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# AN OXAMIDE FROM *PEGANUM HARMALA* SEEDS

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**Key Word Index**—*Peganum harmala*; Zygophyllaceae; seeds; alkaloids; oxamide; structural determination.

**Abstract**—A new oxamide, *N*,*N*′-[(3-hydroxy-5-methyl)phenyl]-oxamide has been isolated from an aqueous extract of *Peganum harmala* seeds. The structure was established together with harmine (7-methoxy-1-methyl-9H-pyrido[3,4-*b*]-indole), harmaline (4,9-dihydro-7-methoxy-1-methyl-3H-pyrido[3,4-*b*]indole) and harmalol (4,9-dihydro-1-methyl-3H-pyrido[3,4-*b*]indol-7-ol).

## INTRODUCTION

Some naturally occurring $\beta$-carbolines such as harmine (**2**) and harmaline (**3**) have been known for a considerable time as central stimulants and amine oxidase inhibitors [1–3]. More recently **2** has been found to have an antiviral effect [4].

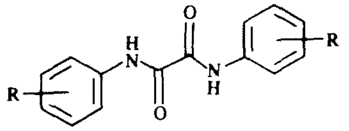
In view of the recent applications of this type of alkaloid, the study of their separation from plants have aroused great interest. The seeds of *Peganum harmala* contain the alkaloid vasicine, which is not an indole, and three other alkaloids, harmine (7-methoxy-1-methyl-9H-pyrido[3,4-b] indole) (**2**), harmaline (4,9-dihydro-7-methoxy-1-methyl-3H-pyrido[3,4-b] indole) (**3**) and harmalol (4,9-dihydro-1-methyl-3H-pyrido[3,4-b] indol-7-ol) (**4**) [5–8], which all contain the indole nucleus. The wide diversity of the products obtained and their variation according to the place of collection led us to study an Iraqi sample collected from Mosul in more detail.

In conjunction with our studies on the separation and characterization of some interesting naturally occurring compounds [9–13], we report here the structure of a new natural compound named *N*,*N*′-[(3-hydroxy-5-methyl)phenyl]-oxamide (**1**) isolated from the aqueous extract of *P. harmala* seeds as well as three other alkaloids, **2**–**4**. The latter have been previously isolated from an ethanolic extract of the seeds [5]. The structure of these compounds was confirmed by UV, IR, $^1$H and $^{13}$C NMR and high resolution mass spectral data.

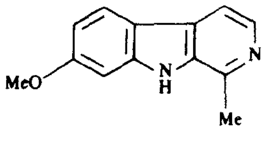
## RESULTS AND DISCUSSION

The work-up of the aqueous extract of *P. harmala* seeds and isolation of compounds is described in the Experimental. The water-soluble fraction was concentrated under reduced pressure and mixed with small amounts of adsorbent and then quantitatively transferred, after drying under vacuum, to the top of the prepared column.

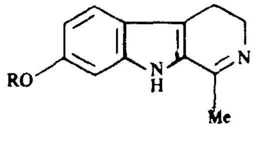
The fluorescent characteristics in UV light are useful in differentiating $\beta$-carbolines from other compounds. The following compounds have been separated. Oxamide (**1**) was obtained as a yellowish solid (0.15% of seed), mp 213–215° from ether–methanol); $R_f$ 0.28 (chloroform–methanol; 10:1); $\lambda_{\max}$ (methanol) 275, 325, 370 and 420 nm. The $M_r$ by mass spectrometry ($[M]^+$, 300.1112) and elemental analysis indicate the molecular formula $C_{16}H_{16}N_2O_4$. The IR spectrum (KBr) showed bands at 1600, 1665 and 3450 cm$^{-1}$ for aromatic, oxamide and hydroxyl group, respectively. The $^1$H NMR spectrum shows signals at $\delta$ 12.2, *s*, 1H and 9.1, *s*, 1H for OH and NH, respectively, ($D_2O$-exchangeable). The presence of a 1,3,5-trisubstituted aryl group was confirmed by the presence of two signals at $\delta$ 7.1, *br*, 1H ($J < 1$ Hz) and 6.9, *br*, 2H ($J < 1$ Hz) in the spectrum. The $^{13}$C NMR spectrum revealed the presence of eight signals: 167.48, *s* (NH–C=O), 152.40 (Ar.C–OH); 141.40, *s* (Ar.C–NH); [120.36, *d*; 119.32, *s*; 117.69, *d*; 114.51, *d*] for aromatic carbons and 14.81, *q* for Me [14]. These data are in agreement with those for the mass spectrum which showed a parent peak at $m/z$ 300 and an $[M/2]^+$ peak at $m/z$ 150 (100%) which support the structure (**1**), i.e. it consists of two symmetrical parts.



**1** R = 3–(OH)–5–(Me)



**2**



**3** R = Me
**4** R = H

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During the separation of compound **1**, three further $\beta$-carboline alkaloids were isolated and their structures confirmed. The first of these was a bright blue fluorescing compound, $C_{13}H_{12}N_2O$, orthorhombic prisms from methanol, mp (259° dec.), UV $\lambda_{max}$ (methanol) 241, 300, 335 nm. Its NMR spectrum (see Experimental) left no doubt that it was harmine hydrochloride (**2**) (lit. mp 262° [15]). The second compound was harmaline (**3**), $C_{13}H_{14}N_2O$, as hydrochloride dihydrate, yellow needles from chloroform, mp 260 (dec.) (lit. mp 265° (dec.) [16]). The third compound was harmalol (**4**), $C_{12}H_{12}N_2O$, a red-orange powder recrystallized from water, mp 211–219° (dec.) (lit. mp 211–219° dec. [15]). The structures of these three compounds was confirmed by comparison of their spectroscopic data with those of authentic samples.

