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LUPIN ALKALOIDS FROM *SOPHORA CHRYSOPHYLLA*

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Key Word Index—*Sophora chrysophylla*; Leguminosae; lupin alkaloid; quinolizidine alkaloid; (−)-mamanine *N*-oxide; (+)-mamanine; (−)-pohakuline; kuraramine; lamprolobine; (−)-lupanine; (+)-matrine; variation of alkaloid content.

Abstract—A new lupin alkaloid, (−)-mamanine *N*-oxide, was isolated from *Sophora chrysophylla* together with 18 known alkaloids including some unusual lupin alkaloids such as kuraramine, lamprolobine, epilamprolobine, epilamprolobine *N*-oxide, (+)-mamanine and (−)-pohakuline. It was also shown that the alkaloid constituents of *S. chrysophylla* differed considerably in the leaves, stems and seeds.

INTRODUCTION

In the course of chemical [1–9] and biosynthetic [10–12] investigations on lupin alkaloids in leguminous plants, we have studied the basic constituents of *Sophora chrysophylla*, the sole *Sophora* plant native to Hawaii. Previous work on the constituents of *S. chrysophylla* indicates the presence of (−)-cytisine and (−)-anagyrine in the seeds [13] and of (−)-cytisine, (+)-matrine, (+)-mamanine and (−)-pohakuline in the bark [14]. This report deals with the isolation of a new lupin alkaloid (**1**) together with 18 known lupin alkaloids from the leaves, stems, seeds and seedlings of *S. chrysophylla* and with the structural elucidation of the new base, which is shown to be the *N*-oxide of (+)-mamanine (**2**). The known alkaloids include unusual lupin alkaloids such as kuraramine [15], lamprolobine [16], epilamprolobine [5] and epilamprolobine *N*-oxide [5]. These may have interesting biosynthetic or metabolic relationships with the common lupin alkaloids [5, 15, 16], as well as with the previously reported alkaloids (+)-mamanine and (−)-pohakuline [14]. The distribution of the various lupin alkaloids in the different organs of the plant is also reported.

RESULTS AND DISCUSSION

The air-dried aerial parts of *S. chrysophylla*, which were collected in December 1979 (fruit-bearing period) in the Hawaiian islands, were separated into leaves, stems and seeds, and each part was then treated as described previously [1–9]. The alkaloid mixture obtained from the stems was subjected to silica gel column chromatography followed by prep. HPLC, to yield the following 15 known lupin alkaloids: (+)-matrine, (+)-matrine *N*-oxide, (−)-lupanine, 5,6-dehydrolupanine, (−)-anagyrine, (−)-baptifoline, (−)-cytisine, (−)-*N*-methylcytisine, (−)-*N*-formylcytisine, (−)-pohakuline, (+)-mamanine, kuraramine, lamprolobine, epilamprolobine and epilamprolobine *N*-oxide and one dipiperidine alkaloid ammodendrine. The alkaloid mixture from the leaves was separated in a similar manner to give a new alkaloid, (−)-mamanine *N*-oxide (**1**), together with 13 known alkaloids (Table 1). The alkaloid mixture from the seeds was subjected to prep. HPLC to yield eight known alkaloids (Table 1). (−)-Rhombifoline was found only in the seeds, 17-oxolupanine only in the leaves.

The new alkaloid (**1**) was obtained from the leaves in a

Table 1. Distribution of lupin alkaloids in the various parts of *S. chrysophylla**

Alkaloids present in	Leaves (515 g)	Stems (890 g)	Seeds (0.4 g)
(+)-Matrine	0.03	0.02	0.05†
(+)-Matrine *N*-oxide	0.01	0.02	0.11
(−)-Lupanine (**5**)	0.37	0.05	—
5,6-Dehydrolupanine	tr	tr	—
17-Oxolupanine	tr	—	—
(−)-Anagyrine (**4**)	0.01	0.01	0.68
(−)-Baptifoline	—	tr	0.26
(−)-Cytisine	tr	0.01	2.15
(−)-*N*-methylcytisine (**7**)	tr	0.01	0.37
(−)-*N*-formylcytisine	—	tr	—
(−)-Rhombifoline	—	—	0.20
(−)-Pohakuline (**3**)	0.03	0.03	—
(+)-Mamanine (**2**)	0.02	0.02	—
(−)-Mamanine *N*-oxide (**1**)	tr	—	—
Kuraramine (**6**)	tr	tr	—
Ammodendrine	0.01	tr	—
Lamprolobine	—	tr	—
Epilamprolobine	—	tr	—
Epilamprolobine *N*-oxide	tr	tr	tr

*Alkaloid content was estimated quantitatively by HPLC as described in Experimental.

