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LUPIN ALKALOIDS FROM *SOPHORA MOLLIS**

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Key Word Index—*Sophora mollis*; Leguminosae; lupin alkaloid; (+)-sparteine; 5,6-dehydrolupanine; lupanine; (−)-cytisine; (−)-anagyrine; (−)-baptifoline; (−)-*N*-formyl-cytisine; rhombifoline; ammodendrine; chemotaxonomy.

Abstract—The major alkaloids of *Sophora mollis* are (+)-sparteine and (−)-cytisine, and the minor ones are also of the sparteine-type (lupanine and 5,6-dehydrolupanine) and the cytisine-type [(−)-anagyrine, (−)-baptifoline, (−)-*N*-methylcytisine, (−)-*N*-formylcytisine and rhombifoline] except for ammodendrine of the dipiperidine-type. No matrine-type alkaloid could be detected in this plant. Thus, the alkaloid composition of *S. mollis* is similar to that of the tribe Genisteae rather than that of the tribe Sophoreae.

INTRODUCTION

Previous study on the basic constituents of *Sophora mollis* (tribe Sophoreae: Leguminosae) has revealed the presence of the cytisine-type lupin alkaloids, cytisine, *N*-methylcytisine and anagyrine [1].

As a continuation of our screening for lupin alkaloids in leguminous plants [2–8], we have further investigated in detail the basic constituents of this plant to isolate the sparteine-type and the dipiperidine-type alkaloids in addition to the cytisine-type alkaloids. The cytisine-type and matrine-type alkaloids are characteristic components of the genus *Sophora*, and the sparteine-type and the dipiperidine-type alkaloids are rarely found in this genus [2–10]. This report describes the isolation of 10 lupin alkaloids from *S. mollis*.

RESULTS AND DISCUSSION

The alkaloid fraction obtained from the air-dried leaves of *S. mollis*, collected at Peshawar in Pakistan in July, was subject to Si gel CC, followed by prep. HPLC to give the 10 known lupin alkaloids, (+)-sparteine, lupanine, 5,6-dehydrolupanine, (−)-anagyrine, (−)-baptifoline, (−)-cytisine, (−)-*N*-methylcytisine, (−)-*N*-formylcytisine, rhombifoline and ammodendrine. These alkaloids were identified by direct comparison (MS, IR, co-TLC and co-HPLC) with authentic samples, obtained as described in our previous papers [2–8]. The presence of the above alkaloids in the stems was confirmed chromatographically.

The major alkaloids of *S. mollis* were (+)-sparteine and (−)-cytisine, which were predominant in the leaves and the stems, respectively, as shown in Table 1. The minor components were also of the sparteine-type alkaloid (lupanine and 5,6-dehydrolupanine) and the cytisine-type alkaloid [(−)-anagyrine, (−)-baptifoline, (−)-*N*-methylcytisine, (−)-*N*-formylcytisine and rhombifoline] except for ammodendrine of the dipiperidine-type. The cytisine-type alkaloids are widespread not only in the members of the tribe Sophoreae but also in the other leguminous plants which have lupin alkaloids, whereas the sparteine-type alkaloids are rarely found in the tribe Sophoreae although they are the universal

Table 1. Distribution of the lupin alkaloids in the leaves and the stems of *Sophora mollis**

Alkaloids	Leaves	Stems
(+)-Sparteine	0.267	0.0035
Lupanine	†	†
5, 6-Dehydrolupanine	†	†
(−)-Anagyrine	0.006	0.065
(−)-Baptifoline	0.0025	0.0053
(−)-Cytisine	0.026	0.248
(−)-*N*-Methylcytisine	0.006	0.0042
(−)-*N*-Formylcytisine	0.003	0.003
Rhombifoline	†	†
Ammodendrine	†	†

*Alkaloid contents were quantitatively estimated by HPLC as described in Experimental and are shown in %/dry plant material.

†Trace.

