STUDIES ON THE CHEMICAL CONSTITUENTS OF THE SEEDS OF PEGANUM HARMALA: ISOLATION AND STRUCTURE ELUCIDATION OF TWO β-CARBOLINES — HARMALACININE AND NORHARMINE

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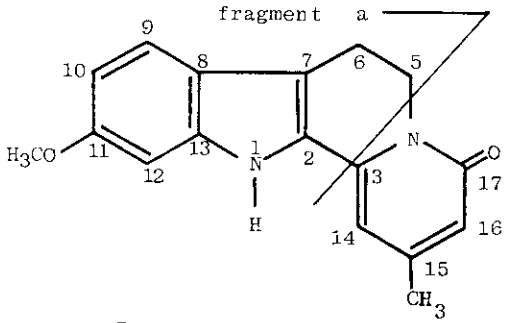
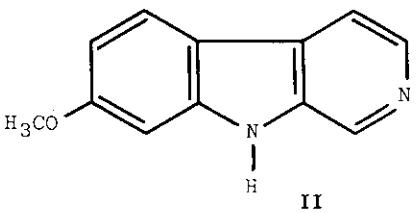
Abstract — A new tetracyclic β-carboline, harmalacinine, has been isolated from the seeds of Peganum harmala, along with norharmine, and their structures elucidated as I and II respectively through spectral studies.

In continuation of studies in the constituents of P.harmala (Zygophylaceae) seeds,[1-3] two minor β-carbolines, harmalacinine and norharmine have been isolated from the neutral fraction. The structures of these constituents have been determined as I and II respectively through spectral studies. Prior to the isolation of I only one tetracyclic β-carboline lactam, harmalanine (III)(16-nor-harmalacinine)has been reported from P.harmala, as a major constituent along with harmalacidine[3] (IV). Previously, simple β-carbolines and quinazoline type of alkaloids were isolated from the plant.[4-6]

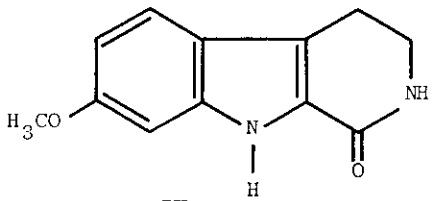
Harmalacinine (I)has molecular formula $C_{17}H_{16}N_2O_2$ (M^+ 280.1213). Its uv spectrum showed maxima at 205, 290, 325, 370 nm indicating the presence of β-carboline skeleton[7] while the ir spectrum displayed peaks at 3450 (NH), 3200 (aromatic ring), 2910, 2850 (C-H) 1680 (amide), 1630 (NH bending) 1460-1600 (aromatic ring) and 1100 (C-O). Its mass spectrum showed significant fragments at m/z 265.0945 and 173.0839 (a) corresponding to the loss of CH_3 and C_6H_5NO. The ^{1}H-nmr spectrum showed a double doublet at δ 6.79 (J=8.5, 2.0) and two doublets at δ 6.81 (J=2.0) and 7.48 (J=8.5) attributable to H-10, H-12 and H-9 respectively. It also exhibited two, two-proton multiplets at δ 3.28 and 4.44 which were ascribed to H-6 and H-5 respectively, and a three-proton singlet at δ 3.86 due to a methoxy group. It further showed a one-proton singlet at δ 7.60 which is exchangeable with D_2O and is thus assigned to the indolic NH. The ^{1}H-nmr spectrum also showed two doublets (J=2.5Hz) at δ 6.05 and 6.71 which were assigned to H-14 and H-16 respectively in ring D. These ^{1}H-nmr spectral data are comparable with those of harmalanine[3] (III) (table 1) and were confirmed through cosy-45 spectrum and ^{1}H-^{1}H homonuclear decoupling experiments. The cosy-45 plot showed through bond interactions of H-9 with H-10, and H-6 with H-5. However, the signal



I



II



IV

of H-15 in harmalanine was replaced by a methyl group singlet at δ 2.48 in **I** which suggested the presence of $-\overset{1}{C}=CH-CCH_3=CH-CO-\overset{1}{N}-$ group in ring-D which was confirmed by the double resonance experiments. Thus irradiation at δ 6.05 resulted in the collapse of the doublet at δ 6.71 to a singlet and vice versa. In the light of these observations and ^{13}C-nmr spectral data structure **I** was assigned to harmalacinine.