†Percent by weight of dry plant material.

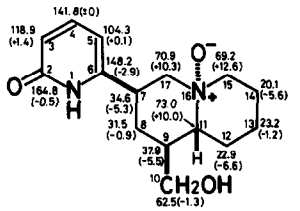
tr, Trace.

yield of 0.0013% of dry wt as a colourless amorphous solid, $[\alpha]_D^{22}$ −9° (EtOH). It had the molecular formula $C_{15}H_{22}N_2O_3$ ($[M]^+$, m/z 278.164, calc. 278.163). Its mass spectrum showed a parent ion at m/z 278 (rel. int. 1%) and fragment ions at m/z 262 (18), 261 (8) and 260 (27) corresponding to $[M-O]^+$, $[M-OH]^+$ and $[M-H_2O]^+$, respectively, which are characteristic for aliphatic amine *N*-oxides [4, 5, 17]. The fragmentation pattern below m/z 260 was similar to that of (+)-mamanine (**2**) which has a base peak at m/z 84.* The UV absorption maxima (EtOH, 228 and 304) and the ^{1}H NMR signals (7.36 (1H, *dd*, J = 8 and 7 Hz, 4-H), 6.44 (1H, *dd*, J = 8 and 1 Hz, 3-H) and 6.10 (1H, *dd*, J = 7 and 1 Hz, 5-H)) due to the pyridone moiety of **1** also resembled those of **2** [14]. Compound **1** was reduced with ferrous sulfate in dilute aqueous ammonia to give **2**, which was reoxidized with *m*-chloroperbenzoic acid to give **1**. The above results indicate that the new base **1** is the *N*-oxide of (+)-mamanine **2**.

Comparison of the ^{13}C NMR spectrum of **1** with that of **2** showed that (−)-mamanine *N*-oxide (**1**), like (+)-mamanine (**2**) [14], contains a *trans*-quinolizidine moiety. The signals of the two methylene carbons and the methine carbon alpha to the *N*-oxide nitrogen were shifted downfield by 10–12 ppm in comparison with those of **2** and the signals due to the carbons at C-7, C-9, C-12 and C-14 were shifted to higher field than those of **2**, as shown in Fig. 1. These shifts are attributable to the α and β-effect of the *N*-oxide bond, respectively [4, 5]. The above data indicate that **1** has a trans-quinolizidine moiety in which the *N*-oxide bond is oriented axially.

The alkaloid constituents and their distribution in *S. chrysophylla* was different in leaves, stems and seeds, as shown in Table 1. The total content (3.7% fr. wt) of alkaloid in the seeds was much higher than that of the leaves (0.66% dry wt) and the stems (0.28% dry wt). (−)-Cytisine, the major alkaloid in the seeds (60% total seed alkaloid), was found only in small amounts in the leaves and stems. (−)-Rhombifoline, present at a relatively high concentration (20% total alkaloid) in the seeds, was absent in the leaves and stems. On the other hand, (−)-lupanine, a rare constituent in *Sophora* species, was the main alkaloid in the leaves and stems but could not be detected in the seeds. In addition, the aerial parts were a rich source of unusual lupin alkaloids such as (+)-mamanine, (−)-pohakuline, kuraramine, epilamprolobine, lamprolobine and epilamprolobine *N*-oxide but the seeds contained only a trace amount of epilamprolobine *N*-oxide. Thus, alkaloid constituents in the seeds of *S. chrysophylla* differ greatly from those of the leaves and stems.

