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LUPIN ALKALOIDS FROM *ECHINOSOPHORA KOREENSIS**

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Key Word Index—*Echinosophora koreensis*; Leguminosae; alkaloid; (−)-cytisine; (−)-*N*-methylcytisine; (−)-*N*-formylcytisine; (−)-*N*-(3-oxobutyl)-cytisine; lupanine; 5, 6-dehydrolupanine; (−)-anagyrine; seasonal variation in alkaloid content.

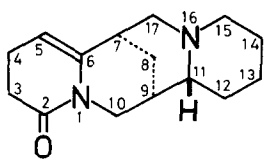
Abstract—The nine known lupin alkaloids, (−)-cytisine, (−)-*N*-methylcytisine, (−)-*N*-(3-oxobutyl)-cytisine, (−)-*N*-formylcytisine, (−)-rhombifoline, (−)-baptifoline, (−)-anagyrine. Lupanine and 5, 6-dehydrolupanine, have been isolated from the freshly harvested leaves, stems and roots of *Echinosophora koreensis*. The seasonal variations in the alkaloid contents of this plant were also examined.

INTRODUCTION

Echinosophora koreensis is a deciduous shrub, which is native in Korea and closely related to the genus *Sophora* (Leguminosae). We have recently isolated a new lupin alkaloid, (−)-*N*-(3-oxobutyl)-cytisine, from the freshly harvested epigeal parts of *E. koreensis* [1]. Further investigation on the alkaloidal constituents in the leaves, stems and roots of *E. koreensis* have resulted in the isolation of eight additional lupin alkaloids. This paper describes the isolation of the lupin alkaloids from *E. koreensis* and the seasonal variations in the alkaloid content of this plant.

RESULTS AND DISCUSSION

From the freshly harvested leaves of *E. koreensis*, eight lupin alkaloids in addition to (−)-*N*-(3-oxobutyl)-cytisine were isolated as listed in Table 1. These known alkaloids were identified by comparing the natural products directly with authentic samples in all measurable respects (MS, IR, co-TLC and co-HPLC), as described in our previous papers [1–7]. The presence of 5, 6-dehydrolupanine (**1**) in this plant, which has been found so far only in *Lupinus* [8] and *Thermopsis* [9] species, was confirmed by comparison of the following spectral and chemical data with those reported originally by Cho and Martin [9]. **1** was a colourless oil and its mass spectrum showed the parent peak at *m/z* (rel. int.) 246 (44) and the fragment ions at *m/z* 245 (15), 134 (9), 98 (100) and 97 (31). The ^{1}H NMR spectrum ($CDCl_3$) of **1** revealed the triplet ($J = 4$ Hz) at δ 4.92 (1H), the doublet ($J = 12$ Hz) at 4.02 (1H) and the double doublet ($J =$ 12 and 5 Hz) at 3.25 (1H) due to H-5, H_α-10 and H_β-10, respectively. In addition, the UV spectrum of **1** at 250 nm (EtOH) was similar to that of aphyllidine [10, 11] and 5, 6-dehydrolupanine [9] and indicative of a vinyl amide grouping as in aphyllidine and 5, 6-dehydrolupanine. The presence of such a grouping in **1** was further suggested by catalytic hydrogenation; **1** was converted with 10% Pd–C into lupanine. These properties of **1** agree with the results of Cho and Martin [9] for the authentic 5, 6-dehydrolupanine. From the above results, it can therefore be presumed that the structure of **1** is 5, 6-dehydrolupanine (**1**).



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The seasonal variations in the alkaloid content of *E. koreensis* were examined using the samples collected at the each stages of pre-flowering (middle of February), flowering (this stage at the beginning of April is accompanied with budding of the leaves), the young fruit-bearing (middle of May), the fructuous (end of June) and at the season immediately before leaf fall (middle of October). The results obtained from the various parts of this plant are shown in Table 1 and Figs. 1–3. The major alkaloids of *E. koreensis* were (−)-cytisine and (−)-*N*-methylcytisine; the former was predominant in the leaves, stems and roots throughout and the latter was a main component in the leaves. The content of both the major alkaloids varied remarkably with growth of the plant. The concentration of (−)-cytisine markedly

*A part of this work was presented at the 26th Annual Meeting of the Japanese Society of Pharmacognosy at Tokyo, 9 November 1979 (*Meeting Abstract* p. 18).

