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(−)-6α-HYDROXYLUPANINE, A LUPIN ALKALOID FROM *LYGOS RAETAM* VAR. *SARCOCARPA*

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Key Word Index—*Lygos raetam* var. *sarcocarpa*; Leguminosae; lupin alkaloid; (−)-6α-hydroxylupanine; absolute configuration.

Abstract—A new lupin alkaloid, (−)-6α-hydroxylupanine, was isolated from aerial parts of *Lygos raetam* var. *sarcocarpa*. Its structure, including absolute configuration, was determined by different spectroscopic methods and by chemical transformation into (+)-5, 6-dehydrolupanine.

INTRODUCTION

Lygos raetam (=*Retama raetam*, *Genista raetam*) var. *sarcocarpa* is a small wild desert shrub [1] growing in Egypt. Previous studies have reported the presence of several lupin alkaloids [2–4]. In the present communication, further investigation of the alkaloid content of aerial parts of this species afforded a new alkaloid, (−)-6α-hydroxylupanine (**1**).

RESULTS AND DISCUSSION

From the 75% ethanol extract of the dried aerial parts of *L. raetam* var. *sarcocarpa*, **1** was isolated as colourless transparent needles in a yield of 0.007% of the dry weight after silica gel and Al_2O_3 column chromatography.

The HR EI mass spectrum of **1** afforded the molecular formula $C_{15}H_{24}H_2O_2$ ($[M]^+$ *m/z* 264.1844, calc. 264.1839). The presence of a hydroxyl group was indicated by fragment ions at *m/z* 247 (21%) and 246 (base peak) in the EI mass spectrum, corresponding to $[M - OH]^+$ and $[M - H_2O]^+$, respectively. In the IR spectrum, the band at 3400 cm^{-1} confirmed the presence of the hydroxyl group and the band at 1640 cm^{-1} indicated the presence of a lactam C=O group, as well *trans*-quinolizidine bands at 2860, 2810 and 2750 cm^{-1}. From these results, **1** could be presumed to be a sparteine-type lupin alkoloid containing lactam and hydroxyl groups in the molecule.

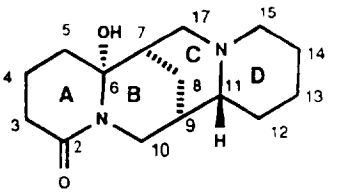
The ^1H NMR spectrum of **1** was complicated because almost all signals are located within a narrow chemical shift range (δ1.0–3.0). Consequently, ^1H–^1H COSY, ^1H–^{13}C COSY and NOESY experiments were applied to make chemical shift assignments and to investigate the conformation of the molecule.

In the ^1H NMR spectrum of **1**, the most down-field proton resonated at δ4.2 (1H, *dt*, *J* = 13.2, 2.2 Hz, H-10α) which was geminally coupled to H-10β (*J* = 13.2) and vicinally coupled to H-9 (*J* = 2.2 Hz) as shown by ^1H–^1H COSY. This assumption was also confirmed by the location of the carbonyl function at position 2, since it has the same chemical shift (δ171.5) as that of (−)-lupanine [2]. Also, the ^1H NMR spectrum exhibited a broad singlet at δ3.95, exchangeable with D_2O, which confirmed the presence of a free hydroxyl group in the molecule. The foregoing results, indicated that **1** is a lupanine-type alkaloid having OH substitution.

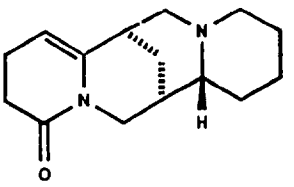
The ^{13}C NMR spectrum of **1** revealed the presence of 15 carbons. Determination of the multiplicity was attained by DEPT experiments, which revealed that **1** has three methine and 10 methylene carbons. The three down-field methine, carbons at δ63.9, 37.8 and 34.5 were assigned to C-11, C-7 and C-9, respectively, while the three methylene carbons at δ55.2, 54.3 and 42.8 were assigned to C-15, C-17 and C-10, respectively. The substitution pattern could be deduced as follows. The carbonyl group is located at position 2 because C-2 has the same chemical shift (δ171.6) as that of (−)-lupanine [2]. The hydroxyl group is established at position 6 on the basis of the lack of a methine C-6 in lupanine which resonated at δ60.9 [2] and, instead, the appearance of a quaternary carbon resonating at δ85.5. This was confirmed by the lack of signals in the region δ3.0–3.5 ppm characteristic of H-6 of lupanine [5]. Therefore, these data indicated unambiguously that the OH group is located at the bridgehead C-6.

