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LUPIN ALKALOIDS FROM FLOWERS OF *ECHINOSOPHORA KOREENSIS*

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Key Word Index—*Echinosophora koreensis*; Leguminosae; flowers; lupin alkaloid; quinolizidine alkaloid; (−)-*N*-ethylcytisine; (−)-methyl 12-cytisineacetate; ethyl 12-cytisineacetate; (−)-12-cytisineacetic acid; (−)-cytisine; variation of alkaloid content.

Abstract—(−)-Methyl 12-cytisineacetate (**2**) was isolated from methanol extracts of fresh flowers of *Echinosophora koreensis* together with seven known lupin alkaloids. Ethyl 12-cytisineacetate (**3**) was also isolated from ethanol extracts of the same flowers. Compounds **2** and **3** were artifacts and (−)-12-cytisineacetic acid (**4**) is assumed to be the principal source of **2** and **3**. The variations in alkaloid content during growth of the flowers and the seedlings were also examined.

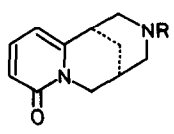
INTRODUCTION

Echinosophora koreensis is a deciduous shrub native to Korea closely related to the genus *Sophora* (Leguminosae). In the course of investigations on lupin alkaloids in legumes [1–3], we have previously reported the presence of (−)-*N*-(3-oxobutyl)cytisine [4, 5], (−)-*N*-ethylcytisine (**1**) [6], (−)-cytisine, (−)-*N*-methylcytisine, (−)-*N*-formylcytisine, (−)-rhombifoline, (−)-baptifoline, (−)-anagyrine, (−)-lupanine and 5,6-dehydrolupanine [5] in the freshly harvested leaves, stems and roots of *E. koreensis*. Further chemical examination of basic constituents of the flowers of *E. koreensis* resulted in the isolation of (−)-methyl 12-cytisineacetate (**2**) together with seven lupin alkaloids, which have been previously isolated from the other parts of this plant. Compound **1** was presumed to be an artifactual product formed from (−)-12-cytisineacetic acid (**4**) during extraction in 75% methanol, by analogy with the case of the isolation of **2** from the epigeal parts of *Euchresta japonica* [7, 8]. The present paper describes the isolation of eight known lupin alkaloids from the fresh flowers of *E. koreensis*. The distribution of the various lupin alkaloids in different organs of the plant is also reported.

RESULTS AND DISCUSSION

The alkaloid mixture (2.5 g) obtained from the 75% methanol extracts of the freshly harvested flowers (422 g) of *E. koreensis* was repeatedly chromatographed on silica gel columns to give seven lupin alkaloids, (−)-methyl 12-cytisine-acetate (**2**), (−)-anagyrine, (−)-*N*-methylcytisine, lupanine, (−)-baptifoline, (−)-*N*-formylcytisine and (−)-cytisine in addition to (−)-*N*-ethylcytisine (**1**). These known alkaloids were identified by comparing the natural products directly with authentic samples in all measurable respects (mass spectrometry, IR, $[\alpha]_D$, co-TLC and co-HPLC) as described in our previous papers [1–8].

Since **2** might be an artifact formed from (−)-12-cytisineacetic acid (**4**) during extraction with 75% methanol, similarly to the isolation of **2** from *Euchresta japonica* [7, 8], newly harvested flowers of *E. koreensis* were extracted with 75% ethanol in place of 75% methanol. The alkaloid mixture obtained from the 75% ethanol extract was treated in a manner similar to the isolation of **2** from the 75% methanol extract. In this case, **2** could not be isolated, but unknown compound **3**, which was not detected in the 75% methanol extract, was obtained, by analogy with the isolation of **2**, from the first effluent of a silica gel column chromatography of the alkaloid mixture together with (−)-*N*-ethylcytisine and (−)-anagyrine. The mass spectrum of **3** showed $[M]^+$ at *m/z* 276 (22% rel. int.) which was 14 mu more than that of **2** and the main fragment ions were similar to those of **2** except for showing an ion at *m/z* 130 instead of the ions at *m/z* 116 in the spectrum of **2**. The ^1H NMR spectrum of **3** was superimposable on that of **2** except that the spectrum of **3** showed signals at δ1.20 (3H, *t*, J = 7 Hz) and δ4.10 (2H, *q*, J = 7 Hz) assigned to a CO_2Et group in the place of the 3H singlet at δ3.08 due to a CO_2Me group in that of **2**. These results indicate that **3** might be ethyl 12-cytisineacetate. The structure of **3** was confirmed by comparison of the spectroscopic data of **3** with those of the synthetic sample prepared from (−)-cytisine and ethyl bromoacetate. Accordingly, **2** and **3** are artifacts and (−)-12-cytisineacetic acid (**4**) is presumed to be the source of both **2** and **3**.



