

NEW ALKALOIDS FROM *LUPINUS ALBUS* L. SEEDS

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تم فصل قلوانيين جديدين ، (+)-14-ديهيدرو-10 α -هيدروكسي ترمسين و (-)-13 α -هيدروكسي-5,6-ديهيدروميتيفلورين - ن - أوكسيد من هذه الخلاصة الكحولية لبذور نبات الليوبينس ألبس بالإضافة الى أربعة قلوانيات معروفة تفصل لأول مرة من هذه البذور وهي (+)-13 α -ألfa (2-ميثيل بيوترييل) - أوكسي ليوبانين و (+) - ألfa أيزوليوبانين و (+)-أمودندرين و (-)-سبارتين. وقد تم التعرف على هذه المركبات بالطرق الطيفية والنصف تخليقية.

Two new lupin alkaloids, (+)-14-dehydro-10 α -hydroxytermisine, (-)-13 α -hydroxy-5,6-dehydromultiflorine N-oxide have been isolated from the ethanolic extract of *Lupinus albus* seeds together with (+)-13 α -(2-methylbutyryl)-oxylupanine, (+)- α -isolupanine, (+)-ammodendrine and (-)-sparteine. The structure elucidation of these alkaloids is based on spectroscopic and semisynthetic methods.

INTRODUCTION

The genus *Lupinus* (Leguminosae) is represented in Egypt by four species.¹ *Lupinus albus* L. (= *L. termis* Forssk) is the widely cultivated one for its edible seeds.¹ We have previously reported the isolation of (-)-5,6-dehydromultiflorine, (-)-5,6-dehydroalbine, (\pm)-termisine, (+)-15 β -hydroxy-17-oxolupanine, (-)-termine and other known lupin alkaloids from the seeds of this plant.²⁻⁶ In the present study, five lupin-type quinolizidine alkaloids were additionally isolated for the first time, from the seeds including two novel lupin alkaloids, (+)-14-dehydro-10 α -hydroxytermisine (1) and (-)-13 α -hydroxy-5,6-dehydromultiflorine N-oxide (2) and a biperidyl alkaloid ammodendrine.

EXPERIMENTAL

Melting points were uncorrected. IR was measured as thin films in KBr or CHCl₃. High and low resolution EIMS were measured on a Hitachi M-60 at 70 eV. ¹H and ¹³C-NMR were recorded on JEOL GSX 400 and JSX 500 spectrometers, respectively, with TMS as internal standard in CD₃OD and CDCl₃. TLC was carried out on silica gel (Kieselgel 60, F

254) of 0.25 mm layer thickness in CH₂Cl₂ - MeOH-28% NH₄OH (90:9:1, 43:6:1). Analytical HPLC was run as described in literature.⁷

Plant materials

The seeds of *Lupinus albus* L. were collected at the Medicinal Plant Experimental Station at Al-Azhar University, Assiut in April 1993. The plant was identified by Prof. A.Fayed (Dept. of Systematic Botany and Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt) and a voucher specimen has been deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

Extraction and isolation

The total basic fractions obtained from the EtOH 75% extracts of seeds (yield = 2.6% fresh weight) by a method previously described.²⁻⁶ The mixture of the bases (20 g) chromatographed on silica gel column (Merck, type 60, 230-400 mesh, 1kg, 7x150 cm) with solvent system CH₂Cl₂ - MeOH-28% NH₄OH to yield the alkaloids as described before.²⁻⁶ (+)-13 α -(2-methylbutyryl)-oxylupanine (11 mg), eluted by 2% MeOH in CH₂Cl₂, (+)- α -isolupanine (17

mg), eluted by 2% MeOH in CH₂Cl₂ (+)-ammodendrine (88 mg), eluted by 9% MeOH in CH₂Cl₂, (-)-13 α -hydroxy-5,6-dromultiflorine (4) (80 mg), eluted by 11% MeOH in CH₂Cl₂. Pure (2) (33 mg), was obtained from fractions eluted by 15% MeOH in CH₂Cl₂, (-)-sparteine (35 mg), eluted by 16% MeOH in CH₂Cl₂. Pure (1) (18 mg) eluted by 18% MeOH in CH₂Cl₂.

