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HARMALIDINE, A β-CARBOLINE ALKALOID FROM *PEGANUM HARMALA*

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Key Word Index—*Peganum harmala*; Zygophyllaceae; β-carboline alkaloid; harmalidine.

Abstract—A new β-carboline alkaloid, harmalidine, has been isolated from the seeds of *Peganum harmala* and its structure elucidated by spectral and chemical studies.

INTRODUCTION

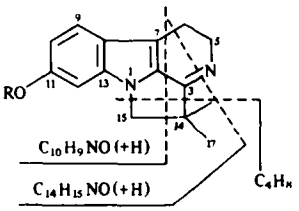
Peganum harmala (Zygophyllaceae), commonly known as harmal, grows wild in semi-arid areas of the Indo-Pakistan subcontinent, Iran and Africa. Its different parts are used in traditional systems of medicine for the treatment of a variety of human ailments [1, 2]. Harmala alkaloids are also reported to possess hypotensive activity [3]. Moreover, β-carbolines have psychotomimetic action [4], and harmine and harmaline are described in the literature as hallucinogens [5]. Recent structure–activity studies of β-carbolines suggested that these bases may act as neuromodulators [3]. In view of the therapeutic importance attributed to *P. harmala*, its different parts have been subjected to chemical studies since 1841 by various groups of workers [6–12], when Goebel for the first time reported the isolation of harmaline from the seeds [13].

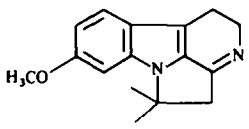
As a result of our present studies on the alkaloidal constituents of *P. harmala* seeds, a new alkaloid harmalidine (**1**) has been isolated and its structure elucidated by spectral and chemical studies. Harmalidine is of potential pharmacological significance since it also possesses a β-carboline skeleton.

RESULTS AND DISCUSSION

The bases obtained from the methanolic extract of whole *P. harmala* seeds were divided into water soluble and insoluble hydroiodides. After usual work up, the base from the soluble hydroiodide fraction was subjected to prep. TLC furnishing harmalidine (**1**) mp 150–152° with a molecular formula $C_{16}H_{18}N_2O$, obtained by high resolution mass spectrometry. The UV spectrum of **1** is characteristic of β-carboline alkaloids, with absorption maxima at 205, 215, 260, 340 and 375 nm. Its IR spectrum showed peaks at 3050 (aromatic ⩾C–H), 2830, 2950 (aliphatic ⩾C–H), 1630 (>C=N–), 1600–1400 (4 peaks, aromatic ring), 1375 (doublet, *gem* dimethyl), 1260 and 1030 (=C–O–C–), 1160 (C–O) and 800 cm^{-1} (substituted aromatic ring). The ^1H NMR spectrum of **1** (Table 1) exhibited a double doublet at δ6.80 (J = 8.9 and 1.8 Hz), two doublets at δ7.08 (J = 1.8 Hz) and 7.42 (J = 8.9 Hz) attributable to H-10, H-12 and H-9, respectively. It also showed two, two-proton triplets at δ3.11 and 3.93 (J = 8.3 Hz) which have been ascribed to H-6 and H-5, respectively, and a three-proton singlet at δ3.85 due to a methoxy group. These assignments were confirmed by ^1H–^1H homonuclear decoupling experiments. Thus, irradiation at δ6.80 collapsed each of the two doublets at δ7.08 and 7.42 into a singlet, while on irradiation at δ7.08 and 7.42 the double doublet at δ6.80 was converted into 2 doublets with J = 8.9 and 1.8 Hz, respectively. Irradiation at δ3.11 resulted in the collapse of the triplet at δ3.93 to a singlet and *vice versa*.

The above ^1H NMR spectral data are comparable with those of harmaline [14] (Table 1); however, in **1** the signals for the C-3 methyl group and the indolic N–H were absent. The absence of the latter was also supported by the





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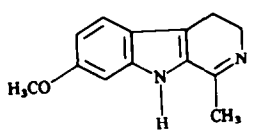
1 R = CH_3
2 R = H
3 R = CH_3, 3,4-dihydro

Fragment a = $C_{10}H_9NO - OCH_3$
Fragment b = $C_{14}H_{15}NO - OCH_3 - CH_3$



Harmaline

Table 1. ^{1}H NMR spectral data of β-carbolines (300 MHz)

