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(-)- TERMINE, A NEW LUPIN ALKALOID FROM THE SEEDS OF *LUPINUS TERMIS*

M.H. Mohamed, A.M.P. Koskinen[1] and M.S. Kamel[2]

Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt
[1] Department of Organic Chemistry, Oulu University, 90570 Linnanma, Oulu, Finland
[2] Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

(-)-ترمين قلوانى جديد من بذور نبات الترمس تم فصله من الخلاصة الكحولية للبذور مع (-)-١٣-ألفا-هيدروكسى سبراتيين. هذا وقد تم التعرف على المركب الجديد بواسطة الطرق الطيفية المختلفة.

A new lupin alkaloid, (-)-termine (1), was isolated from the viable seeds of Lupinus termis, together with (-)-13α-hydroxysparteine. The structure of (1) was determined by spectroscopic methods.

INTRODUCTION

In the course of chemical and biological investigations on lupin alkaloids in leguminous plants, we have already studied the basic constituents of *Lupinus termis* Forssk (*L. albus* L.) cultivated in Egypt[1-7]. Previous work on the constituents of *Lupinus termis* indicates the presence of (-)-5,6-dehydromultiflorine, (-)-5,6-dehydroalbine, (±)-termisine, (+)-15ß-hydroxy-17-oxolupanine in the seeds[1-5], in addition to other known lupin alkaloids. This report deals with the isolation and structure elucidation of a new lupin alkaloid: (-)-13α-hydroxy-5,6-dehydro-4-oxosparteine (termine **1**) together with (-)-13α-hydroxysparteine (**2**) from the viable seeds of *L. termis*.

RESULTS AND DISCUSSION

From the 75% EtOH extract of the crushed seeds of *L. termis*, (-)-termine (**1**) was isolated (0.07% fr. wt.) by silica gel chromatography. The HREIMS spectrum of (**1**) indicated the molecular formula $C_{15}H_{22}N_2O_2$ ($[M]^+$, m/z 262.1789, calcd 262.1682. FAB-MS measurements using glycerol in one experiment and *m*-nitrobenzoic acid in another, revealed a peak at m/z 263 $[M+1]^+$. The presence of a hydroxyl group was indicated by the fragment at m/z 245(14) and 244(16) in the EIMS. These correspond to $[M-OH]^+$ and $[M-H2O]^+$ respectively[8]. The UV spectrum of (**1**) showed absorption at λ_{max} 304 nm (MeOH) indicating presence of a conjugated system. The IR spectrum of (**1**) (KBr) showed the following bands: 3435 cm^{-1} (OH stretching), 1630 cm^{-1} (C=O) and 1580 cm^{-1} (C=C)[9], while *trans*-quinolizidine bands (2800-2600 cm^{-1}) were not observed. From these results (**1**) could be presumed to be a sparteine-type lupin alkaloid containing carbonyl and hydroxyl groups in the molecule.

The ^{13}C-NMR spectrum of (**1**) showed the presence of 15 carbon atoms which could be assigned as shown in Table 1. Determination of the multiplicity was carried out by DEPT experiments, which revealed that (**1**) contains one carbonyl group, one quaternary sp^2 carbon, five methines and eight methylene carbon atoms. This spectrum also indicated the presence of a double bond between a quaternary sp^2 and a teritary sp^2 carbon. The substitution pattern could be deduced as follows: the hydroxyl group

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[2] All correspondence should be addressed to: Dr. M.S. Kamel, Dept. of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

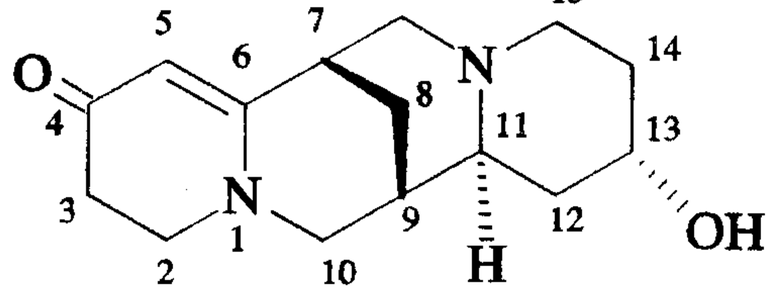
is likely located at position 13 since the signal corresponding to the carbinol carbon at δ 63.8 (d, C-13) is coincident with that of (-)-13α-hydroxysparteine (**2**) and (-)-13α-hydroxy-multiflorine (**3**)[2,10-12]. The carbonyl group is located γ to a teritary nitrogen atom and fits position 4[2,11,13]. The only available position for the double bond is between C-5 and C-6 and can not be present between C-11 and C-12 becaue of the absence of the trans-quinolizidine bands in its IR spectrum[14-16]. The chemical shifts for carbon atoms in rings B, C and D showed similar patterns to (-)-13α-hydroxymultiflorine (**3**) (Table 1).