(+)-14-dehydro-10 α -hydroxytermisine

(1). Pale yellow oil, $[\alpha]_D^{25}$ +24.3° (MeOH; *c* 0.1); EIMS *m/z* (rel. int.): 292 (4), 278 (7), 266 (4), 264 (46), 246 (44), 236 (27), 166 (36), 152 (100), 136 (10), 84 (50), 55 (93), IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3450-2800 (OH and CH), 1692 (COOH), 1647 (amidic C=O), ¹H-NMR (CD₃OD, 500 MHz): δ 9.00 (1H, *s*, H-14), 4.87 (1H, *d*, *J* = 12.7 Hz, partially hidden in CD₃OD, H-10_{eq}), 4.31 (1H, *dd*, *J* = 12.6, 2.2 Hz, H-13_{eq}), 4.22 (1H, *t* like, H-2_{eq}), 4.11 (1H, *d*, *J* = 11.3 Hz, H-11), 3.99 (1H, *dt*, *J* = 12.6, 1.9 Hz, H-13_{ax}), 3.64 (1H, *m*, H-6), 3.55 (1H, *td*, *J* = 12.9, 1.9 Hz, H-2_{ax}), 2.46 (1H, *m*, H-9), 2.35 (1H, *br.s*, H-7), 2.31-2.05 (5H, *m*, 2x H-4', H-2' eq, H-3 eq, H-5 eq), 1.96-1.71 (4H, *m*, H-2' ax, H-8 eq, H-5 ax, H-3 ax), 1.66-1.51 (5H, *m*, H-8 ax, 2x H-4, 2x H-3'), ¹³C-NMR (125 MHz), see Table 1.

(-)-13 α -hydroxy-5,6-dehydromultiflorine

N-oxide (2). Yellow oil; $[\alpha]_D^{25}$ -143°, (MeOH: *c* 0.1) HR in-beam EIMS (rel. int.) *m/z*: 276.1472 ([M]⁺, 12) (calc. for C₁₅H₂₀N₂O₃, 276.1475): 276 (12), 260 (100), 243 (37), 242 (10), 162 (55), 148 (81), 122 (55), 118 (44), 96 (28); UV λ_{\max} 264 nm (MeOH), log ϵ = 3.82; IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3350 (OH), 1640 (C=O), 1560 (C=C), 980 (N⁺-O); ¹H-NMR δ 7.47 (1H, *d*, *J* = 7.7 Hz, H-2), 6.37 (1H, *dd*, *J* = 7.7, 2.75 Hz, H-3), 6.25 (1H, *d*, *J* = 2.75 Hz, H-5), 4.17 (1H, *t*, *J* = 2.67 Hz, H-13), 4.11 (1H, *dd*, *J* = 12.9, 6.1 Hz, H-10_{eq}), 3.91 (1H, *d*, *J* = 12.9 Hz, H-10_{ax}), 3.81 (1H, *dd*, *J* = 12.3, 11 Hz, H-17_{eq}), 3.64 (1H, *ddd*, *J* = 12.7, 12.7, 2.4 Hz, H-15_{ax}), 3.32 (1H, *d*, *J* = 11.2 Hz, H-11), 3.07 (1H, *dd*, *J* = 12.3, 3.2 Hz, H-17_{ax}), 3.01 (1H, *m*, H-15_{eq}), 2.89 (1H, *m*, H-7), 2.42 (1H, *br.s*,

H-9), 2.32 (1H, *m*, H-8_{ax}), 2.11-1.73 (3H, *m*, H-8_{eq}, H-12_{ax}, H-14_{ax}), 1.52-1.43 (2H, *m*, H-12_{eq}, H-14_{eq}), ¹³C-NMR (125 MHz): see Table 1.

The other known compounds were identified by chromatographic and spectroscopic analysis as well as comparison with authentic samples and published data.⁸⁻¹¹

Reduction of (-)-13 α -hydroxy-5,6-dehydromultiflorine N-oxide (2) to (-)-13 α -hydroxy-5,6-dehydromultiflorine (4)¹²⁻¹⁴

Compound 2 (20 mg) was dissolved in 4 ml of EtOH and reduced with SO₂ gas for 30 min at 0°C. The purification by PLC gave 4 mg of pure (4). Compound (4) was identified by direct HPLC comparison with authentic sample.