Protons	Harmaline*	1†	2‡	3†
H-3	—	—	—	3.75 *s*
H-5	3.81 *t* (8.5)	3.93 *t* (8.3)	4.20 *t* (6.5)	3.84 *m*
H-6	2.93 *t* (8.5)	3.11 *t* (8.3)	3.50 *t* (6.5)	3.27 *m*
H-9	7.45 *d* (8.9)	7.42 *d* (8.9)	7.64 *d* (9.0)	7.36 *d* (8.6)
H-10	6.80 *dd* (8.9, 2.1)	6.80 *dd* (8.9, 1.8)	6.83 *dd* (9.0, 1.9)	6.90 *dd* (8.6, 1.7)
H-12	6.93 *d* (2.1)	7.08 *d* (1.8)	7.12 *d* (1.9)	6.96 *d* (1.7)
H-14	2.57 *s*	—	—	—
H-15	—	3.30 *m*	3.31 *m*	3.68 *m*
H-16	—	1.30 *s*	1.29 *s*	1.30 *s*
H-17	—	1.30 *s*	1.29 *s*	1.29 *s*
OCH_3	3.84 *s*	3.85 *s*	—	3.91 *s*
OH	—	—	5.40 *s*	—
NH	8.17 *br s*	—	—	1.72

All values are in δ (ppm) and the coupling constants (in parentheses) are in Hz. Solvents used: *DMSO-d_6; †$CDCl_3$; ‡acetone-d_6.

IR spectrum which did not show a band for N–H stretching. Instead, the presence of an additional ring was indicated by the molecular formula which showed nine double bond equivalence. That this was a five-membered ring was indicated by the ^{1}H NMR spectrum which showed a two-proton multiplet at δ3.30 (H-15) and a six-proton singlet at δ1.30 (H-16 and H-17). These structural features were finally confirmed by the presence of signals at δ43.0 (C-15), 39.7 (C-14) and 27.0, 14.0 (C-16 and C-17), in the ^{13}C NMR spectrum (Table 2) and diagnostic ions at *m/z* 128.0588 (C_9H_6N, fragment a), 167.0715 ($C_{12}H_9N$, fragment b) and 198.0796 resulting from the loss of C_4H_8, in the mass spectrum. A Dreiding model shows that one of the C-14 methyl groups is γ-*trans* to the indolic nitrogen, and this may explain the upfield shift of one of the methyls [15].

Demethylation of **1** with hydroiodic acid yielded **2**, the ^{1}H NMR spectrum (Table 1) of which showed a singlet at δ5.40 for the hydroxyl function while the singlet of the methoxy group disappeared. On hydrogenation (H_2/Pt), **1** furnished the dihydroharmalidine (**3**) which gave a $[M]^+$ at *m/z* 256 and displayed UV absorption at 208, 225, 245, 305 and 345 (sh) nm. The ^{1}H NMR spectrum (Table 1) showed a one-proton singlet at δ3.75 for H-3 and a signal at δ1.72, exchangeable with D_2O, for N_b–H; apart from other signals of harmalidine. The chemical and spectral data recorded above provided the structure of harmalidine as **1**, which was substantiated by ^{13}C NMR chemical shifts (Table 2) and important fragments in the mass spectrum (*vide* Experimental).

It is noteworthy in this context that 3,4-dihydro-β-carbolines are very rare in nature [16–20], although a number of naturally occurring β-carboline alkaloids are reported which are either fully oxidized (harmine type) [20, 21] or fully reduced (tetrahydroharmine type) [20]. Moreover, it may also be mentioned that this is the first instance of a five-membered ring between C-3 and the indolic nitrogen in a β-carboline skeleton. A survey of the literature reveals only one example of a β-carboline with an isobutyl side chain at C-3 with hypothetical biosynthesis from the condensation of a tryptophane (tryptamine) intermediate and C-5 terpenoid unit [22]. The cyclization of this side chain with indolic nitrogen would also lead to a five-membered ring as in **4**. However, the ^{13}C NMR (DEPT) data of harmalidine and ^{1}H NMR of **3** are consistent with structure **1**. The assignment of various carbons of the β-carboline ring system of **1** left two values, *viz* δ43.0 and 39.7, in the downfield region and the former more downfield value was assigned to the carbon attached to the nitrogen atom. The DEPT experiment showed that the carbon at δ43.0 is a methylene carbon while that at δ39.7 is a quaternary carbon. In the alternative structure (**4**), the carbon attached to the nitrogen atom is quaternary and the value δ39.7 seems rather upfield for it. Finally, in the ^{1}H NMR spectrum of the dihydro derivative (**3**) the signal of H-3 appeared at δ3.75 as a one-proton singlet which is possible only in structure **1**. The biosynthesis of **1** may be assumed through condensation of a five-carbon (non-isoprenoid) unit with a tryptophan moiety [23].

