Department of Pharmacognosy[1], Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt and Department of Pharmacognosy[2], Faculty of Pharmacy, Assiut, University, Assiut Egypt

Minor lupin alkaloids from the seeds of *Lupinus varius* and *Lupinus hartwegii*

M. H. Mohamed[1], K. M. Mohamed[2], H. A. Hassanean[2] and S. A. El-Moghazy[2]

A new lupin alkaloid, (−)-13α-hydroxy-4-oxosparteine (**1**), was isolated from the chloroform fraction of an ethanol extract of the seeds of *Lupinus varius* together with (−)-13α−hydroxysparteine. In addition, another new alkaloid, (−)-13α-tigloyloxyaphylline (**2**), was isolated from the chloroform fraction of an ethanol extract of the seeds of *Lupinus hartwegii* together with (−)-17-oxosparteine, (+)-17-oxolupanine and (−)-sparteine. The structure of these alkaloids was established by spectral methods and chemical transformations.

1. Introduction

Various *Lupinus* species (family Leguminosae) growing in Egypt were a subject of interest to our group due to their rich alkaloidal content [1–5]. *Lupinus varius* L. ssp. orientalis Franco et Silva (= *L. digitatus* Forsk, *L. pilosus* L., *L. hispanicus* and *L. micranthus*) is an annual herb, growing in the Mediterranean region [5–7]. Previous work on its alkaloidal constituents indicated the presence of (−)-13β-hydroxymultiflorine, and thirteen known alkaloids [5].

Lupinus hartwegii Lindl. (= *L. mexicanus*) is an annual herb growing in gardens as winter ornamental [5, 8]. Previous investigations on its alkaloidal constituents reported the isolation of (+)-2β-hydroxyaphylline, (+)-13α-hydroxyaphyllidine and eleven known alkaloids [5].

In the present study, the constituents of the seed of *Lupinus varius* and *Lupinus hartwegii* were further examined. A new lupin alkaloid, (−)-13α-hydroxy-4-oxosparteine (**1**) and (−)-13α-hydroxysparteine (**3**) were isolated from the chloroform soluble fraction of the ethanol extract of *Lupinus varius*. Another new alkaloid, (−)-13α-tigloyloxyaphylline (**2**) was isolated from the chloroform soluble fraction of the ethanol extract of *Lupinus hartwegii* together with (−)-17-oxosparteine, (+)-17-oxolupanine and (−)-sparteine.

2. Investigations, results and discussion

2.1. Identification of the alkaloid 1

From the chloroform fraction of the 75% ethanolic extract of the crushed seeds of *Lupinus varius*, **1** was isolated (0.006% fr, wt.) by silica gel column chromatography. The HREIMS spectrum of **1** indicated the molecular formula $C_{15}H_{24}N_2O_2$ ($[M]^+$, m/z 264.1844, calcd 264.1839). The presence of a hydroxyl group was indicated by the fragment at m/z 247 (38) and m/z 246 (46) in the EIMS, corresponding to $[M-OH]^+$ and $[M-H_2O]^+$ respectively [9]. The IR spectrum of **1** showed a band at 3447 cm^{-1} (OH) and a band at 1723 cm^{-1} (C=O) [9–12]. The latter was confirmed by the presence of a signal at δ 206.5 in the ^{13}C NMR spectrum [9, 10, 12]. From these results and other spectral data, **1** could be persumed to be a sparteine-type lupin alkaloid containing carbonyl and hydroxyl moieties.

The DEPT ^{13}C NMR spectrum of **1** showed the presence of 15 carbon atoms (Table); one carbonyl, five methines and nine methylene carbon atoms. The chemical shifts for carbon atoms forming rings B, C and D of **1** showed patterns similar to (−)-13α-hydroxysparteine (**3**) except those of ring A where the carbonyl group is located at C-3 γ to N-1 [12, 13]. The hydroxyl group of **1** was located at C-13 having an α-axial orientation from the ^{13}C NMR signal at δ 63.8 (d, C-13) [5, 13]. This was also established from the ^1H NMR spectrum of **1**, where the H-13 signal appeared at δ 4.17 (1 H, t = 2.81, H-13β). The small coupling constant, multiplicity and chemical shift of H-13 confirmed its β-equatorial nature [5, 14].

