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N-(3-OXOBUTYL)CYTISINE: A NEW LUPIN ALKALOID FROM *ECHINOSOPHORA KOREENSIS**

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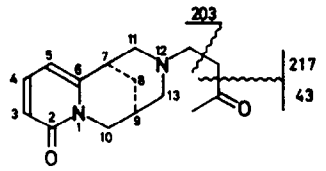
Key Word Index—*Echinosophora koreensis*; Leguminosae; alkaloid; cytisine; *N*-(3-oxobutyl)cytisine.

Abstract—A new lupin alkaloid, *N*-(3-oxobutyl)cytisine, was isolated from the aerial parts of *Echinosophora koreensis*. Its structure was determined by spectrometric data and by direct comparison with a synthetic sample.

As a result of screening plants belonging to the Leguminosae for lupin alkaloids, a new alkaloid, *N*-(3-oxobutyl)-cytisine (**1**), was isolated from the fresh aerial parts of *Echinosophora koreensis* as colourless plates, mp 118°, $[\alpha]_D^{21}$ −211.6°.

Echinosophora koreensis Nakai (Japanese name, inukurara) is a perennial shrub, which is native in Korea and closely related to the genus *Sophora* (Leguminosae). It was growing in the medical plant garden at the University of Chiba.

The structure was suggested as *N*-(3-oxobutyl)-cytisine (**1**) by the IR absorption bands at 2700–2800 (*trans*-quinolizidine), 1650 (α-pyridone) and 1700 cm^{-1} (ketone), and by the MS, M^+ at *m/e* 260 (12), *m/e* 160 (12), 147 (9) and 146 (20) which were characteristic of lupin alkaloids containing a α-pyridone ring, such as cytisine and *N*-methylcytisine, as described in previous papers [1, 2]. The PMR spectrum (100 MHz, C_6D_6) of the new base was essentially superimposable on that of *N*-methylcytisine except for the signals at 2 (A_2B_2, 4H, *m*, N—CH_2—CH_2—CO) and 1.52 (3H, *s*, —CO—Me). The other significant signals were at 2.4 (2H, *br*, *d*) for equatorial H at C-11 and C-13, as well as *N*-methylcytisine. The IR, MS, and PMR clearly indicated the presence of a Me—CO—CH_2—CH_2— function in the N-12 of cytisine. Therefore, the new alkaloid was presumed to be *N*-(3-oxobutyl)cytisine (**1**). Further confirmation of the identity of the new alkaloid was obtained by comparing the natural compound directly with a synthetic sample, prepared from cytisine and methylvinylketone.



(**1**)

Scheme 1. Characteristic fragment ions in the MS of *N*-(3-oxobutyl)cytisine (**1**).

N-(3-Oxobutyl)cytisine might be an intermediate in the biosynthesis of cytisine from the baptifoline-type compounds or *vice versa*. The biosynthesis and the function of **1** in plants are currently being undertaken in our laboratories.

EXPERIMENTAL

Mps were uncorr. UV and IR spectra were determined in EtOH and KBr pellets, respectively. MS were measured at 70 eV, and NMR spectra on a 100 MHz instrument with TMS as an internal standard.

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Isolation of **1**. The alkaloid fraction (2.56 g) obtained from the 75% EtOH extracts of the fresh aerial parts of *E. koreensis* (600 g), collected in September, was chromatographed, essentially as described in refs. [1–3], on a Si gel column (Merck, type 60) using CH_2Cl_2–MeOH–conc NH_4OH (1000:30:3) to CH_2Cl_2–MeOH–conc NH_4OH (90:9:1). **1** appeared in the early eluates under the above chromatographic conditions and was obtained as crystals, mp 118° (C_6H_6–petrol); $[\alpha]_D^{21}$ −211.6 (EtOH, *c* 0.219); λ_{max}^{EtOH} nm (log ε): 232 (3.81), 306 (3.89): found: C, 69.26; H, 7.78; N, 10.67. $C_{15}H_{20}O_2N_2$ requires: C, 69.20; H, 7.74; N, 10.76. IR (KBr) ν max cm^{-1}: 2700–2800 (*trans*-quinolizidine), 1700 (C = 0), 1650 (α-pyridone C = 0). MS (70 eV): *m/e* 260 (M^+, 12), 217 (15), 203 (16), 160 (12), 147 (9), 146 (20), 114 (100), 58 (28), 43 (54). PMR (C_6D_6): δ 1.05 (*m*, 2H, C-8 CH_2), 1.4–2.3 (*m*, 11H containing a *s*, 3H at 1.52 (—CO—Me) and an A_2B_2 *m*, *ca* 4H at 2 (—CO—$CH_2CH_2N<$)), 2.4 (*bd*, 2H, *J* = 10 Hz, equatorial H at C-11 and C-13), 3.65 (*q*, 1H, *J* = 15.5, 7 Hz, C-10 H), 3.99 (*d*, 1H, *J* = 15.5 Hz, C-10 H), 5.37 (*q*, 1H, *J* = 6.5, 1.5 Hz, C-5 H), 6.52 (*q*, 1H, *J* = 9, 1.5 Hz, C-3 H), 6.8 (*q*, 1H, *J* = 9, 6.5 Hz, C-4 H).

