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A NEW LUPINE ALKALOID, (−)-Δ^5-DEHYDROMULTIFLORINE, FROM THE SEEDS OF *LUPINUS TERMIS*

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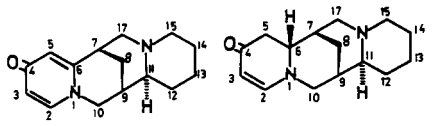
ABSTRACT.—A new lupine alkaloid, (−)-Δ^5-dehydromultiflorine [**1**], was isolated from the viable seeds of *Lupinus termis*. The unusual lupine alkaloids (−)-multiflorine [**2**], (+)-angustifoline and (±)-lupanine *N*-oxide were also isolated, together with (±)-lupanine and (+)-13-hydroxylupanine. The structure of **1** was determined by spectroscopic methods.

As a result of screening plants belonging to the Leguminosae for lupine alkaloids (1–4), a novel lupine alkaloid, (−)-Δ^5-dehydromultiflorine [**1**], was isolated from the viable seeds of the Egyptian lupine, *Lupinus termis* Forsk. *L. termis* is an annual herb which is cultivated in the countries of the Mediterranean region for its edible seeds (5). Previous work on the basic constituents in the seeds of *L. termis* has demonstrated the presence of (±)-lupanine and (+)-13-hydroxylupanine (6–8).

In further investigations, the basic components in the viable seeds of the Egyptian lupine yielded the new lupine alkaloid **1** together with the five known lupine alkaloids (±)-lupanine, (−)-multiflorine [**2**], (+)-angustifoline, (+)-13-hydroxylupanine, and (±)-lupanine *N*-oxide. This is the first isolation of (±)-lupanine *N*-oxide from natural sources, while (+)-lupanine *N*-oxide has already been isolated from *Thermopsis lupinoids* (1).

From the 75% EtOH extract of the seeds, **1**, as a colorless oil, $[\alpha]^{25}D$ −94.4°, was isolated (0.01%/fresh wt) using Si gel chromatography. The hreims spectrum of **1** indicated the molecular formula $C_{15}H_{20}N_2O$ ($[M]^+$ *m/z* 244.1573, calcd 244.1574). The uv spectrum of **1** (λ max 263 nm/MeOH) (log ε = 3.935) suggested the presence of a γ-pyridone ring system (9,10). In the ir spectrum, the bands at 1640 cm^{-1} and 1560 cm^{-1} showed the presence of a conjugated carbonyl and double bonds, but the trans quinolizidine bands (2800–2600 cm^{-1}) were not observed in comparison with those of (−)-multiflorine [**2**] (11–14). These data suggest that **1** is Δ^5-dehydromultiflorine in which ring A is the γ-pyridone type and rings C and D are fused in the cis configuration.



1 **2**

The structure of **1** was confirmed by ^1H-^1H correlation spectroscopy (COSY) and ^{13}C-^1H COSY. The protons at C-2, C-3, and C-5 could be assigned to the signals at δ 7.19 (1H, d, *J* = 7.69 Hz), 6.36 (1H, dd, *J* = 7.69 Hz, 2.75 Hz), and 6.19 (1H, d, *J* = 2.75 Hz), respectively. The ^{13}C nmr showed the presence of a carbonyl group at 178.4 ppm (C-4, s), a quaternary sp^2 carbon at 154.1 ppm (C-6, s), and three tertiary sp^2 carbons at 141.0 ppm (C-2, d), 117.7 ppm (C-3, d), and 116.1 ppm (C-5, d). Comparing the data of ^1H and ^{13}C nmr of **1** with those of (−)-multiflorine [**2**], the signal of the proton at C-11 of **1** was shifted downfield at 2.39 ppm (2.04 ppm in **2**),

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and the signals C-12 and C-14 of **1** were shifted upfield at 22.6 and 18.9 ppm, respectively (Table 1).

TABLE 1. ^{13}C-nmr Data of (−)-Δ^5-Dehydromultiflorine [**1**] and (−)-Multiflorine [**2**].

Carbon	Compound	
	1	**2**
C-2	141.1 (d)	155.6 (d)
C-3	117.7 (d)	99.1 (d)
C-4	178.4 (s)	192.5 (s)
C-5	116.1 (d)	39.3 (t)
C-6	154.1 (s)	60.3 (d)
C-7	34.6 (d)	31.1 (d)
C-8	20.9 (t)	25.7 (t)
C-9	32.7 (d)	34.4 (d)
C-10	57.7 (t)	57.4 (t)
C-11	62.9 (d)	63.6 (d)
C-12	22.6 (t)	31.4 (t)
C-13	25.1 (t)	24.7 (t)
C-14	18.9 (t)	23.6 (t)
C-15	54.4 (t)	55.2 (t)
C-17	52.1 (t)	51.1 (t)

These results indicated that the configuration of rings C and D in the molecule of **1** is a chair-chair cis system. This is also supported by the disappearance of the trans quinolizidine bands in the ir spectrum of **1** (11–14).