Synthesis of (-)-13 α -hydroxy-5,6-dehydromultiflorine N-oxide (2) from (-)-13 α -hydroxy-5,6-dehydromultiflorine (4)¹²⁻¹⁴

Compound (4) (80mg) was dissolved in 15 ml of CH₂Cl₂. *m*-Chloroperoxybenzoic acid (100 mg) in CH₂Cl₂ was added gradually to the former solution. After stirring 6 hours at room temperature, the products were extracted and chromatographed on silica gel column with the solvent system CH₂Cl₂ - MeOH-28% NH₄OH (400: 64:3). Compound 2, $[\alpha]_D^{25}$ -143°, was obtained with a yield of 40 % (33 mg). The structure of the synthetic products was identified by IR and co-HPLC as compound 2.

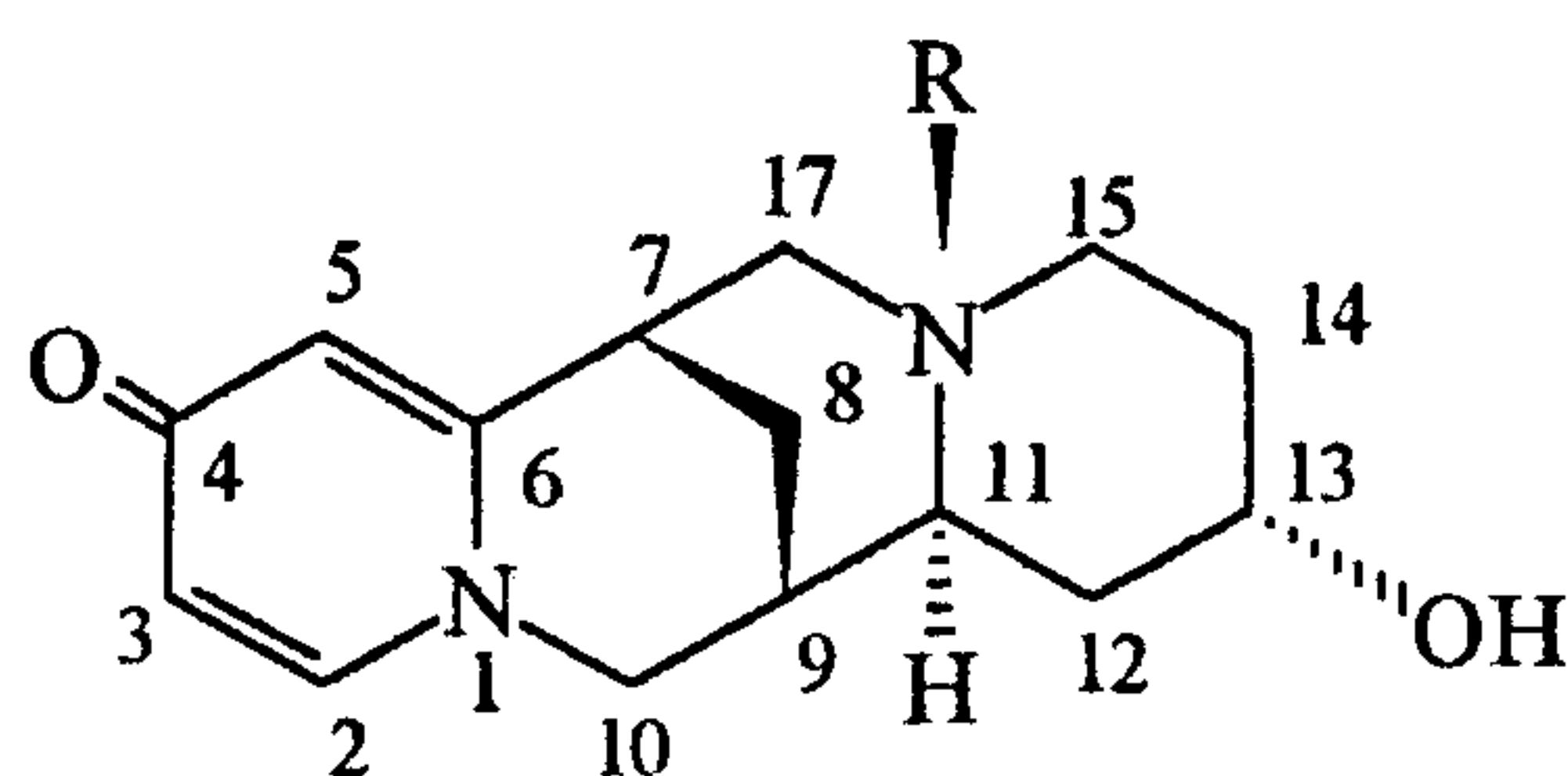
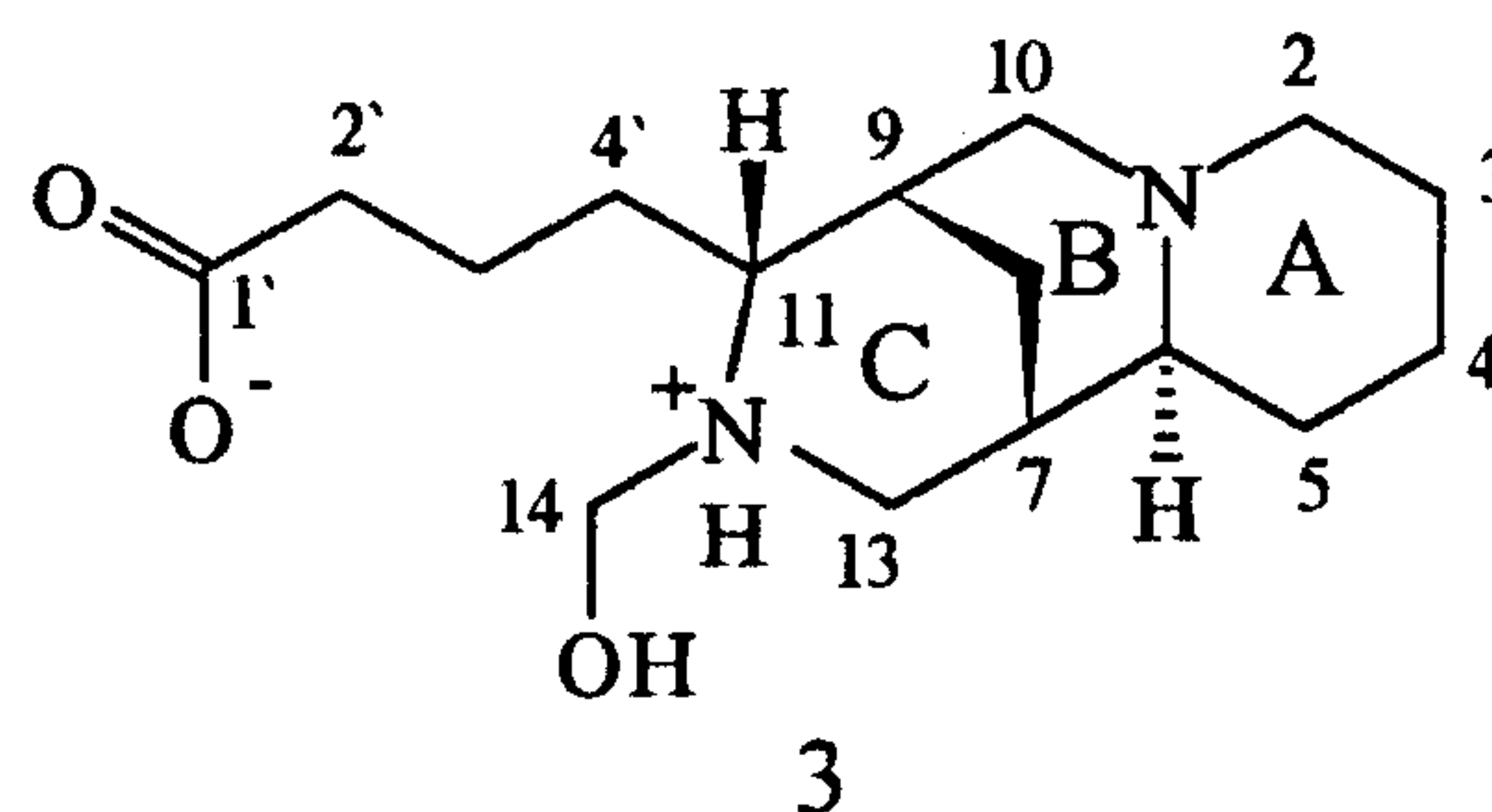
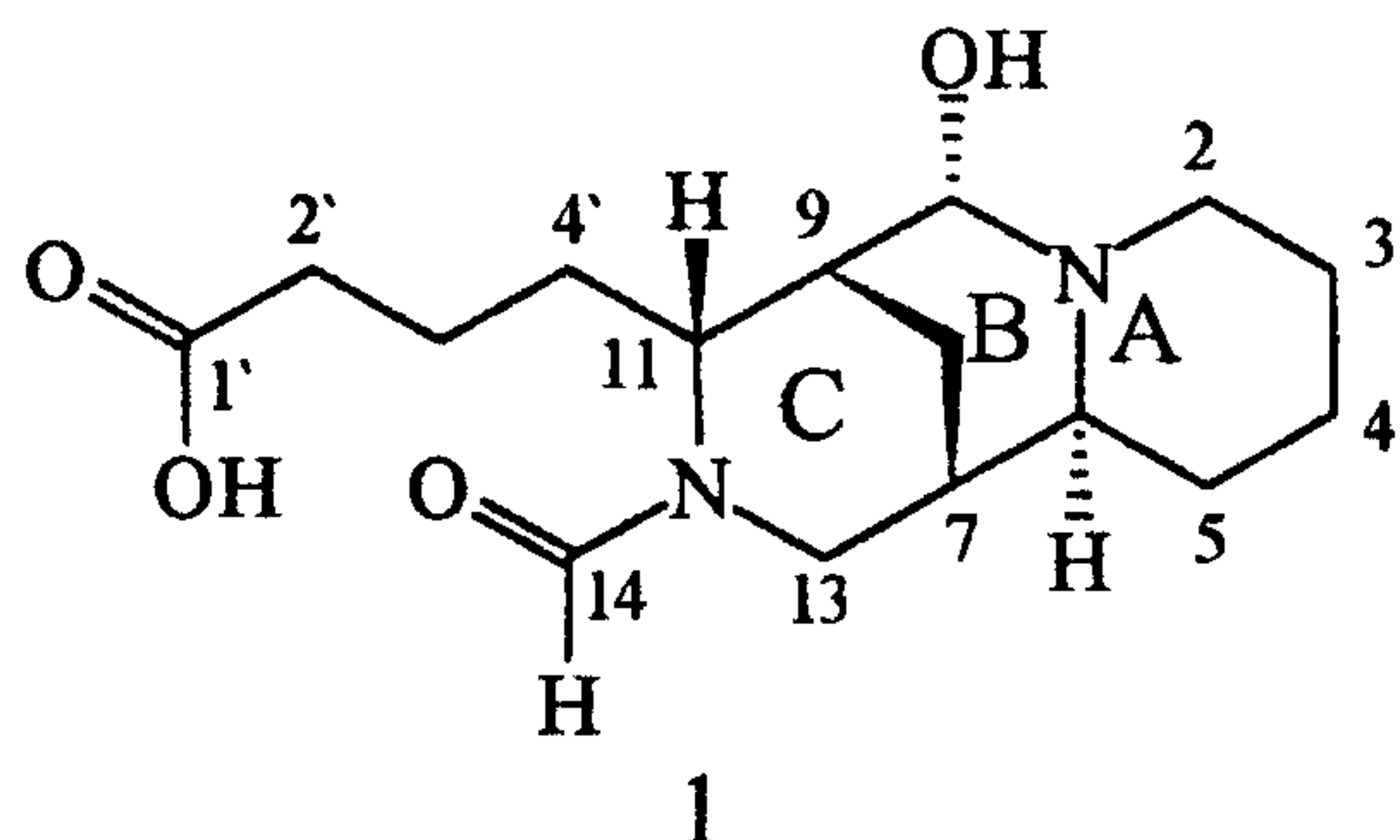
RESULTS AND DISCUSSION

