(±)-Termisine, a Novel Lupine Alkaloid from the Seeds of Lupinus termis

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(±)-TERMISINE, A NOVEL LUPINE ALKALOID FROM THE SEEDS OF *LUPINUS TERMIS*

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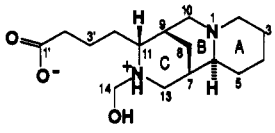
ABSTRACT.—A novel lupine alkaloid, (±)-termisine [**1**], was isolated from the EtOH extract of the crushed seeds of *Lupinus termis*. The structure of **1** was assigned on the basis of its spectroscopic data, in addition to chemical and semisynthetic methods.

In our continuing studies on lupine alkaloids in Egyptian plants (1–5), we have previously reported the presence of (−)-Δ^5-dehydromultiflorine, (−)-Δ^5-dehydroalbine, and nine lupine alkaloids isolated from the viable seeds of *Lupinus termis* Forsk. (Leguminosae) (1,2). *L. termis* is widely cultivated in the Mediterranean area and Egypt for its edible seeds (6). The seeds are also used in traditional medicine for the treatment of diabetes and eczema (7–11). Recently, we demonstrated the hypoglycemic effect of (−)-multiflorine, which was isolated as a major compound from *L. termis* seeds, on streptozotocin-induced diabetic mice (5). Now, we report the isolation and structure determination of the novel lupine alkaloid, (±)-termisine [**1**].

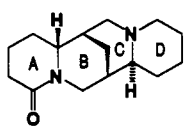
From the 75% EtOH extract of the crushed seeds, (±)-termisine [**1**] was isolated (0.08% fresh wt) by Si gel chromatography. The eims of **1** showed the same fragment ion peaks with almost the same intensities as those of (±)-lupanine [**2**] (1,12,13). An additional peak was found at *m/z* 264 (2%), i.e., 16 units more than that of (±)-lupanine ([M]$^+$ *m/z* 248). Fabms measurements, using glycerol in one experiment and *m*-nitrobenzoic acid in another, showed peaks at *m/z* 297 ([M+1]$^+$, 2%), and at *m/z* 279 ([M−OH]$^+$, 100%) suggesting a mol wt of 296. The fragmentation pattern in the eims indicated a possible lupanine skeleton.

The ir spectrum of **1** (KBr) showed a broad intense band at 3300 cm^{-1} (O-H stretching, intramolecular, hydrogen bonding). Multiple bands at 3100 cm^{-1} extending to 2500 cm^{-1} suggested quaternary nitrogen and combination overtones, embodying the C-H stretching the Bohlmann's bands characterstic for quinolizidine-type alkaloids (14). A strong, sharp absorption band at 1580 cm^{-1} and a moderate one at 1405 cm^{-1}, due to a carboxylate anion, were also observed (15).

The carboxylate anion has two strongly coupled carbon-to-oxygen bonds, with bond strengths intermediate between C=O and C−O (15), which give rise to the two bands at 1580 and 1405 cm^{-1}. In addition, the ammonium band in the 3100–2600 cm^{-1} region gave further evidence for the carboxylate anion, as it can make an endo-salt with the tertiary nitrogen of the compound.



1



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The ^{13}C-nmr spectrum of **1** showed the presence of 16 carbons, which could be assigned as shown in the Experimental section. Determination of the multiplicity was carried out by DEPT experiments, which revealed that **1** has one carbonyl [C=O] at δ 182.3 ppm, four methine carbons, and eleven methylene carbons. One of these methylene groups is highly deshielded at δ 79.4 ppm. Based on empirical calculations, this signal was assigned as a CH_2OH on a quarternary nitrogen atom (16,17). The chemical shift of a carbonyl group at δ 182.3 ppm (s, CD_3OD) could be assigned to a saturated carboxylic acid (18,19), giving additional evidence for the presence of a carboxylic moiety in the structure.

^1H-^1H and ^1H-^{13}C Correlation (COSY) experiments showed that the methylene proton signals at δ 4.60 and δ 4.34 ppm (J=11.7 Hz) were coupled to the carbon at δ 79.4 ppm which belongs to the N-CH_2OH group. ^1H and ^{13}C assignments based on the above experiments are shown in the Experimental section and Table 1 and indicate that **1** is a tricyclic structure substituted at N-12 (CH_2OH) and having a side chain of four carbons at C-11, the terminal carbon being a carboxylic group.