Table 2. ^{13}C NMR spectral data of harmaline and harmalidine

C	Harmaline [24]	Harmalidine (1) ($CDCl_3$)
2	126.6	126.3
3	162.1	161.3*
5	41.6	42.8
6	17.3	19.0
7	114.4	119.1
8	126.6	126.7†
9	121.7	122.1
10	109.3	115.0
11	157.7	144.5*
12	90.7	94.0
13	136.8	124.4†
14	19.1	39.7
15	—	43.0
16	—	27.0‡
17	—	14.0‡
OCH_3	54.6	55.2

Chemical shifts are in δ (ppm); *,†,‡ values may be reversed.

EXPERIMENTAL

Mps are uncorr. MS were recorded on a double focusing instrument connected to a computer system. ^1H and ^{13}C NMR broad band and DEPT spectra were recorded on a 300 MHz instrument. ^{13}C NMR spectral assignments have been made partly through the appearance of signals in the DEPT spectrum and partly through a comparison of the chemical shifts with those of harmaline [24]. The purity of samples was checked on TLC (silica gel S1F-254 precoated aluminium cards).

Whole seeds (4 kg) of *P. harmala* L. collected from non-arable northern areas of Pakistan were percolated × 4 with MeOH at room temp. On removal of the solvent from the combined percolates under red. pres, a dark reddish-brown viscous residue was obtained which was partitioned between EtOAc and 10% HOAc. On addition of KI to the latter fraction, a light brown crystalline ppt. of the HI of the bases was obtained, which was filtered, washed and dried. The HI was subjected to the isolation procedure described in ref. [6] yielding harmine and harmaline. To the filtrate of the HI of the base was added dilute alkali, and the liberated bases were extracted with EtOAc. The residue obtained from the EtOAc extract, after usual work up, was subjected to prep. TLC (silica gel, $CHCl_3$–MeOH, 9:1). As a result, **1** was obtained as a yellow crystalline compound, which on recrystallization from MeOH formed rods, mp 150–152°. HRMS *m/z* (rel. int.): 254.1408 $[M]^+$ (calc. for $C_{16}H_{18}N_2O$: 254.1419) (7), 253.1339 $[M-1]^+$ (7), 239.1184 $[M-Me]^+$ (10), 224.0952 $[M-2\times Me]^+$ (3), 213.1044 $[M-C_3H_5]^+$ (13), 212.1000 $[M-C_3H_6]^+$ (2), 199.0878 $[M-C_4H_7]^+$ (3), 198.0796 $[M-C_4H_8]^+$ (4), 193.0815 $[M-2\times Me-OMe]^+$, 181.0834 $[M-C_3H_6-OMe]^+$, 167.0715 (fragment b) (2), 128.0588 $[C_9H_6N$, fragment a$]^+$ (2), 114.0364 $[C_8H_4N]^+$ (2), 75.0274 $[C_6H_3]^+$ (9) and 56.0625 $[C_4H_8]^+$ (7).

Demethylation of **1**. **1** was refluxed with HI for 15 min. The mixture was then poured into H_2O and extracted with EtOAc. The aq. phase was basified with dilute NH_3 and the liberated base extracted with EtOAc. On usual work-up of the EtOAc phase, chromatographically pure **2** was obtained, mp 210–212°. It is soluble in 1% alkali. UV λ_{max} (MeOH) (nm): 208, 300, 365 and 410. IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1630, 1430–1575 (4 peaks), 1375 (*d*), and 830. EIMS *m/z* (rel. int.): 240 $[M]^+$ (3), 222 $[M-18]^+$ (2), 198 $[M-42]^+$ (22), 184 $[M-56]^+$ (6), 149 $[M-91]^+$ (21), 144 (3), 134 (15), 126 (100), 95 (40) and 91 (15).

Catalytic reduction of **1**. **1** (9 mg) was hydrogenated in EtOH over Pt black at room temp. for 24 hr. After conventional work up and crystallization from $CHCl_3$, **3** was obtained as colourless rods, mp 172–173°. UV λ_{max}^{MeOH} nm: 212, 265 and 295. EIMS *m/z* (rel. int.): 256 $[M]^+$ (5), 229 (10), 213 (10), 195 (2), 173 (6), 171 (3), 158 (6), 151 (6), 112 (15), 105 (18), 98 (30), 97 (50) and 83 (100).

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