The α-configuration of the OH group was proved by NOESY spectral data (Fig. 1), where there is a strong cross-peak between H-10α and the OH proton. Also, there is a cross-peak between H-10α and H-8α indicating that both protons have a 1,3-diaxial relationship.

The final confirmation of the structure of **I**, including absolute configuration, was made by chemical transformation of **1** into (+)-5, 6-dehydrolupanine (**2**) by dehydration with trifloroacetic acid. The product from dehy-



1



2

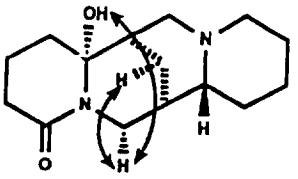


Fig. 1. Observed NOEs of compound 1.

dration was identified by co-chromatography, IR, $[\alpha]_D$, mass spectrometry and NMR with an authentic sample. The absolute coufiguration of (+)-5,6-dehydrolupanine was thus established as 7*R*, 9*R*, 11*R* [6]. These data clearly indicate that **1** and **2** have the same absolute configuration. From these results, it can therefore be established that the new alkaloid is (−)-6α-hydroxylupanine.

The closely related 6β-hydroxylupanine has been isolated from *Bolusanthus speciosus* [7]. However, the mp, $[\alpha]_D$ and absolute configuration were not mentioned and complete proton asignments were not made. It should be noted that **1** might have a significant role in the biosynthetic pathway of quinolizidine alkoloids, because it is likely to be the intermediate between lupanine and 5,6-dehydrolupanine [7].

EXPERIMENTAL

General. Mps uncorr. IR recorded as a thin film from $CHCl_3$ soln. Optical rotation was measured for a path length of 10 cm in EtOH. ^1H and ^{13}C NMR were recorded at 500 and 125 MHz, respectively; TMS was used as an int. standard in $CDCl_3$. EIMS were measured at 70 eV. TLC was carried out on silica gel (Kieselgel 60, F 254) in CH_2Cl_2–MeOH–28% NH_4OH (43:6:1) and on Al_2O_3 (F 254, type E) in benzene–Me_2CO–MeOH (34:3:3). Chromatograms were visualized by spraying with Dragendorff's or iodoplatinate reagents. Analytical GC and HPLC were performed as described in Refs [8, 9].

Extraction and isolation of **1**. The aerial parts of *Lygos raetam* var. *sarcocarpa* (Zoh.) Täckh. et Boulos. was collected in April 1988 near Abo Mady (North Egypt). The species was identified by Prof. N. El Hadidy (Department of Botany, Faculty of Science, Cairo University, Egypt), and a voucher specimen has been deposited in the Department of Pharmacognosy (Faculty of Pharmacy, Mansoura University, Egypt). The total alkaloid fr. from 75% EtOH extracts of dried aerial parts was obtained in yields of 2.5% of the dry using the method of Ref. [10]. The mixt. of bases (10 g) was chromatographed on a silica gel column (Merck, type 60, 230–400 mesh, 300 g, 2.5 × 54 cm) and gradient elution using MeOH in CH_2Cl_2–28% NH_4OH (500:1) as reported previously [2, 4]. The frs enriched in **1** (130 mg) were eluted with 2.5% MeOH in CH_2Cl_2–28% NH_4OH (500:1), together with (−)-lupanine. Pure **1** (70 mg) was obtained by further purification on an Al_2O_3 column (Merck, type 90, mesh 70–230) with benzene–MeOH–Me_2CO (23:1:1).