The ^1H NMR spectrum of **1** showed downfield shifted protons at δ 3.29 (1 H, m, H-6) and δ 2.71 (1 H, br.d, J = 11.7, H-2eq) [15]. The assignments including all the protons and carbons of **1** were confirmed by ^1H-^1H COSY and ^{13}C-^1H COSY. The above data provided further evidence that the skeleton of **1** is (−)-13α-hydroxy-4-oxosparteine.

From the biosynthetic point of view, it can be proposed that both **1** and **3** could be derived from sparteine by specific enzyme oxidation. Compound **1** may also be considered as intermediate between **3** and (−)-13α-hydroxymultiflorine.

Table: Comparison of ^{13}C NMR data of compounds 1–4

C	1	3*	2	4**
2	54.7 (t)	56.2 (t)	42.8 (t)	42.7 (t)
3	41.1 (t)[a]	25.7 (t)	25.0 (t)[b]	25.1 (t)
4	206.5 (s)	24.7 (t)	24.7 (t)[b]	24.8 (t)
5	47.6 (t)	29.3 (t)	29.3 (t)	29.1 (t)
6	64.9 (d)	66.5 (d)	59.9 (d)	59.4 (d)
7	35.2 (d)	35.6 (d)	32.3 (d)	32.4 (d)
8	27.6 (t)	27.4 (t)	22.9 (t)	22.6 (t)
9	33.1 (d)	33.4 (d)	43.8 (d)	43.2 (d)
10	61.0 (t)	61.4 (t)	172.8 (s)	172.9 (s)
11	56.0 (d)	57.2 (d)	53.2 (d)	52.0 (d)
12	40.3 (t)[a]	41.7 (t)	27.2 (t)	29.3 (t)
13	63.8 (d)	64.6 (d)	67.3 (d)	65.3 (d)
14	32.2 (t)	32.8 (t)	23.1 (t)	25.1 (t)
15	50.1 (1)	49.2 (t)	49.1 (t)	47.8 (t)
17	54.7 (t)	53.2 (t)	47.3 (t)	46.1 (t)
1′			167.3 (s)	
2′			128.7 (s)	
3′			138.2 (d)	
4′			14.7 (q)	
5′			12.9 (q)	

* Data from ref. [13]
** Data from ref. [19]
[a, b] These values may be interchangeable