The conformation of the B and A rings of (±)-termisine should be assigned as a boat-chair on the basis of ^1H-nmr analysis in $CDCl_3$. Of the two C-10-attached protons, the pseudo-equatorial proton (more downfield) showed geminal coupling (J=12.1 Hz) and a large vicinal coupling to C-9 (J=12.9 Hz). The more upfield C-10 proton (pseudo-axial) ex-

TABLE 1. Correlation of ^{13}C- and ^1H-nmr Shifts of (±)-Termisine [**1**] Measured in MeOD and $CDCl_3$.

Chemical shift (δ value, J in Hz)		Corresponding C in ^{13}C
CD_3OD	$CDCl_3$	
—	5.30 (1H, s; exchangeable OH)	—
4.60 (1H, d, J=11.7; H_{eq}-14)	4.05 (1H, d, J=11.8)	79.4
4.34 (1H, dd, J=11.8, 2.6; H_{ax}-14)	3.95 (1H, d, J=12.1)	79.4
4.12 (1H, dd, J=12.8, 2.6; H_{eq}-13)	3.72 (1H, dd, J=14.0, 12.1)	51.5
3.72 (1H, d, J=12.8; H_{eq}-11)	3.64 (1H, d, J=11.3)	71.7
—	3.46 (1H, br s; N^+H exchangeable)	—
3.50 (1H, d, J=13.4; H_{eq}-10)	3.30 (1H, t, J=21.1)	60.0
3.36 (1H, d, J=13.4; H_{ax}-10)	3.22 (1H, d, J=12.9)	60.0
3.33 (1H, td, J=12.2, 1.8; H_{ax}-13)	2.98 (1H, d, J=11.9	51.5
3.13 (1H, t, J=9.5; H_{eq}-2)	Em[a]	59.6
3.10 (1H, t, J=6.4; H_{eq}-6)	Em	63.0
2.96 (1H, td, J=13.0, 1.3; H_{ax}-2)	Em	59.6
2.30 (1H, dd, J=10.4, 3.1; H_{eq}-3)	Em	28.7
2.24 (1H, m; H-4′)	Em	25.4
2.19 (1H, dd, J=7.0, 1.3; H-2′)	Em	38.6
1.98 (1H, d, J=13.7; H_{eq}-4)	In[b]	19.6
1.94 (1H, d, J=9.2; H_{ax}-3)	In	28.7
1.79 (2H, br s; H_{ax}-9, H_{eq}-8)	In	30.5
	In	23.3
1.74 (1H, m; H_{eq}-5)	In	30.4
1.73(1H, s; H_{eq}-7)	In	31.5
1.71 (1H, m; H_{eq}-4)	In	19.6
1.68 (1H, m; H_{ax}-8)	In	23.3
1.60 (1H, dd; J=6.4, 1.7; H_{eq}-3′)	In	24.0
1.54 (3H, m; H_{ax}-3′	In	24.0
H_{ax}-4′,	In	25.4
H_{ax}-5)	In	30.4

[a]Em=Embodied in a multiplet integrating five protons centered at δ 2.14.
[b]In=Included in multiple peaks at δ 1.45 to δ 1.98.

hibited a geminal interaction, but a much smaller vicinal coupling. These values were compatible only with a boat conformation of ring B, in which the dihedral angle between the pseudo-equatorial H-10 and the equatorial H-9 is small.

Since compound **1** had been proved by spectral data to contain both basic (N) and acidic (COOH) groups, it is amphoteric and exists as a dipolar ion (20,21).

The structure of **1** was confirmed by chemical synthesis from (±)-lupanine [**2**] (22,23). In the plant, (±)-termisine [**1**] may possibly be biosynthesized by hydrolysis of (±)-lupanine [**2**] followed by one carbon addition and hydroxylation of the Me group thus formed.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. Ir spectra were recorded in $CHCl_3$ and KBr. ^{1}H-nmr (500 MHz), ^{13}C-nmr (67.8 Mz), 2D ^{1}H- ^{1}H, and ^{13}C- ^{1}H COSY nmr spectra were recorded in $CDCl_3$ and CD_3OD on a JEOL GSX 500 spectrometer, using TMS as internal standard. Eims was measured on a Hitachi M-60 at 70 eV. Fabms using glycerol and *m*-nitrobenzoic acid was measured at room temperature. Tlc was carried out in Si gel (Merck) plates (5 mm) in the following sytems: CH_2Cl_2-MeOH-28% NH_4OH (90:9:1, 80:18:1, and 70:30:1).

