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(−)-12-CYTISINEACETIC ACID, A NEW LUPIN ALKALOID IN *EUCHRESTA JAPONICA**

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Key Word Index—*Euchresta japonica*; Leguminosae; alkaloids; methyl 12-cytisineacetate; 12-cytisineacetic acid; cytisine.

Abstract—A new lupin alkaloid, methyl 12-cytisineacetate **1**, was isolated from the MeOH extract of *Euchresta japonica*. Its structure was confirmed by spectrometric data and by direct comparison with a synthetic sample. However, **1** is an artifact product and 12-cytisineacetic acid (**2**) is assumed to be the principal source of **1**.

INTRODUCTION

In our continuing studies on the lupin alkaloids in the legume [1–8], we have previously reported the presence of a new alkaloid, 5,17-dehydromatrine *N*-oxide, in *Euchresta japonica* [6]. We now report the isolation of one more new lupin alkaloid, methyl 12-cytisineacetate **1**, from the MeOH extract of the same plant. However, **1** appears to be an artifact which arises from 12-cytisineacetic acid **2** during extraction in a widely used MeOH solvent system.

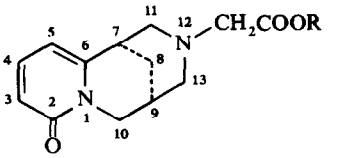
RESULTS AND DISCUSSION

Compound (**1**), recrystallized from *n*-hexane as colourless crystals, mp 107–109°, $[\alpha]_D^{15}$ −174.2°. The molcular formula, $C_{14}H_{18}O_3N_2$, was established by high resolution MS.

The structure was suggested as methyl 12-cytisineacetate (**1**) by its IR absorption bands at 1740 (ester-CO), 1655 (α-pyridone), and by the MS, M^+ at *m/e* 262 (22%), 160 (22) and 146 (23) which are characteristic of lupin alkaloids having an α-pyridone-ring [1, 2, 4]. The PMR spectrum ($CDCl_3$) of **1**, except for the two isolated signals at δ 3.18 (2H, *s*, $-CH_2-$) and 3.63 (3H, *s*, CH_3), was essentially superimposable on that of *N*-methylcytisine, viz. δ 1.84 (2H, *m*, C-8 $-CH_2-$), 2.43 (1H, *br*, C-9 H), a set of δ 3.85 and 4.08 (*dd* and *bd*, C-10 $-CH_2-$), together with the downfield signals due to the α-pyridone ring.

In view of the above results, the structure of the new alkaloid was presumed to be methyl 12-cytisineacetate (**1**). Further confirmation of the identity of the new alkaloid as **1** was obtained by comparing the natural product directly with a synthetic sample, prepared from (−)-cytisine and methyl bromoacetate.

Methyl 12-cytisineacetate (**1**) might be an artifact arising from an esterification of 12-cytisineacetic acid (**2**), since the extraction was performed by using 75% MeOH. Therefore, the presence of **2** in the fresh plant



1 R = Me
2 R = H

was examined. Three 75% EtOH extracts from the fresh leaves, stems and roots of *E. japonica* were separately treated with IR-120B (H^+ form) and each fraction containing the zwitter-ion compounds was directly subjected to TLC for the presence of **2**. TLC analyses of all the fractions revealed clearly the presence of **2** in all of the five solvent systems used (see Experimental). Accordingly, the occurrence of the free carboxylic acid (**2**) in the whole parts of *E. japonica* can be considered to be the normal form in the intact plant and not the new alkaloid (**1**).

EXPERIMENTAL

MPs are uncorr. The high and low resolution MS were measured at 70 eV. The PMR (100 MHz) was recorded using TMS as internal standard.