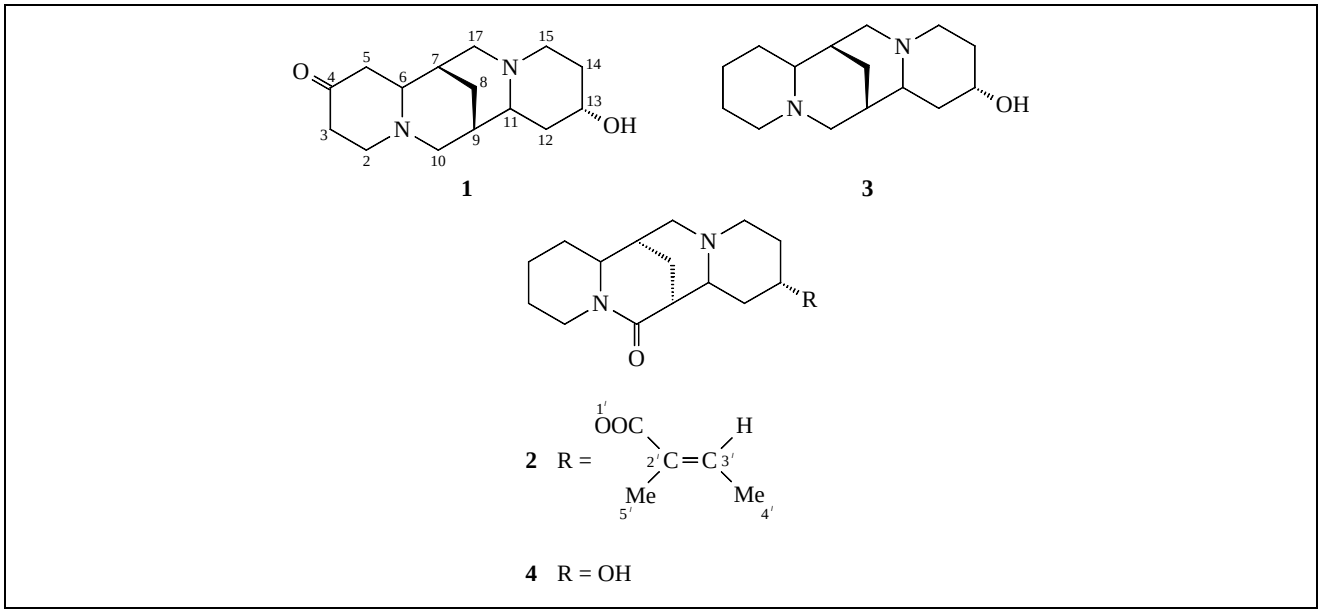

Fig. Structure of compounds 1–4

2.2. Identification of the alkaloid 1

The CIMS of compound **2** exhibited $[M+1]^+$ at m/z 347 which is consistent with the molecular formula $C_{20}H_{30}N_2O_3$. It was evident from the fragmentation pattern of **2** that the molecule consisted of a substituted aphylline skeleton and ^{1}H NMR spectrum showed the presence of the tigloyl moiety [16–18]. The latter was confirmed by the fragment at m/z 246 [M^+-100 (100)] in the EIMS due to the loss of tigloyl moiety from M^+ [16–18]. The IR spectrum of **2** showed bands at 2950–2700 cm^{-1} (Bohlmann bands), 1705 (ester), 1633 cm^{-1} (lactam carbonyl) [9, 16–18]. The amide and ester carbonyl groups were confirmed by signals at 172.8 and 167.3 respectively in its ^{13}C NMR spectrum [5, 9, 10].

The ^{13}C NMR spectrum of **2** showed the presence of 20 carbons which were assigned as shown in the Table. The chemical shifts of the carbon atoms forming rings A, B and C of **2** were similar to those reported for 13α-hydroxyaphylline (**4**) and the chemical shift of the side chain was almost typical for the tigloyl moiety as side chain [5, 16–19]. However, the chemical shifts of ring D carbons of 2 showed marked difference to that of **4** indicating its substitution with a tigloyl moiety. The presence of the tigloyl moiety was confirmed by the five ^{13}C NMR signals: the ester carbonyl at δ 167.3 (s, C-1′), two carbons at δ 128.7 (s, C-2′), 138.2 (d, C-3′) indicating the double bond and the two methyl carbons at δ 14.7 (q, C-4′) and 12.9 (q, C-5′) [16–18]. The latter two methyl carbons clearly indicated the tigloyl moiety (c.f. the angloyl isomer) [19]. This is also further supported by the ^{1}H NMR signal at δ 6.79 (1 H, q, J = 7.9 Hz, H-3′) [19]. It is noteworthy to mention that traces of the angeloyl isomer were detected in the ^{1}N NMR spectrum.

The attachment and stereochemistry of the tigloyl moiety at C-13 as α-axial was determined from the chemical shift δ 67.3 (d, C-13) in the ^{13}C NMR [16–18]. This is further supported by the ^{1}H NMR signal at δ 5.14 (1 H, t, J = 2.71 Hz, H-13eq) [17]. The small coupling constant, multiplicity and chemical shift of H-13 confirmed its α-equatorial orientation and accordingly the tigloyl moiety affixed an α-axial orientation [5, 14].