Table 1. Distribution of lupin alkaloids in leaves, stems and roots of *Echinosophora koreensis**

Alkaloids†	Leaves	Stems	Roots
Total base	1.11	0.53	0.51
(−)-Cytisine	0.200	0.410	0.40
(−)-*N*-Formylcytisine	0.0045	0.0058	0.0082
(−)-*N*-Methylcytisine	0.670	0.055	0.014
(−)-Rhombifoline	tr	tr	tr
(−)-*N*-(3-Oxobutyl)-cytisine	tr	tr	tr
(−)-Anagyrine	0.002	0.0016	0.0061
(−)-Baptifoline	0.026	0.061	0.061
Lupanine‡	tr	tr	tr
5, 6-Dehydrolupanine (**1**)‡	tr	tr	tr

*The plant materials were collected in April at flowering in the medicinal plant garden at the Chiba University.

†Alkaloid contents were quantitatively estimated by HPLC as described in the Experimental and are shown in %/fr. wt.

‡Optical rotation not determined due to shortage of material.

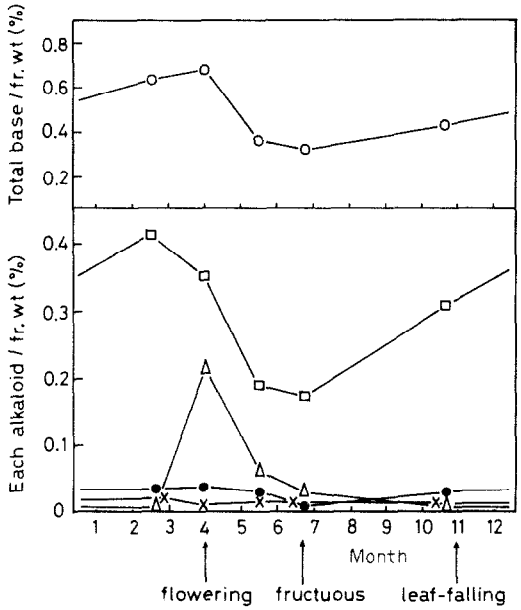

Fig. 1. Variations in alkaloid content in whole plants of *Echinosophora koreensis* during growth. (○), total base; (□), (−)-cytisine; (△), (−)-*N*-methylcytisine; (×), (−)-anagyrine; (●), (−)-baptifoline.

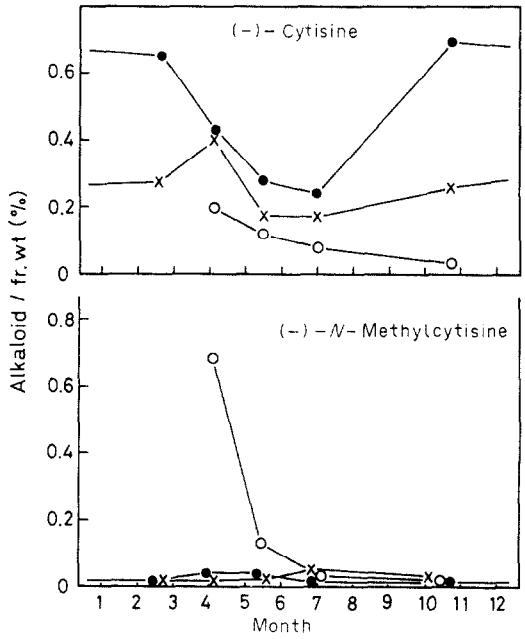

Fig. 2. Variations in content of (−)-cytisine and (−)-*N*-methylcytisine in leaves (○), stems (●) and roots (×) of *Echinosophora koreensis* during growth.

