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ALKALOIDS OF *THERMOPSIS LUPINOIDES*

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Key Word Index—*Thermopsis lupinoides*, Leguminosae, lupin alkaloids, (+)-lupanine, (+)-17-oxolupanine, (−)-anagyrine, (−)-cytisine, ammodendrine, absolute configuration, alkaloid content

Abstract—Ammodendrine, together with seven other known lupin alkaloids, was isolated from *Thermopsis lupinoides* (+)-Lupanine and (+)-17-oxolupanine occurred together with (−)-anagyrine, (−)-baptifoline, (−)-cytisine, (−)-*N*-methylcytisine and (−)-*N*-formylcytisine These alkaloids have the opposite stereochemistry to that of (+)-lupanine and (+)-17-oxolupanine The distribution of alkaloids in fresh flowers, leaves, stems and roots of this plant was also examined

INTRODUCTION

The plants of the genus *Thermopsis* (Leguminosae) are known to be a rich source of lupin alkaloids [1] The two *Thermopsis* species, *T chinensis* (Japanese name, kusoendo) and *T lupinoides* (Japanese name, sendai-hagi) grow in Japan As part of investigations on lupin alkaloids in leguminous plants native to Japan [2–10], we previously reported the isolation of five basic constituents from the roots of *T chinensis* [2] This report describes the isolation of eight known lupin alkaloids from the other Japanese *Thermopsis* species, *T lupinoides*, and their distribution in flowers, leaves, stems and roots

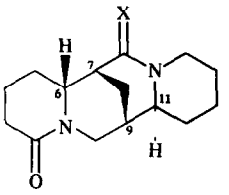
RESULTS AND DISCUSSION

Alkaloid fractions were obtained from freshly harvested flowers, leaves, stems and roots of *T lupinoides* which were collected in May (flowering period) The crude alkaloid mixture obtained from the stems was repeatedly chromatographed on alumina and silica gel columns to yield eight known lupin alkaloids, (+)-lupanine (**1**), (+)-17-oxolupanine (**2**), (−)-anagyrine (**3**), (−)-baptifoline (**4**), (−)-cytisine (**5**), (−)-*N*-methylcytisine (**6**), (−)-*N*-formylcytisine (**7**) and ammodendrine The main alkaloids of *T lupinoides*, (+)-lupanine (**1**) and (−)-*N*-methylcytisine (**6**) were present in all four parts of the plants (+)-Lupanine was the major alkaloid, constituting more than half of the total alkaloid in all parts The other alkaloids were minor components (< 5% of total alkaloid) and their distribution differed in flowers, leaves, stems and roots (Table 1)

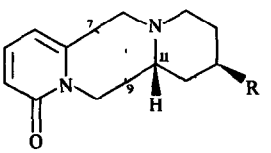
Although the sparteine type alkaloids (**1** and **2**) occurred together with the anagyrine-cytisine type alkaloids (**3**–**7**) in *T lupinoides*, the absolute configuration of the methylene bridge of **1** (7*S* 9*S*) and **2** (7*R* 9*S*) is opposite to that of **3** (7*R* 9*R*), **4** (7*R* 9*R*), **5** (7*R* 9*S*), **6** (7*R* 9*S*) and **7** (7*R* 9*S*) [11, 12] In general, lupin alkaloids which have the same absolute configuration, such as (+)-sparteine, (−)-lupanine, (−)-anagyrine, (−)-baptifoline, (−)-cytisine, (−)-*N*-methylcytisine etc, are found together among the legumes [1, 12] Thus, *T lupinoides* is exceptional, in that alkaloids having the opposite absolute configuration occur together Another plant in which alkaloids of opposite stereochemistry have been reported to occur in is *Genista aetnensis* DC, in which (−)-sparteine is accompanied by (+)-retamine and (−)-cytisine, both of which have the opposite stereochemistry to that of (−)-sparteine [13, 14]

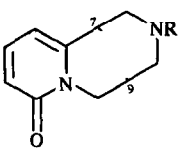
EXPERIMENTAL

General methods Mps are uncorr TLC was carried out on silica gel plates in the following solvent systems 1, CH_2Cl_2–MeOH–28% NH_4OH (90 9 1), 2, CH_2Cl_2–MeOH (4 1), and on Al_2O_3 plates in 3, C_6H_6–MeOH–Me_2CO (34 3 3) Analytical HPLC was performed with 4, 15% MeOH in Et_2O–2 5% NH_4OH (50 1), 5, 15% MeOH in Et_2O–H_2O –25% NH_4OH (500 10 3) using LiChrosorb SI 100 (Merck,



1 X = H_2 (6*R* 7*S* 9*S* 11*S*)
2 X = O (6*R* 7*R* 9*S* 11*S*)





3 R = H (7*R* 9*R* 11*R*)
4 R = OH (7*R* 9*R* 11*R*)

5 R = H (7*R* 9*S*)
6 R = Me (7*R* 9*S*)
7 R = CHO (7*R* 9*S*)

Table 1 Distribution of lupin alkaloids in flowers, leaves, stems and roots of *Thermopsis lupinoides**