*A part of this work was presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan at Tokyo, 5 April, 1980 (Meeting Abstract, p. 230).

constituents in *Genista*, *Lupinus*, *Cytisus* and *Spartium* of the tribe Genisteae [1–10]. The tribe Sophoreae, and especially the genus *Sophora*, is also an exclusive source of matrine-type alkaloids [1–10]. However, no matrine-type alkaloid could be found in *S. mollis*. The dipiperidine-type alkaloids such as ammodendrine and histrine so far have not been found to accumulate in the *Sophora* species, although ammodendrine and (+)-kuraramine have recently been isolated from *S. franchetiana* [4, 5] and *S. tomentosa* [3], and from *S. flavescens* [2], respectively, as minor basic constituents. These dipiperidine-type alkaloids mainly occur in the plants of *Genista*, *Adenocarpus* (tribe Genisteae) and *Ammodendron* (tribe Sophoreae). Thus, it is of chemotaxonomic interest that the alkaloidal composition of *S. mollis* leaves is more similar to those of members of the tribe Genisteae than to members of the tribe Sophoreae.

EXPERIMENTAL

Mps are uncorr. IR spectra were measured in KBr discs or $CHCl_3$ solution. MS spectra were obtained by direct inlet, at 70 eV. TLC were carried out on Si gel plates in the following solvent systems: 1, CH_2Cl_2–MeOH–28% NH_4OH (90 : 9 : 1); 2, CH_2Cl_2–MeOH–28% NH_4OH (43 : 6 : 1); 3, CH_2Cl_2–MeOH (4 : 1) and on Al_2O_3 plates in 4, C_6H_6–MeOH–Me_2CO (34 : 3 : 3). Analytical HPLC were performed with solvents 5, 15% MeOH in Et_2O–2.5% NH_4OH (50 : 1); 6, 15% MeOH in Et_2O–H_2O–25% NH_4OH (500 : 10 : 3); 7, 25% MeOH in Et_2O–H_2O–25% NH_4OH (100 : 4 : 3), using LiChrosorb SI 100 (Merck, 10 μm, 0.3 × 50 cm) column employing a monitoring flow system (220 or 310 nm) at a flow rate of 1 ml/min. Prep. HPLC was carried out with the solvents 5–7 on LiChrosorb SI 100 (Merck, 10 μm, 0.5 × 50 cm) column monitoring with a UV detector (220 or 310 nm).

Isolation of alkaloids. The aerial parts of *S. mollis* were collected in June in the suburbs of Peshawar in Pakistan and divided into the leaves and stems. The air-dried, powdered leaves were extracted with EtOH ×3 at room temp. extending over 6 days. The combined extracts were concentrated to a semi-solid mass under reduced pressure. The mass was suspended in 10% acetic acid and the insoluble materials were filtered off. The acid extract was washed with Et_2O ×2, basified with 25% NH_4OH to pH 11 and extracted with $CHCl_3$ repeatedly until it became negative to Dragendorff's reagent. The $CHCl_3$ extracts were combined, dried on anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The crude alkaloid was obtained as a pale brown oil in a 0.5% yield of the dry plant material. The stems also were treated with the same procedure as described for the leaves to give the crude alkaloid in a 0.35% yield of the dry plant material. The crude alkaloid (2.0 g) from the leaves was subjected to Si gel CC (Merck, type 60, 70–230 mesh, 3 × 18 cm) using 5% MeOH in CH_2Cl_2–28% NH_4OH (500 : 1) as an eluting solvent and 80-ml fractions were collected. Each fraction was screened by HPLC and TLC and similar samples were combined to give fractions A (fraction Nos. 1–2), B (3–4), C (5), D(6), E (7–8), F (9–13) and G (14–16). Fraction A on prep. HPLC separation with solvent 5 yielded rhombifoline (1.5 mg) and 5, 6-dehydrolupanine (1 mg). Fraction B was separated by prep. HPLC with solvent 5 to give lupanine (0.5 mg), (−)-anagyrine (23 mg) and (−)-*N*-methylcytisine (19 mg). Fraction C was purified by prep. HPLC with solvent 5 to yield (−)-baptifoline (8 mg). Fraction D was crystallized from benzene to give (−)-cytisine (66 mg). Fraction E on prep. HPLC separation with solvent 5 yielded (−)-*N*-formylcytisine (8 mg), ammodendrine (1.5 mg) and further amount of (−)-cytisine (25 mg). (+)-Sparteine (954 mg) was obtained from fraction F as an oily product, which was almost pure. Fraction G contained mainly (+)-sparteine. The physical and chromatographic properties of the alkaloids from the leaves of *S. mollis* are shown in Table 2.