EXTRACTION AND ISOLATION OF (±)-TERMISINE [**1**].—The seeds of *L. termis* were collected at the Medicinal Plant Experimental Station at Assiut University, Assiut, Egypt. A voucher specimen was identified by Prof. Dr. Kamal El-Batanony (Department of Systematic Botany, Faculty of Science, Cairo University, Cairo, Egypt) and is deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

EXTRACTION AND ISOLATION OF ALKALOIDS.—A total basic fraction (22 g, fraction A) was obtained from the 75% EtOH extracts of the air-dried seeds (1 kg) by a previously described method (1,2). The aqueous layer remaining was made strongly basic by addition of K_2CO_3 under ice-cooling and was extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried (K_2CO_3), and concentrated to dryness to yield fraction B (5.1 g).

Fraction B (5.1 g) was chromatographed on a Si gel column (Merck, type 60, 230–400 mesh, 350 g) using CH_2Cl_2/MeOH/28% NH_4OH to yield the alkaloids as follows: (±)-lupanine [800 mg, mp 98°, $[\alpha]^{25}D$ 0° (c=0.1 MeOH), eluted by 4% MeOH/CH_2Cl_2]; (−)-multiflorine [200 mg, oil, $[\alpha]^{25}D$ −299° (c=0.1, MeOH), eluted by 8% MeOH/CH_2Cl_2]; (+)-angustifoline [150 mg, oil, $[\alpha]^{25}D$ +5.2° (c=0.1, MeOH), eluted by 8% MeOH/CH_2Cl_2]; (−)-albine [200 mg, oil $[\alpha]^{25}D$ −103° (c=0.1, MeOH), eluted by 10% MeOH/CH_2Cl_2]; (+)-13-hydroxylupanine [350 mg, colorless needles, mp 174°, $[\alpha]^{25}D$ +45.5° (c=0.1, MeOH), eluted by 12% MeOH/CH_2Cl_2]; and (±)-termisine [800 mg, yellow needles, mp 98–99°, $[\alpha]^{25}D$ 0° (c=0.1, MeOH), eluted by 25% MeOH/CH_2Cl_2].

(±)-TERMISINE [**1**].—Fine yellow crystals: mp 98–99°; $[\alpha]^{25}D$ 0° (c=0.1, MeOH); eims *m/z* (%) 264 (2), 249 (12), 248 (65), 247 (40), 219 (9), 151 (11), 150 (42), 149 (55), 148 (18), 136 (100), 134 (20), 124 (10), 112 (16), 110 (26), 98 (30), 84 (26), 55 (28), 41 (24); ir (KBr) λ max cm^{-1} 3650, 3300 (OH), 3100–2500 (N^+ and C-H), 1580, 1405 [$C(—O)_2^-$]; ^{1}H nmr and ^{1}H-^{13}C COSY (CD_3OD and $CDCl_3$) see Table 1; ^{13}C nmr (CD_3OD, 67.8 MHz) δ 182.3 (s, C-1′), 79.4 (t, C-14), 71.7 (d, C-11), 63.0 (d, C-6), 60.0 (t, C-10), 59.6 (t, C-2), 51.5 (t, C-13), 38.6 (t, C-2′), 31.5 (d, C-7), 30.5 (d, C-9), 30.4 (t, C-5), 28.7 (t, C-3), 25.4 (t, C-4′), 24.0 (t, C-3′), 23.3 (t, C-8), 19.6 (t, C-4); pK_{a1} (COOH)=1.8, pK_{a2} (quaternary N)=9.4 and pK_{a3} (further part of the molecule)=10.9; pI (the isoelectric point)=6.3

(±)-Termisine was detected by tlc as a minor spot in fraction A and as a major spot in fraction B.

SYNTHESIS OF (±)-TERMISINE [**1**] FROM (±)-LUPANINE [**2**].—*Acid induced ring opening of (±)-lupanine.*—(±)-Lupanine [**2**] (150 mg) was dissolved in HCl-saturated EtOH (30 ml). The reaction mixture was allowed to stir at room temperature for 72 h and then refluxed for 2 h. Usual workup by cc yielded 55 mg (35%) of the open-structured intermediate, mp 138–140°, R_f 0.35 [$CHCl_3$−MeOH−NH_4OH(80:20:1)]. Lupanine [**2**], 80 mg (60%), R_f 0.75 was recovered.

Formylation and hydrolysis of the intermediate.—To the open-structured intermediate obtained from the above-mentioned reaction (35 mg) and dissolved in EtOH (10 ml), was added formalin solution (37%, 2 ml). The reaction mixture was allowed to stir at room temperature overnight. HCl (1N, 10 ml) was added, and the reaction mixture was allowed to stir for a further 6 h at room temperature. After neutralization by NH_4OH (10%) and workup with $CHCl_3$ and H_2O, the organic layer was concentrated, and the residue was purified by cc to yield **1** (30 mg, 88%).

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