(−)-6α-*Hydroxylupanine* (**1**). Crystals, mp 116–117°. $[\alpha]_D^{23}$ − 73.5° (EtOH, *c* 0.17). HR EIMS *m/z* (rel. int.): $[M]^+$ 264.1844 (24) (calcd for $C_{15}H_{24}N_2O_2$, 264.1839), 247 (21), 246 (100), 190 (6), 164 (7), 150 (9), 136 (21), 134 (13), 110 (10), 98 (89), 97 (40), 84 (13). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 *br* (OH), 2860, 2810, 2750 (Bohlmann bands), 1640 (lactam C=O). ^1H ^{13}C NMR in Table 1.

Dehydration of **1**. Compound **1** (25 mg) was dissolved in 2 ml of CH_2Cl_2 and an equimolar vol. of TFA added to the soln, stirring for 2 hr at 0–5°. The reaction mixt. was evapd, then basified with 10% $NaHCO_3$ and the free base extracted with CH_2Cl_2 which was then evapd under red. pres. The product of dehydration was purified by silica gel CC, eluting with 3% MeOH in Et_2O–28% NH_4OH (500:1). A transparent oily substance (18 mg) was obtained, which was identified as 5,6-dehydrolupanine. $[\alpha]_D^{23}$ + 37 (EtOH; *c* 0.15). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2850, 200 (Bohlmann bands), 1640 (lactam C=O). EIMS *m/z* (rel. int.): 246 ($[M]^+$ 25), 163 (8), 148 (7), 136 (15), 135 (9), 134 (14), 98 (100), 97 (38), 41 (25). ^1H NMR (500 MHz, $CDCl_3$): δ4.93 (1H, *dd*, *J* = 5.6, 3.4 Hz, H-5), 3.98 (1H, *d*,

Table 1. ^{13}C NMR, hetero COSY and ^1H NMR* spectra of compound **1** in $CDCl_3$

Position	Carbon chemical shift	Multiplicities (DEPT)	Hetero COSY	Multiplicities ^1H–^1H, J (Hz)
2	171.6			
3	33.1	CH_2	2.39 (H-3α)	*dt*, 3α, 3β (6.4)
				3α, 4α (2.5)
			2.36 (H-3β)	*dt*, 3β, 4α (5.4)
				3β, 3α (7.4)
4	19.4	CH_2	1.94 (H-4α)	*m*
			1.83 (H-4β)	*m*
5	32.4	CH_2	1.43 (H-5α)	*m*
			1.35 (H-5β)	*m*
6	85.5			
7	37.8	CH	2.04 (H-7α)	*d*, 7α, 17β (7.7)
8	15.8	CH_2	1.88 (H-8β)	*m*
			1.62 (H-8α)	*m*
9	34.5	CH	1.54 (H-9α)	*br s*
10	42.8	CH_2	4.12 (H-10α)	*dt*, 10α, 10β (13.2)
				10α, 9α (2.2)
			2.9 (H-10β)	*m*
11	63.9	CH	1.59 (H-11β)	*br s*
12	34.1	CH_2	1.83 (H-12β)	*m*
			1.62 (H-12α)	*m*
13	24.4	CH2	1.62 (H-13α)	*m*
			1.20 (H-13β)	*m*
14	24.6	CH_3	1.43 (H-14α,14β)	*m*
15	55.2	CH_2	2.7 (H-15α)	*br d* 15α, 15β (11.5)
			1.9 (H-15β)	*d* 15β, 14α (3.5)
17	54.3	CH_2	2.87 (H-17α)	*m*
			1.80 (H-17β)	*m*

*The OH proton resonated at δ3.9 as *br s*, disappearing on addition of D_2O.

J = 13.4 Hz, H-10α). ^{13}C NMR ($CDCl_3$, 125 MHz): 170.9 (*s*, C-2), 31.7 (*t*, C-3), 19.2 (*t*, C-4), 102.2 (*d*, C-5), 143.0 (*s*, C-6), 34.1 (C-7), 25.3 (*t*, C-8), 33.2 (*d*, C-9), 48.2 (*t*, C-10), 63.3 (*d*, C-11), 27.4 (*t*, C-12), 21.4 (*t*, C-13), 22.8 (*t*, C-14), 56.5 (*t*, C-15), 54.7 (*t*, C-17).

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