The final confirmation of the structure of **2** was made by its hydrolysis to **4**, which was confirmed by direct comparison with an authentic sample (m.p., IR and co-chromatography).

These data established unequivocally that the skeleton of **2** is (−)-13α-tigloyloxyaphylline.

The other known compounds were identified by comparison of their physical and spectral parameters with published data and authentic samples [1–5, 15, 20].

3. Experimental

3.1. Plant material

The seeds of *L. varius* L. were collected from plants which grow in the Sinai region near El Arish in April 1992 and the seeds of *L. hartwegii* were supplied from Prof. N. El-Keltawy (Dept. of Horticulture, Faculty of Agriculture, Assiut University, Assiut, Egypt). Both seeds were cultivated at the Medicinal Plant Experimental Station of Al-Azhar University, Assiut in October 1993 and collected in April 1994. The plants were identified by Prof. A. Fayed (Dept. of Systematic Botany and Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt).

3.2. Instruments

High and low resolution EIMS and CIMS were measured on a Kratos 50 spectrometer at 70 eV. Mps: uncorr.; IR: thin films of $CHCl_3$ and KBr on a Unicam SP-3-1025 (Pye-unicam LTD cambridge, England) spectrophotometer: Optical rotations: 10 cm path length in the solvents stated using Perkin Elmer 141 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker WH 400 and 500 spectrometers, respectively. TMS was used as internal standard in $CDCl_3$. TLC; silica gel (Kieselgel 60, F 254, E. Merck) of 0.25 mm layer thickness in $CHCl_3$/MeOH/28% NH_4OH (90 : 9 : 1, 80 : 18 : 1). The chromatograms were visualized by spraying with Dragendorff's reagent.

3.3. Extraction and isolation of 1

The total alkaloidal fraction from the 75% EtOH extracts of the finely ground seeds of *L. varius* (1.3 kg) was obtained in a yield of 1.9% of the fresh weight using a method described before [1–5]. The mixture of bases (27 g) was chromatographed on a silica gel column (Merck, type 60, 230 to 400 mesh, 1 kg, 7 × 150 cm) and gradient elution using MeOH in $CHCl_3$/28% NH_4OH to yield the alkaloids as follows: (−)-13α-hydroxysparteine, oil, (11 mg), $[\alpha]_D^{25}$ −87° (C = 0.1, EtOH) eluted by 17% MeOH, pure **1** amorphous, (16 mg), $[\alpha]_D^{25}$ −131° (C=0.1, MeOH), eluted by 19% MeOH in $CHCl_3$.

3.4. Identification of 1

EIMS, m/z 264 (M+, 52), 247 (38), 246 (46), 165 (38), 152 (100), 134 (32), 55 (31), IR ν_{max}^{KBr} 3447 (OH), 2900–2750 (Bohlmann bands), 1723 (C=O), ^{1}H NMR ($CDCl_3$) δ 4.17 (1 H, t, J = 2.81, H-$13_{eq.}$) 3.29 (1 H, m, H-6), 2.71 (1 H, br. d. J = 11.7, H-2_{eq}), 2.36 (1 H, dd, J = 11.4, 2.9 Hz, H-17_{ax}), 2.49 (1 H, dd, 10.3, 2.4 Hz, H-10_{eq}), 2.41 (1 H, ddd, J = 13.3, 13.3,

2.9, H-15$_{ax}$), 2.23 (1 H, br.d, J = Hz, H-15$_{eq}$), 2.13 (1 H, d, J = 11.9, H-11), 2.07 (1 H, ddd, J = 13.2, 2.7, 2.7 Hz, H-3$_{ax}$), 2.01 (1 H, ddd, J = 13.5, 10.7, 2.1 Hz, H-3$_{eq}$), 1.97 (1 H, td, J = 12.3 H-z, H-2$_{ax}$), 1.95–1.92 (3 H, m, H-10$_{ax}$, H-7, H-8$_{ax}$), 1.88–1.81 (2 H, m, H-5$_{ax}$, H-17$_{eq}$), 1.69 (1 H, m, H-14$_{ax}$), 1.61 (1 H, m, H-14$_{eq}$), 1.52–1.50 (2 H, m, H-12$_{ax}$, H-5$_{eq.}$), 1.44–1.41 (2 H, m, H-12$_{eq.}$, H-9) 1.21 (1 H, br.s., H-8$_{eq}$). ^{13}C NMR ($CDCl_3$) see Table.