Alkaloids present in	Flowers (81 g)	Leaves (1 4 kg)	Stems (870 g)	Roots (750 g)
Total base	0 25	0 50	0 20	0 23
(+)-Lupanine (1)	0 16	0 31	0 13	0 15
(+)-17-Oxolupanine (2)	trace	trace	trace	trace
(−)-Anagyrine (3)	0 001	0 006	0 012	0 001
(−)-Baptifoline (4)	trace	trace	0 001	0 003
(−)-Cytisine (5)	0 006	0 005	0 014	0 011
(−)-*N*-Methylcytisine (6)	0 029	0 097	0 021	0 025
(−)-*N*-Formylcytisine (7)	0 001	trace	0 001	0 001
Ammodendrine	trace	trace	0 002	0 004

* %Fr wt

Table 2 Physical constants and chromatographic behaviour of lupin alkaloids isolated from *Thermopsis lupinoides*

Alkaloids	mp°	$[\alpha]_D^{\circ}$*	R_f on TLC† 1	2	3	R_t (min) on HPLC† 4	5	R_t (min) on GC
(+)-Lupanine (1)	oil	+83	0 67	0 26	0 71	15 0	—	7 5
(+)-17-Oxolupanine (2)	154	+140	0 63	0 71	—	9 6	—	11 7
(−)-Anagyrine (3)	oil	−164	0 65	0 57	0 70	8 4	—	11 4
(−)-Baptifoline (4)	210	−135	0 30	0 27	0 24	24 3	16 0	21 2
(−)-Cytisine (5)	155	−117	0 35	0 16	—	47 5	29 3	6 6
(−)-*N*-Methylcytisine (6)	137	−222	0 61	0 52	0 64	10 5	—	5 9
(−)-*N*-Formylcytisine (7)	172	−233	0 42	0 52	0 27	35 8	30 5	13 8
Ammodendrine	oil	‡	0 38	0 17	—	51 7	22 5	4 2

*EtOH solution
†Solvents 1–5 for TLC and HPLC are described in Experimental
‡Optical rotation not measured due to shortage of material

10 μm, 0 3 × 50 cm) column employing a UV monitoring flow system (220 or 310 nm) at a flow rate of 1 ml/min Prep HPLC were carried out with solvents 4, 5 and 6, 10% MeOH in Et_2O–H_2O–2 5% NH_4OH (500 5 1) on LiChrosorb SI 100 (Merck, 10 μm, 0 5 × 50 cm) column monitoring with a UV detector (220 or 310 nm) Analytical GC was performed with a glass column (2 m × 3 mm i d) packed with 2% OV-17 on 80–100 mesh Gas Chrom Q, using N_2 as carrier gas (40 ml/min) The column was programmed from 220° to 280° at 8°/min and afterwards isothermally For GC/MS the same chromatographic system, except for the use of He as carrier gas, was coupled with a MS

Plant material T lupinoides which was grown in Miyagi-prefecture, Japan was collected in May (flowering period) The plant was identified by Prof J Haginiwa, Faculty of Pharmaceutical Sciences, Chiba University and voucher specimens have been deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Chiba University, Chiba, Japan

Extraction and isolation of alkaloids Fresh plant material was separated into flowers, leaves, stems and roots, from 75% MeOH extracts of which the total alkaloids were obtained in yields of 0 25, 0 50, 0 20 and 0 23% of fr wt, respectively, by a method reported previously [2–10] The mixture of bases (2 1 g) from the stems was chromatographed on Al_2O_3 (Merck, active grade II–III, 1 5 × 32 cm) The column was eluted with C_6H_6, followed by C_6H_6 containing increasing amounts of MeOH, 100 ml fractions being collected 1, C_6H_6 (fractions 1–15), 2, 0 2% MeOH in C_6H_6 (fractions 16–22), 3, 0 5% MeOH in C_6H_6 (fractions 23–33), 4, 1% MeOH in C_6H_6 (fractions 34–38), 5, 1 5% MeOH in C_6H_6 (fractions 39–46), 6, 2% MeOH in C_6H_6 (fractions 47–50), 7, 5 0% MeOH in C_6H_6 (fractions 51–53) (+)-Lupanine (1, 1 05 g) was obtained from fractions 2–24 as a colourless oil in almost pure form Prep HPLC separation of fraction 25 with solvent 7 gave (−)-anagyrine (3, 45 mg) and (+)-17-oxolupanine (2, 3 mg) Fractions 26–32 contained 3 and (−)-*N*-methylcytisine (6) Crystallization of fractions 33–37 from *n*-hexane yielded colourless needles of (−)-*N*-methylcytisine (6, 104 mg) (−)-Cytisine (5, 114 mg) was obtained from fractions 38–47 by crystallization from C_6H_6 Prep HPLC separation of fractions 48–50 using solvent 5 gave (−)-*N*-formylcytisine (7, 8 mg) Fractions 51–53 were subjected to prep HPLC with solvent 5 to yield (−)-baptifoline (4, 9 mg) Ammodendrine was obtained directly from the crude mixture of bases extracted from the stems by prep HPLC separation using solvent 5 The alkaloids were identified by comparison of mp, TLC, HPLC, IR, MS and ^1H NMR with those of authentic samples, as described in our previous papers [2–10] Some physical constants and chromatographic behaviour of the alkaloids are listed in Table 2

The crude alkaloid mixtures obtained from flowers, leaves and roots were separated by GC and all alkaloids involved in this study were identified by GC/MS The alkaloid content of flowers, leaves, stems and roots was estimated quantitatively by HPLC

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