1 R = Et
2 R = COOMe
3 R = COOEt
4 R = COOH

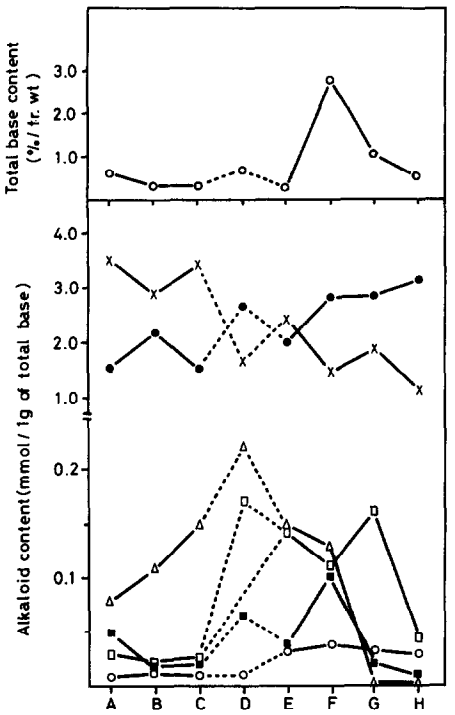


Fig. 1. Variations of alkaloid content in the flowers, seeds and seedlings of *Echinosophora koreensis* at various stages during growth. A, budding; B, full; C, end; D, immature pods; E, immature seeds; F, mature seeds; G, 4-day-old seedlings; H, 8-day-old seedlings. Seedlings were grown at 27–28° in the dark. (○), (−)-*N*-Ethylcytisine; (□), (−)-anagyrine; (■), (−)-baptifoline; (△), (−)-*N*-formylcytisine; (×), (−)-*N*-methylcytisine; (●), (−)-cytisine.

Variations in alkaloid content at various stages of the flower and seedling growth are shown in Fig. 1.

EXPERIMENTAL

General. Mps are uncorr. MS were measured at 70 eV using a direct inlet system. ^{1}H NMR spectra were determined at 100 MHz in $CDCl_3$ with TMS as int. standard. TLC was performed on silica gel (Merck, GF_{254}, type 60) with solvents: 1, CH_2Cl_2–MeOH–28% NH_4OH (90:9:1); 2, Et_2O–MeOH–28% NH_4OH (40:2:1); 3, 10% MeOH in Et_2O–28% NH_4OH–H_2O (500:5:1). Analytical HPLC was carried out with solvent 4, 15% MeOH in Et_2O–2.5% NH_4OH (50:1) using a LiChrosorb SI 100 (Merck, 10 μm, 3 × 500 mm) column employing a monitoring flow system (220 nm) at a flow rate of 1 ml/min. Kiesel gel 60 (Merck, 70–230 mesh) was used for CC.

Plant material. Flowers of *E. koreensis* Nakai were collected in April at the Medicinal Plant Garden of Chiba University. Voucher specimens have been deposited in the Herbarium of Faculty of Pharmaceutical Sciences, Chiba University, Chiba, Japan.

Isolation of alkaloids. Freshly harvested flowers (422 g) were extracted × 3 with 75% MeOH at room temp over a period of 10 days. The alkaloid mixture (2.5 g) obtained was chromatographed on a silica gel (150 g) column with solvent 2, 30 ml fractions being collected. Fractions 19–30 was subjected to HPLC using solvent 3 to give (−)-*N*-ethylcytisine (**1**, 4.7 mg) and (−)-methyl 12-cytisineacetate (**2**, 2.5 mg). CC separation of fractions 31–41 on silica gel with solvent 4 yielded (−)-anagyrine (2 mg), (−)-*N*-methylcytisine (35 mg) and lupanine (0.5 mg). Crystallization of fractions 42–60 from *n*-hexane gave colourless needles of a further amount of (−)-*N*-methylcytisine (2.0 g). Fractions 61–70 was re-chromatographed on silica gel column with solvent 4 to give (−)-baptifoline (4 mg), (−)-*N*-formylcytisine (5 mg) and (−)-cytisine (190 mg).

Isolation of ethyl 12-cytisineacetate (**3**). Fresh flowers (500 g) harvested in April were extracted with 75% EtOH as described above. The alkaloid mixture (2.95 g) obtained from the 75% EtOH extract was subjected to silica gel (150 g) CC eluted with solvent 2 as described above and 30 ml fractions were collected. Ethyl 12-cytisineacetate (**3**) appeared in fractions 25–38 together with (−)-*N*-ethylcytisine and (−)-anagyrine. The fraction was separated by prep. HPLC with solvent 4 to give 1.2 mg of ethyl 12-cytisineacetate (**3**); EIMS *m/z* (rel. int.): 276 $[M]^+$ (22), 230 (10), 203 (100), 160 (16), 146 (16), 130 (24), 58 (52). ^{1}H NMR ($CDCl_3$): δ1.20 (3H, *t*, *J* = 7 Hz, $-COOCH_2\underline{CH}_3$), 3.18 (2H, *s*, $>NCH_2CO-$), 3.75–4.15 (2H, *m*, 10-H_2), 4.10 (2H, *q*, *J* = 7 Hz, $-COO\underline{CH}_2CH_3$), 5.98 (1H, *dd*, *J* = 7 and 1.5 Hz, H-5), 6.44 (1H, *dd*, *J* = 9 and 1.5 Hz, H-3), 7.28 (1H, *dd*, *J* = 9 and 7 Hz, H-4).

Synthesis of **3**. To a soln of (−)-cytisine (57 mg, 0.30 mmol), isolated from *E. koreensis*, in C_6H_6 (5 ml) were added Et bromoacetate (84 mg, 0.50 mmol) and Et_3NH (0.5 ml). The reaction mixture was refluxed for 1.5 hr. After cooling, the solvent was removed *in vacuo* and the residue purified by silica gel CC with solvent 2 to give a colourless oil (45 mg) of **3**. The synthetic product was identical with the natural product (IR, MS, ^{1}H NMR).

Estimation of alkaloid content. Samples of flowers and seeds were collected during the periods of flower-budding, flowering, immediately after petal fall, seed-growth, which was divided into the seeds and pods, and maturity of seeds. Skinned seeds were germinated and grown on moistened vermiculite in the dark at 28°. Samples of seedlings were collected after 4 and 8 days of germination. Individual alkaloid mixtures were extracted in a similar manner to that described previously [1, 5]. The content of individual alkaloids were estimated by HPLC using solvent 4.

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