Two new lupin alkaloids, (+)-14-dehydro-10 α -hydroxytermisine (1) and (-)-13 α -hydroxy-5,6-dehydromultiflorine N-oxide (2) were isolated from the basic chloroform extract of the seeds of *L. albus*, in addition to, three known alkaloids, (+)-13 α -(2-methylbutyryl)-oxylupanine, (+)- α -isolupanine, (-)-sparteine and a bipiperidyl alkaloid (+)-ammodendrine.

The FAB-mass spectrum of (1) exhibited a protonated [M]⁺ at *m/z* 311 and the DEPT spectrum gave the molecular formula C₁₆H₂₆N₂O₄. The EI-Mass spectrum revealed the

Table 1: Comparison of the ^{13}C -NMR data of compounds 1-4 in CD_3OD .

Carbon	Compound		Carbon	Compound		Δ 2-4
	1	3		2	4	
C-2	58.4(t)	59.6(t)	C-2	142.5(d)	142.8(d)	-0.3
C-3	30.8(t)	28.7(t)	C-3	117.9(d)	117.2(d)	+0.7
C-4	21.4(t)	19.6(t)	C-4	179.2(s)	178.9(s)	+0.3
C-5	31.5(t)	30.4(t)	C-5	115.9(d)	116.2(d)	+0.3
C-6	62.1(d)	63.0(d)	C-6	155.9(s)	154.7(s)	+1.2
C-7	33.0(d)	31.5(d)	C-7	31.6(d)	33.4(d)	-1.8
C-8	28.8(t)	23.5(t)	C-8	20.2(t)	21.6(t)	-1.4
C-9	40.5(d)	30.5(d)	C-9	32.1(d)	35.2(d)	-3.1
C-10	64.0(d)	60.0(t)	C-10	61.1(t)	59.1(t)	+2.0
C-11	63.8(d)	71.7(d)	C-11	64.1(d)	57.2(d)	+6.9
C-13	49.2(t)	51.5(t)	C-12	25.1(t)	29.9(t)	-4.8
C-14	173.4(d)	79.4(t)	C-13	63.9(d)	65.3(d)	-1.4
C-1'	180.4(s)	182.3(s)	C-14	23.4(t)	26.0(t)	-2.6
C-2'	41.1(t)	38.6(t)	C-15	64.0(t)	49.1(t)	+14.9
C-3'	23.6(t)	24.0(t)	C-17	68.2(t)	52.4(t)	+15.8
C-4'	29.5(t)	25.4(t)				



2 R = O

4 R = lone pair

fragments at m/z 292 (loss of H_2O) and m/z 266 (loss of CO_2), suggesting the presence of hydroxyl and carboxyl groups.¹⁵ Generally, the fragmentation pattern in the EIMS indicated a lupanine-type skeleton.

The IR spectrum of (1) showed an intense multiple broad bands combination at 3450-2800 cm^{-1} indicative of the presence of hydrogen bonding hydroxyl groups and C-H stretching.¹⁵ A strong absorption bands at 1692 and 1647 cm^{-1} suggesting the presence of both carboxylic and amidic carbonyl groups respectively which were confirmed by the ^{13}C -NMR signals at δ 180.4 (COOH) and at δ 173.4 (amidic $C=O$).^{15,16}

DEPT experiments (Table 1) showed that (1) has six methine, and nine methylene carbons, in addition to the carboxylic carbonyl carbon. The most downfield methine carbon at δ 173.4 is assigned for C-14 and the other three downfield methine carbons at δ 64.0, 63.8 and 62.1 are assigned for C-10, C-11 and C-6, respectively, guided by 1H - ^{13}C -Correlation Spectroscopy (COSY) experiments. The upfield methine carbons at δ 40.5 and 33.0 are assigned to C-9 and C-7, respectively. The chemical shift of C-9 was in accordance with its calculated value.^{15,16}

In the 1H -NMR spectrum of (1), the most downfield shifted proton resonated at δ 9.00 (1H, *s*, H-14), this aldehydic proton was shown to be connected to the carbon at δ 173.4 (C-14).^{15,16} The other downfield proton resonated at δ 4.87 (1H, *d*, $J=12.7$ Hz, H-10_{eq}) and was shown to be connected to the carbon at δ 64.0 (C-10) which bears the hydroxyl substituent. Homonuclear spin decoupling experiments and 1H - 1H -COSY helped to determine the connectivities. Thus, H-10_{eq} (δ 4.87) collapsed to a singlet upon saturation and was coupled with the multiplet at δ 2.46 (H-9). Further evidence was obtained from 1H - 1H -COSY, where H-10_{eq} showed a strong cross-peaks with H-9.