decreased during the growing season from pre-flowering to fructuous, while (−)-*N*-methylcytisine increased rapidly at flowering. In the later stages of flowering, the concentration of (−)-*N*-methylcytisine diminished and it again became a major component particularly in the leaves (Figs. 1 and 2). A significant decrease in (−)-cytisine content in the stems at the early stages of plant growth seems to be associated with the transient rapid accumulation of (−)-*N*-methylcytisine in the young leaves. Such a complementary variation in the content of (−)-cytisine and (−)-*N*-methylcytisine has also been observed during seedling growth of *Thermopsis chinensis* [12] and *Sophora tomentosa* [13], in which cytisine *N*-methyltransferase, an enzyme catalysing the transformation of (−)-cytisine into (−)-*N*-methylcytisine, has been found by Murakoshi *et al.* [12]. These facts strongly suggest that (−)-cytisine in the stems is transformed into (−)-*N*-methylcytisine, which is accumulated in the young leaves, at the early stage of growth of *E. koreensis*. Thus, it is particularly interesting from the viewpoint of the physiological role of the lupin alkaloids in the plants that the complemen-

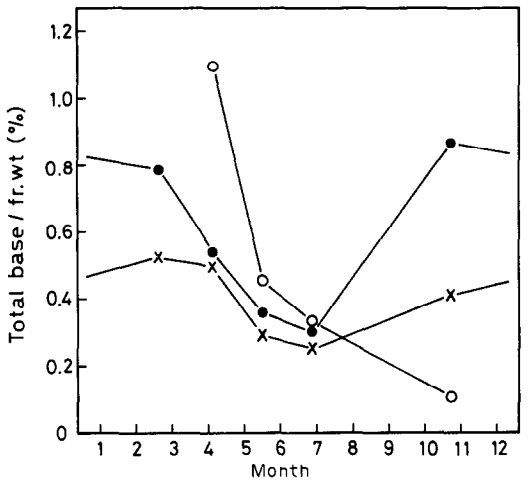

Fig. 3. Variations of total alkaloid concentration in leaves (○), stems (●) and roots (×) of *Echinosophora koreensis* during growth.

tary variation in the content of (−)-cytisine and (−)-*N*-methylcytisine corresponds to the biosynthetical interconversion and takes place mainly at the stage when the plants are actively growing.

The concentrations of alkaloid in the leaves, stems and roots of this plant decreased remarkably during the growing season in parallel with the variation of the major alkaloid in the individual parts, namely with the variation of (−)-cytisine in the stems and roots and that of (−)-*N*-methylcytisine in the leaves (Figs. 1 and 3).

EXPERIMENTAL

General methods. Mps are uncorr. MS were measured at 70 eV using a direct inlet system. The ^{1}H NMR (100 MHz) spectra were recorded with TMS as int. standard. Analytical TLC was 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); (4) Et_2O–MeOH–28% NH_4OH (40:2:1); (5) Et_2O–MeOH–28% NH_4OH (70:30:1) and on Al_2O_3 plates in (6) C_6H_6–MeOH–Me_2CO (34:3:3). Analytical HPLC was performed with solvents: (7) 15% MeOH in Et_2O–2.5% NH_4OH (50:1); (8) 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:10:3); (9) 3% MeOH in CH_2Cl_2–25% NH_4OH (500:1) using a LiChrosorb SI 100 (Merck, particle size 10 μm, 0.3 × 50 cm) column employing a monitoring flow system (220 and 310 nm) at a flow rate of 1 ml/min. The chromatographic behaviour of the alkaloids is summarized in Table 2.