A variation in the alkaloid content was also observed during the growth of the seedlings of *S. chrysophylla* as shown in Figs 2 and 3. The total base content of the seedlings at first decreased in the early stages of growth (Fig. 2), with (−)-rhombifoline decreasing to almost zero within 8 days of germination (Fig. 3). Also, an increase in (−)-*N*-methylcytisine content during growth was accompanied by a decrease in (−)-cytisine content (Fig. 3). A similar change has been observed in the seedlings of some other leguminous plants [11, 12].



1

Fig. 1. Signal assignment and substituent effects of *N*-oxide bond on ^{13}C NMR spectrum of (−)-mamanine *N*-oxide (**1**).

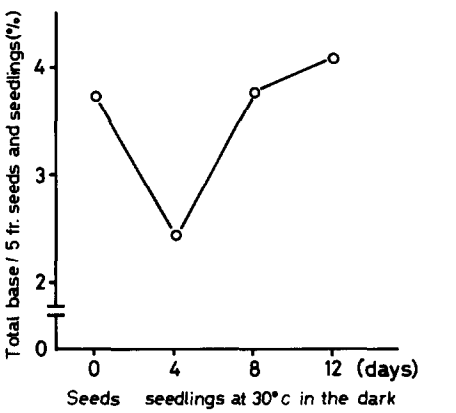


Fig. 2. Total base contents at various stages of the seedlings of *Sophora chrysophylla* during growth.

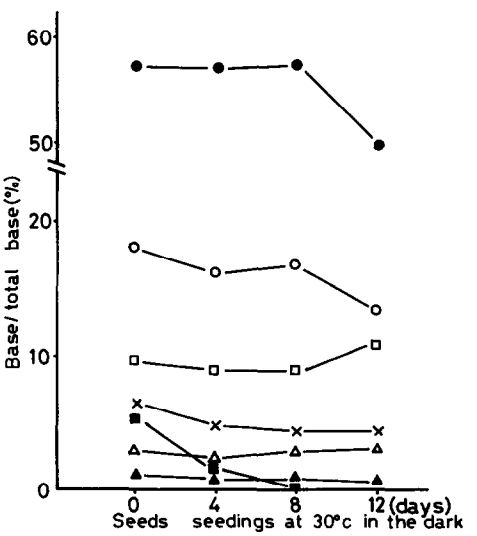


Fig. 3. Variation in the alkaloid content at various stages of the seedlings of *Sophora chrysophylla* during growth. (●), (−)-cytisine; (○), (−)-anagyrine; (□), (−)-*N*-methylcytisine; (×), (−)-baptifoline; (■), (−)-rhombifoline; (△), (+)-matrine *N*-oxide; (▲), (+)-matrine.

*The mass spectrum of **2** was recorded on a double focussing spectrometer at 70eV. The relative intensities [262 $[M]^+$ (25), 245 (5), 231 (12), 204 (33), 122 (23), 121 (28), 96 (20), 84 (100), 83 (31)] were different from those of the reported spectrum [14] which was measured on a single focussing spectrometer.

The structure of (+)-mamanine (**2**) and (−)-pohakuline (**3**) correspond to oxidative products derived from the N_1–C_{10} bond cleavage of (−)-anagyrine (**4**) and (−)-lupanine (**5**), respectively, coexisting in the same plant (Fig. 4). The structure of (+)-kuraramine (**6**), which was isolated first by Murakoshi *et al.* [15] from the flowers of *S. flavescens*, could also be expected to be related to an oxidative product of (−)-*N*-methylcytisine (**7**), coexisting in the same plant, at the same N_1–C_{10} position (Fig. 4).