The α -axial orientation of the 10-hydroxyl group was determined on the basis of 1H -NMR analysis. The presence of large vicinal coupling (12.7 Hz) between H-10 as an equatorial proton with the vicinal H-9 and the lack of small vicinal

coupling between H-10 and H-9 established the equatorial orientation of H-10 (consequently the axial orientation of the hydroxyl group), where the dihedral angle between the pseudo-equatorial H-10 and the equatorial H-9 is small.⁴ The NOE spectral data of (1) further support the α -axial orientation of the hydroxyl at C-10, where a cross peak between H-10_{eq} and H-8_{ax}. The above data was only compatible with the boat conformation of ring B.⁴

1H - ^{13}C -COSY spectrum confirmed the assignments of the protons and their corresponding carbons indicating that (1) is a tricyclic structure substituted at N-12 (CHO), at C-10 (axial OH) and at C-11 having a side chain of four carbons, the terminal of which is a carboxylic group. This structure can be directly related to termisine (3).⁴

Although (1) had been proved to contain two nitrogens and a carboxylic group, it didn't have a dipole ion structure (zwitterion), since both nitrogens are adjacent to electron withdrawing groups ($HC=O$, and OH) decreasing their basic characters (c.f. termisine 3).⁴

The in-beam HRMS of (2) indicated the molecular formula $C_{15}H_{20}N_2O_3$ ($[M]^+$, m/z 276.1472, calc. 276.1475). The fragmentation pattern of (2) was similar to that of (-)-13 α -hydroxy-5,6-dehydromultiflorine (4)⁸ and the UV spectrum of (2) (λ_{max} 264 nm / MeOH) ($\log \epsilon = 3.82$) suggested the presence of γ -pyridone ring system.^{2,3,9} The IR spectrum of (2) showed bands at 3350 cm^{-1} (OH-stretching), two bands at 1640 and 1560 cm^{-1} for conjugated carbonyl and double bonds and an N-oxide bond at 980 cm^{-1} .¹⁵ Preliminary analysis of the spectral data of (2) suggested that it was an N-oxide of (-)-13 α -hydroxy-5,6-dehydromultiflorine (4) which has been recently isolated and fully characterized from *L. albus* seeds.^{8,9}

In the 1H -NMR spectrum of (2), the protons at C-2, C-3 and C-5 could be assigned at δ 7.47 (1H, *d*, $J=7.7$ Hz), 6.37 (1H, *dd*, $J=7.7, 2.75$ Hz) and 6.25 (1H, *d*, $J=2.75$ Hz), respectively (4).^{2,3,8} The proton at δ 4.17 could be assigned as (1H, *t*, $J=2.67$, H-13 β).^{10,17} The protons at C-11, C-15 and C-17 appeared in downfield

region between δ 3.8 and 3.0 (δ 3.3-2.4 in 4) because of the substituent effects of the N-oxide.¹²⁻¹⁴

The ¹³C-NMR spectrum of (2) showed 15 carbon atoms which were assigned as shown (Table 1). The signals of C-11, C-15 and C-17 of (2) were shifted downfield in a range of 7-16 ppm compared to those of (4) due to the steric effects of the axial N-oxide. The shifts of these signals of the carbons and protons adjacent to the tertiary nitrogen atom were in good agreement with the substituent effects of N-oxides.¹²⁻¹⁴

The α -axial configuration of the hydroxyl group at position 13 was established from the chemical shift, multiplicity and magnitude coupling of H-13 in the ¹H-NMR¹⁸⁻²⁰ which was also confirmed by the chemical shift of C-13 in the ¹³C-NMR δ 63.9 (*d*, C-13).²¹ The NOE spectral data of (2) gave further evidence for the axial orientation of the hydroxyl group at C-13, where irradiation at H-13 gave negative effects on H-11 and both the protons at C-15.^{17,22} Thus the structure of (2) was determined as (-)-13 α -hydroxy-5,6-dehydromultiflorine N-oxide.

The final confirmation of the structure of (2) was performed by chemical conversions between (2) and (4). Compound (2) was reduced by sulphur dioxide to give (4). Furthermore, (2) was synthesized from (4) by oxidation with *m*-chloroperoxybenzoic acid.¹²⁻¹⁴

The assignments including all the protons and carbons of compounds (1) and (2) were confirmed by ¹H-¹H and ¹³C-¹H COSY.

All the other known alkaloids were identified by comparison of their physical and spectral parameters with published data and available authentic samples.⁸⁻¹¹

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