Isolation and characterization of alkaloids. The alkaloid fraction (0.6 g) obtained from the 75% EtOH extract of fresh leaves (180 g) of *E. koreensis* Nakai, collected in June at the Medicinal Plant Gardens of Chiba University, was extracted with petrol. The petrol insoluble fraction (0.20 g) was chromatographed on a Si gel column (Merck, type 60, 160 g, 2 × 62 cm) using solvent 1 to give (−)-*N*-formylcytisine (3 mg), (−)-cytisine (130 mg) and (−)-baptifoline (1 mg) in order of elution. The petrol soluble fraction (0.4 g) was dissolved in boiling *n*-hexane and then stood at room temp. The crystalline ppts obtained from the *n*-hexane solution were recrystallized from *n*-hexane to give colourless crystals of (−)-*N*-methylcytisine (30 mg). The mother liquor (0.35 g) was applied to a Si gel (Merck, type 60, 2 × 62 cm) column eluting with solvent 4 and 40 ml fractions were collected. (−)-Rhombifoline (2 mg) was isolated from fractions 23–33 by prep. TLC in solvent 4. Prep. TLC of fractions 49–66 with solvent 4 gave (−)-anagyrine (27 mg) and 5, 6-dehydrolupanine (**1**, 6 mg). Fractions 67–72 was subject to prep. TLC in solvent 4 to give a further amount of **1** (4 mg) and lupanine (3 mg). Fractions 105–111 were crystallized from C_6H_6–petrol to give colourless crystals of (−)-*N*-(3-oxobutyl)-cytisine (2 mg). 5, 6-Dehydrolupanine (**1**): colourless oil, UV λ_{max}^{EtOH} 250 nm, MS (70 eV) *m/z* (rel. int.); 246 $[M]^+$ (44), 245 (15), 134 (9), 98 (100), 97 (31). ^{1}H NMR ($CDCl_3$) δ 4.92 (1H, *t*, *J* = 4 Hz, H-5), 4.02 (1H, *d*, *J* = 12 Hz, H_α-10), 3.25 (1H, *dd*, *J* = 12 and 5 Hz, H_β-10). **1** was quantitatively hydrogenated over 10% Pd–C in EtOH under atmospheric pressure at room temp. to give lupanine, which was identified by direct comparison with the authentic sample (MS, co-TLC and co-HPLC). The product of the reaction of **1** with nihydrin reagent was pale red, which subsequently turned to red–brown.

Estimation of alkaloid content. Samples of *E. koreensis* were collected the middle of February (pre-flowering), beginning of April (flowering and leaf-budding), middle of

Table 2. Physical properties and chromatographic behaviour of lupin alkaloids isolated from *Echinosophora koreensis*

Alkaloids	mp(°)	$[\alpha]_D^{\circ}$	R_f TLC*						R_t(min)HPLC*		
			1	2	3	4	5	6	7	8	9
(−)-Cytisine	155	−116	0.35	0.57	0.16	—	0.18	—	47.5	29.3	40.8
(−)-*N*-Formylcytisine	170–172	−233	0.42	0.60	0.52	—	0.21	0.27	35.8	30.5	16.1
(−)-*N*-Methylcytisine	137	−223	0.61	—	0.52	0.26	0.59	0.64	10.5	7.5	13.5
(−)-Rhombifoline	oil	−232	0.67	—	0.76	0.49	0.76	0.73	4.5	—	—
(−)-*N*-(3-Oxobutyl)cytisine	118	−212	0.65	—	0.70	0.18	0.66	0.60	7.7	6.5	10.5
(−)-Anagyrine	oil	−165	0.65	—	0.57	0.34	0.63	0.70	8.4	5.6	12.3
(−)-Baptifoline	210	−137	0.30	0.45	0.27	—	0.33	0.24	24.3	17.0	—
Lupanine	oil	†	0.67	—	0.26	0.41	0.42	0.71	15.0	10.5	—
5, 6-Dehydrolupanine	oil	†	0.72	—	0.49	0.65	0.68	0.76	5.5	—	—

*Solvents 1–9 for TLC and HPLC are described in the Experimental.
†Optical rotations not measured.

May (young fruit-bearing), end of June (fruiting) and end of October (immediately before leaf-fall), and each sample was separated into leaves, stems and roots. The freshly harvested plant materials were homogenized in 75% EtOH and extracted $\times 3$ with 75% EtOH at room temp. The combined extracts were concd *in vacuo* until the organic solvent was completely removed, acidified with dilute HCl and filtered. The filtrate was extracted twice with Et_2O, made alkaline with K_2CO_3 at 0° and extracted $\times 5$ with CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried (K_2CO_3) and evaporated to dryness *in vacuo*. The wt of residue was the total alkaloid concn. The contents of individual alkaloids in various fresh parts of the plant were estimated by HPLC using solvents 7, 8 and 9, as described in previous papers [1–6, 12, 13].

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