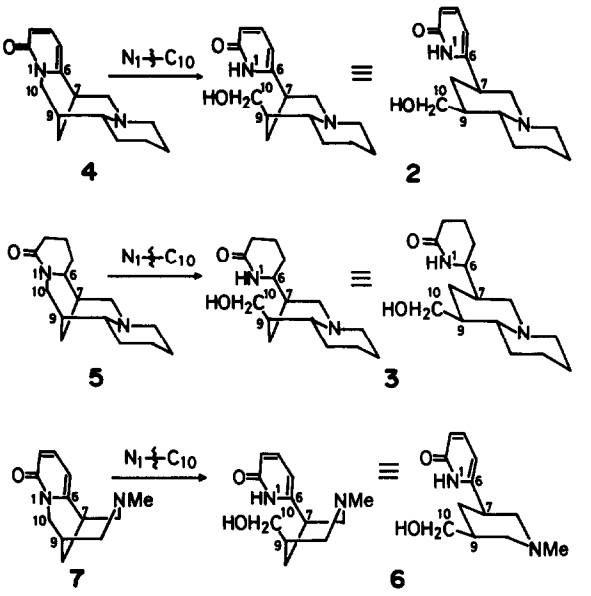


Fig. 4. Structural correlations of (+)-mamanine (**2**), (−)-pohakuline (**3**) and (+)-kuraramine (**6**) with (−)-anagyrine (**4**), (−)-lupanine (**5**) and (−)-*N*-methylcytisine (**7**), respectively, co-existing in the aerial parts of *Sophora chrysophylla*.

EXPERIMENTAL

General. High and low resolution MS were measured at 70 eV using a direct inlet system. The ^{1}H NMR (100 MHz) and ^{13}C NMR (25 MHz) spectra were recorded using TMS as int. standard. Analytical TLC was carried out in the following solvent systems: (A) CH_2Cl_2–MeOH–28% NH_4OH (90:9:1); (B) CH_2Cl_2–MeOH–28% NH_4OH (43:6:1); (C) CH_2Cl_2–MeOH (4:1); (D) Et_2O–MeOH–28% NH_4OH (40:2:1); (E) Et_2O–MeOH–28% NH_4OH (90:20:1). Analytical HPLC was performed with the following solvent systems: (F) 15% MeOH in Et_2O–2.5% NH_4OH (50:1); (G) 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:10:3); (H) 25% MeOH in Et_2O–H_2O–25% NH_4OH (500:20:15) using a LiChrosorb SI 100 (Merck, 10 μm, 0.3 × 50 cm) column employing a monitoring flow system (220 and 310 nm) at a flow rate of 1 ml/min. Prep. HPLC was carried out on a LiChrosorb SI 100 (Merck, 5 μm, 0.8 × 50 cm) column with the solvent systems F, G, H and I (25% MeOH in Et_2O–H_2O–2.5% NH_4OH (500:4:1) monitoring with an UV detector. The chromatographic behaviour and some physical constants of the alkaloids are summarized in Table 2.

Extraction and isolation of alkaloids. The aerial parts of *S. chrysophylla* were collected in the Hawaiian Islands in December 1979 (fruit bearing period), air dried and separated into leaves, stems and seeds. The EtOH extracts of the leaves (515 g) and the stems (890 g) were treated as described previously [1–9] to give the crude alkaloid mixture in a yield of 0.66% (3.4 g) and 0.28%

Table 2. Physical constants and chromatographic behaviour of lupin alkaloids isolated from *S. chrysophylla*

			R_f on TLC*					R_t (min) on HPLC*		
Alkaloids	mp (°)	$[\alpha]_D^{\circ}$†	A	B	C	D	E	F	G	H
(+)-Matrine	76	+40	0.70		0.51	0.49		6.0		
(+)-Matrine *N*-oxide	206	+46	0.27	0.44	0.11					20.3
(−)-Lupanine (**5**)	oil	−58	0.67		0.26	0.24		15.0	10.5	
5,6-Dehydrolupanine	oil	‡	0.72		0.49			5.5		
17-Oxolupanine	154	‡	0.58		0.51			9.6		
(−)-Anagyrine (**4**)	oil	−165	0.65		0.57	0.38		8.4		
(−)-Baptifoline	210	−137	0.30	0.45	0.27	0.14	0.39	24.3	16.0	
(−)-Cytisine	155	−117	0.35	0.65	0.16	0.08	0.21	47.5	29.3	
(−)-*N*-methylcytisine (**7**)	137	−223	0.61		0.52	0.30		10.5		
(−)-*N*-formylcytisine	172	−233	0.42		0.52			35.8	30.5	
(−)-Rhombifoline	oil	−232	0.67		0.76	0.58		4.5		
(−)-Pohakuline (**3**)	170–171	−17	0.20	0.40			0.29	52.3	23.0	7.4
(+)-Mamanine (**2**)	170–172	+31	0.20	0.35	0.17	0.14	0.40	22.0		5.8
(−)-Mamanine *N*-oxide (**1**)	amorphous	−9		0.21						20.0
Kuraramine (**6**)	amorphous	‡	0.13	0.20				56.7	43.1	
Ammodendrine	oil	‡	0.38		0.17			51.7	22.5	
Lamprolobine	oil	‡	0.65					10.3		
Epilamprolobine	101.5	‡	0.65		0.28			9.8		
Epilamprolobine *N*-oxide	amorphous	‡	0.20		0.12					12.5

*Solvents A–H for TLC and HPLC are described in the Experimental.