3.5. Extraction and isolation of 2

The total basic fraction from the 75% EtOH extracts of the finely ground seeds of *L. hartwegii* (1.2 kg) was obtained in a yield of 1.8% of the fresh weight using a method described before [1–5]. The mixture of bases (24 g) was chromatographed on a silica gel column (Merck, type 60, 230–400 mesh, 1 kg, 7 × 150 cm) and gradient elution using MeOH in $CHCl_3$/28% NH_4OH to yield the alkaloids as follows: (−)-17-oxosparteine, (19 mg), needles, m.p. 83° $[\alpha]_D^{25}$ −139° (C = 0.1, EtOH) eluted by 1% MeOH in $CHCl_3$, pure **2**, (24 mg), oil, $[\alpha]_D^{25}$ −17.3°, (C = 0.1, $CHCl_3$); eluted by 2% MeOH in $CHCl_3$, (+)-17-oxolupanine, (14 mg), needles, m.p. 154–155 °C $[\alpha]_D^{25}$ −19° (C = 0.1, EtOH) eluted also by 2% MeOH in $CHCl_3$ (−)-spartaine, oil, (61 mg), $[\alpha]_D^{25}$ −17 ° (C = 0.1, MeOH) eluted by 16% MeOH in $CHCl_3$.

3.6. Identification of 2

EIMs m/z (rel int.): $[M]^+$ not detected, m/z (rel int): 247 (43), 246 (100), 220 (33), 191 (22), 137 (44), 136 (22), 97 (43), 84 (32), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 2950–2700 (Bohlmann bands), 1705, 1633 (ester and amide C=O), 1560 (C=C); ^{1}H NMR ($CDCl_3$) δ 6.79 (1 H, q, J = 8 Hz, H-3′), 5.14 (1 H, t, J = 2.71, H-13$_{eq}$), 4.79 (1 H, dd, J = 14.3, 4.1, H-2$_{eq}$), 3.26 (1 H, dd, J = 12.1, 8.2, H-17$_{eq}$), 3.21 (1 H, m, H-6), 3.11 (1 H, dd, 12.1, 3.2, H-17$_{ax}$), 2.77 (1 H, dd, J = 12.7, 3.4, H-15$_{ax}$), 2.71 (1 H, 12.7, 8.1, H-15$_{eq}$), 2.41 (1 H, dd, J = 14.4, 6.3, H-2$_{ax}$), 2.31 (1 H, m, H-9), 1.91 (1 H, m, H-11), 1.86 (3 H, s, Me-5′), 1.79 (3 H, m, Me-4′).

3.7. Identification of the known alkaloids

These compounds were identified by comparison with authentic samples as well as all means of chromatographic and spectroscopic methods. For full data see ref.s [1–5, 8, 14, 20].

3.8. Hydrolysis of 2 to 13α-hydroxyaphylline (4)

Compound **2** (24 mg) was dissolved in 4 ml 5% KOH/MeOH (1 : 1) and refluxed at 100 °C for 6 h. The residue was dissolved in 3 ml H_2O and extracted with $CHCl_3$ (10 ml × 4) to give a product of hydrolysis (12 mg, needles) which was identified by m.p. (249 °C), $[\alpha]_D$-46° and R_f value as 13α-hydroxyaphylline [5, 17].

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Mahmoud Hamed Mohamed Ph. D.
Faculty of Pharmacy Dean
Al-Azhar University
Assiut 71524
Assiut, Egypt