Table 1: ^{13}C-NMR data of (-)-termine (**1**) and (-)-13α-hydroxymultiflorine (**3**).

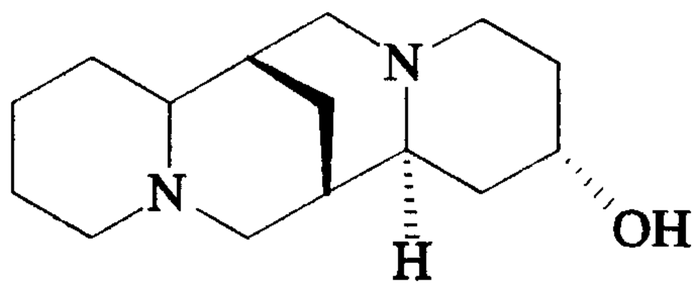
C	**1** (CD_3OD)	**3*** ($CDCl_3$)
2	63.0(t)	155.6(d)
3	56.9(t)	98.0(d)
4	196.4(s)	192.4(s)
5	159.8(d)	39.0(t)
6	176.2(s)	59.8(d)
7	29.6(d)	31.0(d)
8	34.4(t)	25.4(t)
9	32.8(d)	33.6(d)
10	62.1(t)	57.2(t)
11	58.0(d)	56.1(d)
12	33.4(t)	37.0(t)
13	63.8(d)	64.3(d)
14	29.3(t)	30.0(t)
15	49.1(t)	48.4(t)
17	52.7(t)	50.1(t)

* Data from ref. 2.

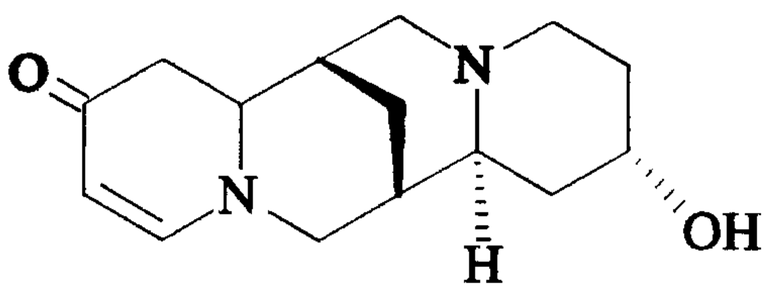
In the ^{1}H-NMR spectrum, the downfield shifted proton of (**1**) resonates at δ 7.14 (1H, s, H-5) because of the conjugation between N-1, C-6, C-5 and C=O in addition to the anisotropic effect of the carbonyl group as shown in Fig. 1. The other downfield proton resonates at δ 4.18 (1H, s, H-13ß)[2,10,11]. The assignment of the protons and carbons was confirmed by ^{1}H-^{1}H Correlation Spectroscopy (COSY) and ^{13}C-^{1}H COSY. The above data provided further evidence that the sparteine skeleton of (**1**) is substituted by a carbonyl and hydroxyl groups at C-4 and C-13 respectively and a double bond between C-5 and C-6.



(**1**)



(**2**)



(**3**)

One of the most important diagnostic values characteristic for conformation of ring C is the ^{13}C chemical shift of bridge C-8[12,17,18] and therefore the conformation of rings C and D in the molecule is *chair-chair*, which is also supported by the absence of the cross peak between C-11 and C-9 protons in the ^{1}H-^{1}H COSY spectrum[4,5].

From the biosynthetic point of view, we propose that both (**1**) and (-)-13α-hydroxy-multiflorine (**3**) could be derived from (-)-13α-hydroxysparteine (**2**) by oxidation and specific enzyme dehydrogenation.