†EtOH solution.

‡Optical rotation was not measured due to the shortage of material. The alkaloids was identifed by co-TLC, co-HPLC and by comparison of MS data with those of authentic compounds.

(2.5 g) of dry wt, respectively.

(i) *Isolation of alkaloids from stems.* Part of the crude alkaloid mixture (0.9 g) from the stems was applied to a silica gel (Merck, type 60, 230–400 mesh, 30 g, 1.5 × 30 cm) column and developed successively with the following solvent systems, 50 ml fractions being collected: 1, 1.5% MeOH in CH_2Cl_2–28% NH_4OH (500:0.5, fractions 1–24); 2, 2.3% MeOH in CH_2Cl_2–28% NH_4OH (500:0.75, fractions 25–36); 3, 3% MeOH in CH_2Cl_2–28% NH_4OH (500:1, fractions 37–50); 4, 4% MeOH in CH_2Cl_2–28% NH_4OH (500:1.5, fractions 51–62); 5, 5% MeOH in CH_2Cl_2–28% NH_4OH (500:2, fractions 63–76); 6, 6.5% MeOH in CH_2Cl_2–28% NH_4OH (500:3, fractions 77–88); 7, 8% MeOH in CH_2Cl_2–28% NH_4OH (500:4, fractions 89–108). Prep. HPLC separation of fractions 1–24 using solvent F gave (+)-matrine (58 mg) and (−)-*N*-methylcytisine (4 mg). Fractions 25–29 were separated by prep. HPLC with solvent F to give 5,6-dehydrolupanine (1 mg), (−)-anagyrine (35 mg), a mixed fraction of two alkaloids and (−)-lupanine (132 mg), in that order of elution. The mixed fraction was further separated by prep. HPLC using solvent I to yield a further amount of (−)-*N*-methylcytisine (21 mg) and lamprolobine (2 mg). Fractions 30–50 were subjected to prep. HPLC using solvent G to give (−)-cytisine and a mixed fraction, which was further separated by prep. HPLC using solvent I to give lamprolobine (1 mg) and epilamprolobine (3 mg). Ammodendrine (3 mg) was obtained from fractions 54–59 by prep. HPLC purification with solvent G. Fractions 60–65 were purified by prep. HPLC using solvent G to give (−)-baptifoline (5 mg). Prep. HPLC separation of fractions 66–69 with solvent H gave epilamprolobine *N*-oxide (3 mg) and matrine *N*-oxide (48 mg). (+)-Mamanine (43 mg) and (−)-pohakuline (21 mg) were obtained from fractions 70–76 by prep. HPLC separation with solvent H, together with a further amount of (+)-matrine *N*-oxide (32 mg). Fractions 77–83 contained a mixture of (+)-mamanine and (−)-pohakuline. Fractions 84–108 were applied to prep. HPLC using solvent H to give kuraramine (5 mg). (ii) *Isolation of* (−)-*mamanine* N-*oxide* (**1**) *and* 17-*oxolupanine from leaves.* Part of the crude alkaloid mixture (1.27 g) obtained from the leaves was extracted with *n*-hexane. The *n*-hexane insoluble fraction (0.75 g) was applied to a silica gel (Merck, type 60, 230–400 mesh, 150 g, 2.5 × 60 cm) column and developed successively with CH_2Cl_2–MeOH (4:1, 1.8 l), CH_2Cl_2–MeOH–28% NH_4OH (90:9:1, 1.8 l) and CH_2Cl_2–MeOH–28% NH_4OH (70:40:5, 0.6 l), 25 ml fractions being collected. Fractions 50–66 were applied to prep. HPLC with solvent H to give **1** (2.5 mg, 0.0013%/dry wt) as a colourless amorphous solid, $[\alpha]_D^{22}$ −9° (*c* 0.003, EtOH). EIMS *m/z* (rel. int.): 278.164 $[M]^+$, calc. for $C_{15}H_{22}N_2O_3$ 278.163, (1), 262 $[M-O]^+$ (18), 261 $[M-OH]^+$ (8), 260 $[M-H_2O]^+$ (27), 231 (12), 229 (25), 204 (27), 122 (31), 121 (36), 96 (28), 84 (100), 83 (42). UV λ_{max}^{EtOH} nm (log ε): 228 (3.95), 304 (4.01). ^1H NMR (5% CD_3OD in $CDCl_3$): δ7.36 (1H, *dd*, *J* = 8 and 7 Hz, H-4), 6.44 (1H, *dd*, *J* = 8 and 1 Hz, H-3), 6.10 (1H, *dd*, *J* = 7 and 1 Hz, H-5). ^{13}C NMR (5% CD_3OD in $CDCl_3$): δ164.8 (*s*, C-2), 118.9 (*d*, C-3), 141.8 (*d*, C-4), 104.3 (*d*, C-5), 148.2 (*s*, C-6), 34.6 (*d*, C-7), 31.5 (*t*, C-8), 37.9 (*d*, C-9), 62.5 (*t*, C-10), 73.0 (*d*, C-11), 22.9 (*t*, C-12), 23.2 (*t*, C-13), 20.1 (*t*, C-14), 69.2 (*t*, C-15), 70.9 (*t*, C-17). The *n*-hexane soluble fraction (0.52 g) was chromatographed on a silica gel (Merck, type 60, 230–400 mesh, 50 g, 1.8 × 40 cm) column which was developed with Et_2O–MeOH–28% NH_4OH (40:2:1), 30 ml fractions were collected. Fractions 36–39 were applied to prep. HPLC with solvent F to give 17-oxolupanine (3 mg). (iii) *Extraction and isolation of alkaloids from seeds.* Seeds (0.4 g) were skinned, swollen in H_2O and then homogenized in EtOH. The EtOH extract obtained was treated in a similar manner as described above to give 15 mg (3.75%/fr wt) crude alkaloid mixture. This mixture was subjected to prep. HPLC and developed successively with solvents F and H. (−)-Rhombifoline, (+)-matrine, (−)-anagyrine, (−)-*N*-methylcytisine, (−)-baptifoline and (−)-cytisine were eluted with solvent F in that order, and (+)-matrine *N*-oxide was obtained as sole component with solvent H.