## EXPERIMENTAL

IR spectra were obtained in KBr, UV spectra in MeOH. NMR spectra were recorded at 250 MHz with TMS as int std, $^{13}$C NMR spectra at 22 MHz. MS were measured at 70 eV in the EI mode. CC on silica gel was performed using Merck Kieselgel 60. TLC was carried out on commercially prepared plates coated with Merck Kieselgel $GF_{254}$.

*Extraction and isolation of alkaloids.* Fr. seeds (500 g) collected in June 1986 were crushed and covered with twice their wt of dist. $H_2O$ (1 l). The seeds swelled as they absorbed $H_2O$ and formed a thick dough which was filtered the next day. The soaking process was repeated and the liquid again filtered off. The combined liquors were concd *in vacuo* (50°) to give *ca* 30 g of a crude residue as a dark red gum. The residue was mixed with a small amount of silica gel (20 g) and then quantitatively transferred to the top of a prepared silica gel (70–230 mesh) column. The column was eluted with $CHCl_3$ containing increasing proportions of MeOH. The compounds were eluted from the column in the order **3**, **2**, **4** and **1**. Frs containing mixts of compounds were detected using HPLC. Alkaloids in fractions were visualized as differently, coloured spots by spraying TLC plates with Dragendorff's reagent. The following compounds were sepd.

N,N′-[(3-*hydroxy*-5-*methyl*) *phenyl*]-*Oxamide* (**1**). (0.15%) 75 mg, yellowish solid, mp 213–215° (Found: C, 63.64; H, 5.41, N, 9.01% $C_{16}H_{16}N_2O_4$ requires: C, 64.00; H, 5.53; N, 9.33%); MS *m/z* 300.1112 ([M]$^+$, 100%, $C_{16}H_{16}N_2O_4$); 150 (100%, $C_8H_8NO_2$, [M/2]$^+$). 107 (29%, $C_7H_7O$, [M/2−HCNO]$^+$); UV: $\lambda_{max}^{MeOH}$ 275, 325, 370 and 420 nm; IR $\nu_{max}^{KBr}$ 1600, 1665 and 3450 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$): $\delta_H$ 12.2 (*s*, 1H), 9.1 (*s*, 1H) $D_2O$-exch., 7.1 (*br*, 1H, $J < 1$ Hz), 6.9 (*br*, 2H, $J < 1$ Hz), 2.4 (*s*, 3H); $^{13}$C NMR: $\delta_c$ 167.48 (C=O), 152.40 (C-3), 141.40 (C-1), 120.36, 119.32, 117.69, 114.51 (C-6, C-5, C-4 and C-2), 14.81 (Me).

*Harmine hydrochloride* (**2**.HCl). Orthorhombic prisms (MeOH) (1.3%). mp 259° (dec.), (Found C, 73.41; H, 5.23; N, 13.51, $C_{13}H_{12}N_2O$ requires: C, 73.58; H, 5.66; N, 13.20%); UV $\lambda_{max}^{MeOH}$ 241, 300 and 335 nm, IR $\nu_{max}^{KBr}$ 1613 and 1630 cm$^{-1}$; NMR (DMSO-$d_6$): $\delta_H$ 7.45 (*d*, 1H, $J = 4$ Hz, H-3), 7.35 (*d*, 1H, $J = 6$ Hz, H-5), 7.1 (*d*, 1H, $J = 4$ Hz, H-4), 6.2 (*dd*, 1H, $J_1 = 6$ Hz, $J_2 = 4$ Hz, H-6), 6.75 (*d*, 1H, $J = 1$ Hz, H-8), 3.5 (*s*, 3H, 7-OMe) and 2.45 (*s*, 3H, 1-Me).

*Harmaline hydrochloride* (**3**. HCl). Yellow needles ($CHCl_3$) (1.92%). mp 260° (dec.), Found C, 72.52; H, 6.72; N, 13.13. $C_{13}H_{14}N_2O$ requires: C, 72. 89, H, 6.54; N, 13.08%); UV $\lambda_{max}^{MeOH}$ 216, 262 and 374 nm; IR $\nu_{max}^{KBr}$ (1610 and 1635 cm$^{-1}$; NMR (DMSO-$d_6$): $\delta$7.3 (*d*, 1H, $J = 6$ Hz, H-5), 6.6 (*dd*, 1H, $J_1 = 6$ Hz, $J_2 = 1$ Hz; H-6), 6.5 (*d*, 1H, $J = 1$ Hz, H-8), 3.8 (*s*, 3H, 7-OMe), 3.7 (*t*, 2H, H-3), 2.8 (*t*, 2H, H-4), 2.7 (*s*, 3H, 1-Me).

*Harmalol* (**4**) ($\sim$0.2% by HPLC) was identified by comparison of TLC, HPLC and UV data with those of an authentic sample.

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