Isolation of **1** was as described in previous papers [1–8]. The alkaloid fraction (9.5 g) obtained from the 75% MeOH extracts of the fresh aerial parts of *E. japonica* collected in July at Kagoshima area, Japan, was chromatographed on a Si gel column (Merck, type 60, 3 × 96 cm) with solvents of increasing alkalinity from [1.5% MeOH. CH_2Cl_2]–28% NH_4OH (1000:1) to [11% MeOH . CH_2Cl_2]–28% NH_4OH (1000:11). **1** appeared as a single compound on elution with [3% MeOH . CH_2Cl_2]–28% NH_4OH (1000:2). The fractions containing **1** were further purified on a Si gel column to give colourless crystals (45 mg), mp 107–9° (*n*-hexane). **1**. $[\alpha]_D^{15}$ −174.2° (*c* = 0.19, EtOH); IR λ_{max}^{KBr} cm^{-1}: 1740 (ester C=O), 1655 (α-pyridone C=O). [1, 2, 4]; MS: *m/e* 262.1315 (M^+, $C_{14}H_{18}O_3N_2$ requires 262.1313, *m/e* 262 (M^+, 22%), 203 (68), 160 (22), 146 (23), 116 (53), 58 (100) [1, 2, 4]; PMR ($CDCl_3$): δ1.84 (*m*, 2H, C-8 H_2), 2.43 (*bm* 1H, C-9 H), 2.6–3.1 (*m*, 5H, C-11 and C-13 H_2, C-7 H), 3.18 (*s*, 2H, N—CH_2—C=O), 3.63 (*s*, 3H, $-COOCH_3$), 3.85 (*dd*,

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1H, J = 15.5 and 6 Hz, C-10 H), 4.08 (*bd*, 1H, J = 15.5, C-10 H), 5.98 (*dd*, 1H, J = 7 and 1.5 Hz, C-5), 6.44 (*dd*, 1H, J = 9 and 1.5 Hz, C-3 H), 7.28 (*dd*, 1H, J = 9 and 7 Hz, C-4 H).

Synthesis of **1**. A soln of cytisine (57 mg, 0.3 mmol), isolated from *Sophora* and *Thermopsis* spp., methyl bromoacetate (69 mg, 0.45 mmol) and triethylamine (0.5 ml) in C_6H_6 (5 ml) was refluxed for 1.5 hr. After removing the solvent *in vacuo*, the residue was purified by Si gel column chromatography using MeOH–CH_2Cl_2 (1:49). **1** was recrystallized from *n*-hexane as colourless crystals, mp 107–109°, $[\alpha]_D^{15}$ −175.1° (c = 0.27, EtOH). The synthetic product was found to be identical with the natural product (IR, MS, PMR and chromatography).

Hydrolysis of **1** *to* (−)-12-*cytisineacetic acid* (**2**). **1** (50 mg) was heated with 5% NaOH (5 ml) at 60° for 2 hr. The reaction mixture was neutralized with dil. HCl, treated with IR-120B (H^+ form) to remove the yielded salts and the resulting product, after removal of the solvent, was recrystallized from EtOH–H_2O to give colourless crystals. Yield, 41 mg (87%). **2**, mp 234–5° (decomp); $[\alpha]_D^{15}$ −200.1° (c = 0.34, H_2O): IR λ_{max}^{KBr} cm^{-1}: 3000–2500, 1710 (COOH), 1635 (α-pyridone C=O). **2** exhibited on TLC positive reactions with Dragendorff's and iodoplatinate reagents.

Tentative identification of **2** *in* E. japonica. Well-chopped fresh leaves (7 g), stems (12 g) and roots (32 g) of *E. japonica*, collected in August at Kagoshima aeria in Japan, were separately soaked in 75% EtOH and extracted 5× with the same solvent for 10 days. Each extract was reduced to 1/5 in vol., adjusted to pH 5 with dil. HCl and passed through a column of IR-120B (H^+ form, 2 × 30 cm). The resin columns were washed with 50% EtOH and H_2O and the basic compounds were then eluted with 3% NH_4OH. The eluates, basified further with 28% NH_4OH to pH 10.5–11, were extracted with CH_2Cl_2 to remove the basic constituents and the aq. layers were concd to dryness *in vacuo*. By this means 570, 305 and 250 mg of the CH_2Cl_2-insoluble fraction (Zwitterion compounds) were obtained from the parts of leaves, stems and roots, respectively.

For the identification of **2** in the CH_2Cl_2-insoluble fraction, analytical TLC was performed on Si gel in the following solvent systems: (1) BuOH–HOAc–H_2O (4:1:1), (2) 96% EtOH–28% NH_4OH (7:3), (3) *n*-PrOH–28% NH_4OH (7:3), (4) $CHCl_3$–MeOH–17% NH_4OH (2:2:1), (5) $CHCl_3$–MeOH–17% NH_4-OH (11:8:1). R_f values for **2** on Si gel TLC in these solvents were 0.08, 0.73, 0.50, 0.92 and 0.41, respectively. **1** was not identified in the CH_2Cl_2 extracts.

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