From the above results, it can be presumed that the structure of the new alkaloid is (−)-Δ^5-dehydromultiflorine [**1**].

Compound **1** has been previously reported as an unexpected intermediate in a catalytic hydrogenation (PtO_2 in H_2O) of (−)-multiflorine [**2**] (9), and its structure has been confirmed by X-ray analysis (15). However, this is the first report of the isolation and full characterization of **1** from a natural source.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The high and low resolution eims were measured on a Hitachi M-60 at 70 eV. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL GSX 400 and GSX 500 spectrometers, respectively. TMS was used as an internal standard in $CDCl_3$. Tlc was carried out on Si gel plates in CH_2Cl_2-MeOH-28% NH_4OH (90:9:1). Analytical hplc was performed as described in our previous papers (2, 16–21).

EXTRACTION AND ISOLATION OF (−)-Δ^5-DEHYDROMULTIFLORINE [**1**].—The seeds of *L. termis* were collected at the Medicinal Plant Experimental Station at Assiut University, Egypt, in October 1987. A voucher specimen has been identified by Prof. Kamal El-Batanoumy, Department of Systematic Botany, Faculty of Science, Cairo University, Egypt and has been deposited in the herbarium of Chiba University, Japan.

The total basic fraction was obtained from the 75% EtOH extracts of the viable seeds in a yield of 2.7%. The crude base (27 g) was chromatographed on a Si gel column (Merck, type 60, 230–400 mesh, 1 kg, 7 × 150 cm) using 10% MeOH in CH_2Cl_2-28% NH_4OH (500:1) as the solvent. The fractions richest in **1** (150 mg) were eluted together with a trace of 13-hydroxylupanine. This fraction was further purified using a Si gel column and cyclohexane-diethylamine (7:3) to separate only a trace amount of 13-hydroxylupanine, and then pure **1** was eluted with MeOH.

(−)-Δ^5-*Dehydromultiflorine* [**1**].—Colorless oil, $[\alpha]^{25}D$ −94.4° (c = 0.015, CH_2Cl_2); hreims *m/z* (%) 244.1573 (100) (calcd for $C_{15}H_{20}N_2O$, 244.1474), 203 (13), 163 (31), 162 (84), 148 (27), 146 (30), 134 (20), 118 (16), 98 (37), 97 (34), 96 (89), 57 (17), 41 (35); ir ν max ($CHCl_3$) cm^{-1} 1640 (pyridone C=O), 1560 (C=C); uv λ max 263 nm (MeOH) (log ϵ = 3.935); ^{1}H nmr δ 7.19 (1H, d, J = 7.69 Hz, H-2), 6.36 (1H, dd, J = 7.69, 2.75 Hz, H-3), 6.19 (1H, d, J = 2.75 Hz, H-5), 4.12 (1H, dd, J = 12.65, 6.33 Hz, H-10β), 3.92 (1H, d, J = 12.65 Hz, H-10α), 3.35 (1H, dd, J = 11.0, 2.75 Hz, H-17), 2.93 (1H, d, J = 11.83 Hz, H-11), 2.90 (1H, m, H-7), 2.76 (1H, ddd, J = 13.75, 13.75, 2.75 Hz, H-15), 2.68 (1H, ddd, J = 13.75, 1.92, 1.92 Hz, H-15), 2.49 (1H, d, J = 8.25 Hz, H-17), 2.05 (1H, brs, H-15), 2.0 (1H, brs, H-8); ^{13}C nmr see Table 1.

ISOLATION AND IDENTIFICATION OF KNOWN ALKALOIDS.—A mixture of the crude alkaloids (27 g) was chromatographed on a Si gel column with CH_2Cl_2/MeOH/28%NH_4OH as described previously (19–21) to yield the known compounds as follows: (±)-lupanine (4 g), colorless needles, mp 98°, $[\alpha]^{25}D$ 0 (c = 0.1, MeOH) eluted by 5% MeOH/CH_2Cl_2/28%NH_4OH; (−)-multiflorine (1.5 g), oil, $[\alpha]^{25}D$ −299° (c = 0.1, MeOH) eluted by 6% MeOH/CH_2Cl_2/28%NH_4OH; (+)-angustifoline (1.3 g), oil, $[\alpha]^{25}D$ +5.2° (c = 0.1, MeOH) eluted by 8% MeOH/CH_2Cl_2/28%NH_4OH; (+)-13-hydroxylupanine (3.1 g), colorless needles, mp 174°, $[\alpha]^{25}D$ +45.5° c = 0.1, MeOH) eluted by 10% MeOH/CH_2Cl_2/28%NH_4OH; (±)-lupanine *N*-

oxide (100 mg), oil, $[\alpha]^{25}D$ 0 ($c = 0.1$, MeOH), eluted by 11% MeOH/CH_2Cl_2/28%NH_4OH. These alkaloids were identified by spectroscopic and chromatographic comparisons with authentic samples.

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