Synthesis of **1**. A mixture of cytisine (60 mg, 0.32 mmol), isolated from *Sophora* and *Thermopsis* sp., and methylvinylketone (76 μl, 0.96 mmol) in C_6H_6 (3 ml) was heated at 60° for 3 hr. After evaporating the solvent *in vacuo*, the residue was purified by Si gel column chromatography, developed with MeOH–CH_2Cl_2 (49:1). **1** was recrystallized from C_6H_6–petrol to give colourless plates, mp 118°, $[\alpha]_D^{19}$ −210° (EtOH, *c* 0.31). The synthetic product was found to be completely identical with those of the natural product by IR, MS, PMR, and chromatographic comparisons.

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METABOLISM OF VINDOLINE, CATHARANTHINE HCl, AND VINCALEUKOBLASTINE SULFATE BY SUSPENSION CULTURES OF *CATHARANTHUS ROSEUS*

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Key Word Index—*Catharanthus roseus*; Apocynaceae; suspension culture; alkaloids; vindoline; catharathine HCl; vincaleukoblastine sulphate; metabolites.

Callus tissue of *Catharanthus roseus* (L.) G. (Don.), culture line Wc13S, [1] continuously subcultured since 1961, was subcultured onto a chemically defined agar medium (PRL1) [2] in the fall of 1972. Suspension cultures were initiated as needed from three week old subcultures of this PRL1 callus line and grown in PRL1 liquid medium. Suspension cultures were grown in 100 ml of medium in 500 ml flasks. The cultures were shaken at 135 rpm in the dark at 24 ± 1°. Cultures were incubated for 14 to 21 days prior to addition of the alkaloid substrate for metabolism studies. Alkaloids were added aseptically to the cultures as separate 70% ethanol solutions. Each alkaloid was added separately at a concentration of 10 mg per 100 ml for vindoline and catharanthine HCl and 9.4 mg for vincaleukoblastine sulphate (VLB.SO_4). Each metabolism experiment consisted of 10 replicate flasks. In an additional experiment, vindoline and catharanthine HCl were added simultaneously, each to a final concentration of 100 mg/l. to 10 replicate flasks. Alkaloid containing cultures were incubated for 1, 3, or 7 days prior to harvest. Suspension tissue plus medium was extracted [3] and examined using multiple TLC and PLC chromatographic systems for expected metabolites. Metabolites were visualized using Dragendorff's and ceric ammonium sulfate spray reagents [4]. Identification of metabolites was based on TLC (five solvent systems), and MS comparison to reference compounds. The solvent systems utilized were EtOAc, EtOAc–EtOH (1:1), EtOAc–EtOH (3:1, *n*-BuOH–HOAc–H_2O (4:4:1), and MeOH dried over Na_2SO_4. Appropriate control experiments were conducted to ensure that isolated compounds were not artifacts.

Only two metabolites of vindoline were present on the chromatograms. These were identified as desacetylvindoline (DAV) and dihydrovindoline (DHV). No metabolite of catharanthine HCl was detected. Metabolites isolated from the cultures fed both vindoline and catharanthine HCl consisted only of DAV and DHV. Three metabolites were isolated from extracts of tissue which had been fed VLB sulfate (as well as unmetabolized VLB sulfate). Preliminary MS investigations indiate that two of these are dimeric alkaloids (*m/e*'s greater than 750). The third does not show an *m/e*