The basic fraction from the stems was also investigated in the same manner.

REFERENCES

1. Izaddoost, M. (1975) *Phytochemistry* **14**, 203.
2. Murakoshi, I., Kidoguchi, E., Haginiwa, J., Ohmiya, S., Higashiyama, K. and Otomasu, H. (1981) *Phytochemistry*, **20**, 1407.
3. Murakoshi, I., Kidoguchi, E., Nakamura, M., Haginiwa, J., Ohmiya, S., Higashiyama, K. and Otomasu, H. (1981) *Phytochemistry* **20**, 1725.

Table 2. Physical constants and chromatographic behaviour of the alkaloids isolated from *Sophora mollis*

Alkaloids	Mp(°)	$[\alpha]_D^{\circ}$†	R_f on TLC*				R_t (min) on HPLC*		
			1	2	3	4	5	6	7
(+)-Sparteine	oil	+17.0	0.10	0.19				26.0	5.5
Lupanine‡	oil		0.67		0.26	0.71	15.0	10.5	
5,6-Dehydrolupanine‡	oil		0.72		0.49	0.76	5.5		
(−)-Anagyrine	oil	−165.3	0.65		0.57	0.70	8.4	5.6	
(−)-Baptifoline	210	−137.2	0.30	0.45	0.27	0.24	24.3	17.0	6.3
(−)-Cytisine	155	−116.7	0.35	0.57	0.16		47.5	29.3	7.9
(−)-*N*-Methylcytisine	137	−223.4	0.61		0.52	0.64	10.5	7.5	
(−)-*N*-Formylcytisine	172	−232.6	0.42	0.60	0.52	0.27	35.8	30.5	8.7
Rhombifoline‡	oil		0.67		0.76	0.73	4.5		
Ammodendrine‡	oil		0.38		0.17		51.7	22.5	

*Solvents 1–4 for TLC and 5–7 for HPLC are described in the Experimental.
†All $[\alpha]_D$ were measured in EtOH.
‡Their $[\alpha]_D$ could not be measured because of the shortage of the sample.

4. Ohmiya, S., Higashiyama, K., Otomasu, H., Haginiwa, J. and Murakoshi, I. (1981) *Phytochemistry* **20**, 1997.
5. Bordner, J., Ohmiya, S., Otomasu, H., Haginiwa, J. and Murakoshi, I. (1980) *Chem. Pharm. Bull.* **28**, 1965.
6. Ohmiya, S., Otomasu, H., Haginiwa, J. and Murakoshi, I. (1980) *Chem. Pharm. Bull.* **28**, 546.
7. Ohmiya, S., Higashiyama, K., Otomasu, H., Murakoshi, I. and Haginiwa, J. (1979) *Phytochemistry* **18**, 645.
8. Murakoshi, I., Toriizuka, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1979) *Chem. Pharm. Bull.* **27**, 144.
9. Mears, J. A. and Mabry, T. J. (1971) *Chemotaxonomy of the Leguminosae* (Harborne, J. B., Boulter, D. and Turner, B. L., eds.), pp. 73–178. Academic Press, New York.
10. Salatino, A. and Gottlieb, O. R. (1980) *Biochem. System. Ecol.* **8**, 133.