Estimation of alkaloid content in seedlings. The skinned seeds of *S. chrysophylla* were swollen in H_2O for 6 hr and then germinated and grown on moistened vermiculite in the dark at 30° for 4 days, 8 days and 12 days, respectively. After harvesting, each sample of fresh seedlings was homogenized in 75% EtOH. Each EtOH extract was treated by the method reported previously [8], to give a mixture of bases. The content of individual alkaloid in the mixture of bases was determined by HPLC.

Reduction of **1** *to* **2**. Compound **1** (1 mg) was reduced with an equimolar amount of $FeSO_4 \cdot 7H_2O$ (1 mg) in 0.5 ml of MeOH–28% NH_4OH (20:1) at 60° for 5 min. After evaporation of MeOH, the reaction mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried (K_2CO_3) and concd to dryness *in vacuo*. The residue was purified by prep. HPLC with solvent F. The product (0.4 mg) was identical with **2** (co-HPLC, co-TLC and MS).

N-*Oxidation of* **2** *to* **1**. To a soln of **2** (4 mg) in 1 ml of CH_2Cl_2 was added a soln containing 3.4 mg of *m*-chloroperbenzoic acid in CH_2Cl_2 (0.5 ml) at room temp. with stirring. After stirring for a few min, the reaction mixture was washed with 5% Na_2CO_3 soln, dried (K_2CO_3) and concd to dryness *in vacuo*. The residue was purified by prep. HPLC with solvent H. The product (1.5 mg) was identical with **1** (co-HPLC, co-TLC and MS).

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