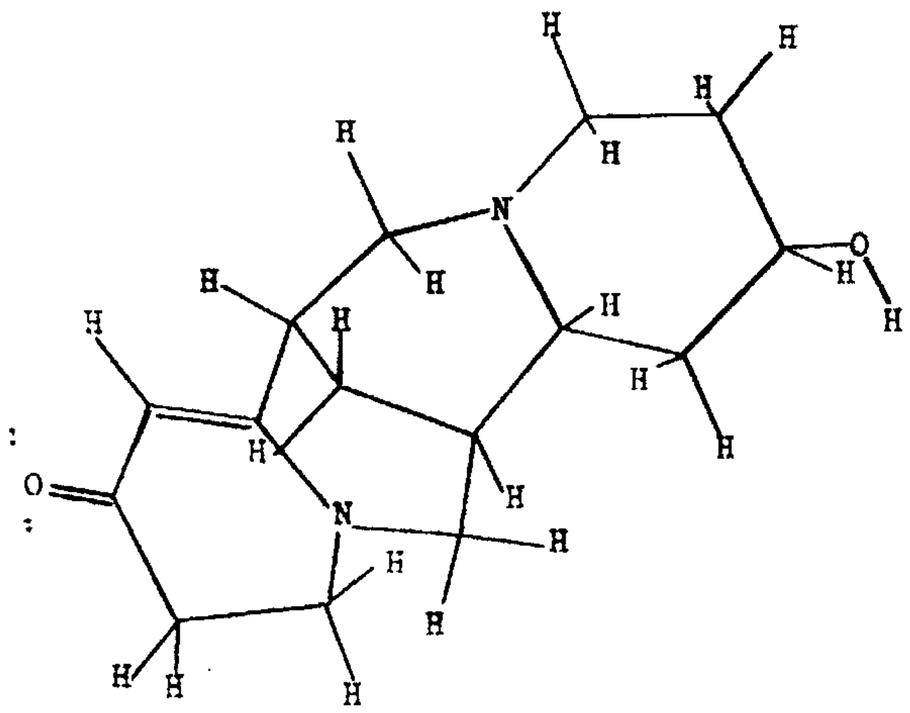


Fig. 1: ORTEP structural formula of compound (**1**) at full minimization by MM2 energy computer programme.

EXPERIMENTAL

The high and low resolution EIMS were measured on a Hitachi M-60 spectrometer at 70 ev. FAB-MS using glycerol and *m*-nitrobenzoic acid were measured at room temperature. ^{1}H and ^{13}C-NMR spectra were recorded on JEOL GSX 400 and GSX 500 spectrometers respectively. TMS was used as internal standard in CD_3OD and $CDCl_3$. TLC was carried out on silica gel plates in CH_2Cl_2-MeOH - 28% NH_4OH (90:9:1, 80:18:1 and 70:30:1). Analytical HPLC was performed as previously described[19,20].

Plant materials

The seeds of *L. termis* were collected at the Medicinal Plant Experimental Station at Al-Azhar University, Assiut in May 1992. The voucher specimen was identified by Prof. A.Fayed (Dept. of Systematic Botany and Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt) and the voucher specimen has been deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

Extraction and isolation

A total basic fractions (22 g, fraction A) and (5.1 g, fraction B) were obtained from the 75% EtOH extracts of the air dried seeds (1 kg) by a previously described method[3]. The aqueous layer remaining was made strongly basic by addition of a sufficient amount of powdered potassium carbonate under ice cooling and extracted with CH_2Cl_2. The organic extracts were combined, dried and concentrated to dryness to yield fraction B (5.1 g). The latter was chromatographed on silica gel column (Merck, type 60, 230-400 mesh, 400 g) using CH_2Cl_2-MeOH - 28% NH_4OH to yield six known alkaloids as follows:(±)-lupanine, (900 mg) m.p 98°, $[\alpha]_D^{25}$ 0°, (C= 0.1, MeOH), eluted by 4% MeOH-CH_2Cl_2; (-)-multiflorine (250 mg), oil, $[\alpha]_D^{25}$ -299° (C= 0.1, MeOH), eluted by 6% MeOH-CH_2Cl_2; (+)-angustifoline (180 mg), oil, $[\alpha]_D^{25}$ +5.3° (C= 0.1, MeOH) eluted by 8% MeOH-CH_2Cl_2; (-)-albine (222 mg), oil, $[\alpha]_D^{25}$ -103° (C= 0.1, MeOH), eluted by 10% MeOH-CH_2Cl_2; (+)-13α-hydroxylupanine (380 mg), m.p. 174°, $[\alpha]_D^{25}$ +45.5° (C= 0.1, MeOH) eluted by 12% MeOH-CH_2Cl_2; (-)-13-α-hydroxysparteine (**2**), oil, $[\alpha]_D^{25}$ -87° (C= 0.1, MeOH) eluted by 16% MeOH in CH_2Cl_2, this compound was identified by all means of chromatographic and spectroscopic methods[21,22].

(-)-Termnie (1), yellow oil, $[\alpha]_D^{25}$ -14.7°, (C= 0.1, MeOH) eluted by 18% MeOH-CH_2Cl_2, EIMS: m/z 262(23), 245(14), 244(16), 219(7), 150(100), 136(35), 110(17) and 55(41), UV (γ_{max} 304 nm, MeOH, log ϵ= 3.114), IR (KBr), cm^{-1}: 3435 (OH), 1630 (C=O) and 1580 (C=C), ^{1}H-NMR δ 7.14 (1H, s, 5-H), 4.18 (1H, s, 13ß-H), 3.73 (1H, br.d, J=17.1, 15α-H), 3.44 (2H, m, 2ß-H, 17α-H), 2.82 (2H, m, 10ß-H, 17ß-H), 2.41 (1H, m, 10α-H), 2.34 (2H, br.d., J=13.9, 2α-H, 15ß-H), 2.28 (1H, m, 11α-H), 2.19 (1H, br.d., J= 16.9, 7-H), 2.03 (1H, m, 3ß-H), 1.88 (1H, m, 9-H) , 1.77-1.62 (4H, m, 12-H, 14α-H, 3α-H), 1.56-1.49 (2H, m, 8α-H, 14ß-H), 1.25 (1H, m, 8-ß-H). ^{13}C-NMR data are recorded in Table 1.

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