [M^+ –54; 63], 159 [M^+ –1; 50], 80 [M^+ –80; 49], 82 [M^+ –78; 32]. 2,2′-Bipyridyl, GC/MS *m/z* (rel. int.): 156 [M^+] (100), 155 [M^+ –1; 75], 130 [M^+ –26; 30], 78 [M^+ –78; 25] and anatalline [16]. GC/MS *m/z* (rel. int.): 239 [M^+] (8), 161 [M^+ –78; 24]. The MS in the region below 162 closely resembled that of anabasine.

Roots. Hyoscyamine. The powdered roots (500 g) were extrd and yielded 2.4 g of a crude extract which was fractionated into seven fractions. Hyoscyamine was the major alkaloid in fractions 3–7. The base (0.4 g) from fraction 4 gave hyoscyamine picrate, mp 166°. (Found: C, 53.28; H, 5.02%.) GC/MS *m/z* (rel. int.): 289 [M^+] (14), 124 (100), 82 (100), 82 (60), 94 (60), 83 (33), 96 (28). The IR spectrum of the picrate agreed with that of an authentic sample. *Hyoscine and nicotine.* Fraction 3 (0.6 g) was subjected to an Al_2O_3 column developed with Et_2O–EtOH mixtures. The hyoscine and nicotine mixture (0.05 g) was eluted with Et_2O–EtOH (9:1). The two alkaloids were sepd by repeating the column chromatography. Nicotine (0.17 g) was eluted by Et_2O–EtOH (97.5:2.5) and hyoscine (75 mg) by Et_2O–EtOH (19:1). The hyoscine neutralized with aq. picric acid gave hyoscine picrate mp 188°. (Found: C, 51.92; H, 4.66. Calc. for $C_{23}H_{24}O_{11}N_4$: C, 51.87; H, 4.51%) GC/MS *m/z* (rel. int.): 303 [M^+] (5), 94 (100), 42 (62), 138 (42), 108 (50), 154 (25). The IR spectrum of hyoscine picrate was prepared and agreed with that of an authentic sample. *Nornicotine.* Fractions 5–7 were combined (0.75 g) and subfractionated into seven subfractions. The last three were combined (0.5 g) and chromatographed on kieselguhr containing 10 ml of 0.5 M Pi buffer, pH 5.7. An initial $CHCl_3$ eluate contained hyoscyamine (0.17 g); a later $CHCl_3$ eluate yielded nornicotine (75 mg). *Other minor alkaloids.* The first fraction was examined by GC/MS which revealed myosmine and *N*-formylnornicotine in addition to the major alkaloids nicotine and hyoscine.

Western Australian sample. Leaves. The three samples were milled and extrd as previously described. Examination by TLC revealed the presence of nicotine and nornicotine in all samples. Hyoscyamine was present in the narrow leaf extract to a greater extent than the other two extracts. The narrow leaf sample also contained the highest alkaloid content of 3.69%. Therefore, the extract of the narrow leaf sample was selected for further investigation. *Nicotine.* The crude extract was fractionated into five fractions. Fractions 2, 3 and 4 were combined (0.755 g) and chromatographed on Si gel, developed with 300 ml of $CHCl_3$–MeOH–NH_3 (84:15:1). Nicotine (0.533 g) was eluted in the first portion. GC/MS *m/z* (rel. int.): 162 [M^+] (20), 84 (100), 133 (38), 161 (18). Optical rotation $[\alpha]_D^{20}$ −130.82° (EtOH; *c* 1.46). *Metanicotine.* Upon standing, fraction 5 deposited a few white hemispherical crystals which did not dissolve easily in $CHCl_3$ but readily dissolved in hot EtOH. The alkaloid gave a characteristic picrate with aq. picric acid, mp 173–175°. GC/MS *m/z* (rel. int.): 162 [M^+] (10), 84 (100), 133 (15), 130 (10), 161 (10). The IR spectrum was prepared from its picrate and agreed with that of metanicotine picrate [17]. *Nornicotine and hyoscyamine.* Fraction 5 (0.732 g) was chromatographed on Al_2O_3, developed with Et_2O–EtOH (19:1) and (9:1) and finally EtOH containing 1% NH_3 soln. Nornicotine (0.146 g) was eluted in the Et_2O–EtOH (9:1) fraction. GC/MS *m/z* (rel. int.): 148 [M^+] (28), 119 (100), 70 (85), 147 (30). The last fraction contained a mixture of nornicotine and hyoscyamine (71 mg). The mixture (30 mg) was subjected to prep. TLC on Si gel (25 μm thickness) developed with $CHCl_3$–MeOH–NH_3 (84:15:1). The zones of alkaloids were detected with I_2 in CCl_4, scraped off and Soxhlet extrd with EtOH for 3 hr. Hyoscyamine (7 mg) afforded a picrate as needles, mp 173.5–174.5°. The IR spectrum complied with that of an authentic sample. *Other minor alkaloids.* Myosmine was detected in the mixture of nornicotine and hyoscyamine obtained from the initial column chromatography of fraction 5. Fraction 1 was examined by GC/MS and revealed cotinine; *m/z* (rel. int.): 176 [M^+] (45), 98 (100), 42 (40) and *N*-formylnornicotine; *m/z* (rel. int.): 176 [M^+], 147 (100), 119 (65), 70 (32). *Root.* Powdered roots (500 g) were extrd as previously described and yielded 0.9 g of crude extract. The residue was subjected to CC on kieselguhr (50 g) containing 35 ml of 0.5 M Pi buffer pH 6.75. The column was developed with Et_2O and $CHCl_3$. An early Et_2O fraction (3–10) contained unresolved bases (0.5 g). A later fraction (11–20) contained nornicotine (85 mg), which gave a needle picrate, mp 191–192°. The $CHCl_3$ eluates contained unresolved bases (0.15 g).

The early Et_2O fraction (3–10) was rechromatographed on a smaller column of kieselguhr (15 g) containing 7 ml of 0.5 M Pi buffer pH 6.1. Et_2O eluted traces of nicotine. Fraction 15–30 eluted in $CHCl_3$ contained *N*-formylnornicotine and other minor alkaloids identified by GC/MS as cotinine and *N*-acetylnornicotine; *m/z* (rel. int.): 190 [M^+] (20), 147 (100), 70 (72), 120 (70), 119 (45), 175 (25). The $CHCl_3$ eluates from the initial CC was rechromatographed on Al_2O_3 and developed with 400 ml of Et_2O–EtOH (9:1). Myosmine (3 mg) was eluted first and then hyoscyamine (25 mg) which gave hyoscyamine picrate mp and mmp identical with an authentic sample, (166–167°).

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