β-Carboline (**II**) has the composition $C_{12}H_{10}N_2O$ on the basis of mass spectrometery (M^+ m/e 198.0793). Its uv spectrum showed maxima at 225, 256, 285, 325, and 370 nm characteristic of β-carbolines.[7] Its ir spectrum displayed peaks at 3000 (aromatic CH), 2920 (aliphatic CH), 1615 (NH-bending), 1450-1600 (4 peaks, aromatic ring) and 1100 (C-O). All the 9 double bond equivalents indicated by the molecular formula were accounted for by the β-carboline skeleton. The ^1H-nmr spectrum showed two one proton doublets at δ 8.48 (J = 4.9Hz) and 8.01 (J=4.9 Hz) for H-5 and H-6 respectively. It also exhibited a double doubelt at δ 6.91 (J=8.3, 2.0), and two doublets at δ 6.98 (J=2.0Hz) and 7.99 (J=8.3Hz) attributable to H-10, H-12 and H-9 respectively and a three-proton singlet at δ 3.93 due to the methoxy group. The

^{1}H-nmr spectral data are comparable with those of harmine and harmol (table 1), however, in **II** the signal for C-3 methyl group is absent, and instead the presence of a proton (δ 8.50s) at C-3 was exhibited by the ^{1}H-nmr spectrum. On the basis of these spectral data and mass fragments (see experimental) structure **II** has been assigned to this β-carboline which is identical with norharmine. It is the first instance of isolation of norharmine from a natural source although a number of 3-nor-β-carbolines have been reported as natural products.[8-13] Norharmine has previously been obtained as a synthetic product.[14,15]

The position of methoxy group at C-11 in harmalacidine (**IV**), reported earlier,[3,16,17] has been further confirmed through NOE difference spectroscopy. Thus irradiation of H-6 (δ 2.92) gave the signal of H-9 (δ 7.44, 1.52% NOE) and H-5 (δ 3.61); in a reverse experiment, on irradiation at δ 7.44, the same enhancement (1.52%) was observed for H-6, while H-10 (δ 6.80) was enhanced to 4.78%. Irradiation at δ 3.90 (OCH_3) gave the signals of both the protons at C-10 (δ 6.80) and C-12 (δ 6.85).

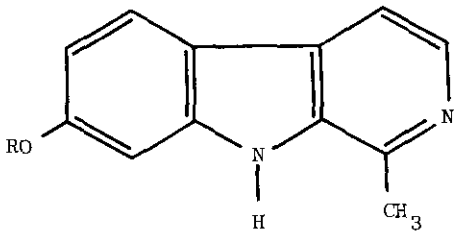
For an exact assignment of nmr chemical shifts for C-5/H-5 and C-6/H-6 as well as other protons and carbons in this series of β-carbolines, heterocosy spectra of readily available alkaloids harmine and harmaline have been recorded (table 2). In the heterocosy spectrum of harmine the most downfield signal at δ 8.19 showed connectivity with δ 137.5 which is the reported chemical shift of C-5,[18] while the proton at δ 7.80 was related with carbon at δ 112.0 assigned to C-6. Similarly the heterocosy spectrum of harmaline showed that the downfield protons (δ 3.65) are linked with the carbon at δ 48.0 which is attributable to C-5 (reported value 41.6)[18] while the aromatic methylene protons (δ 2.70) showed through bond connectivity with the carbon at δ 19.1 (i.e. C-6). In the light of these observations the more downfield chemical shift of the two methylene signals has been assigned to H-5 while the protons resonating at relatively upfield have been attributed to H-6. It may be noted that in the case of harmaline, both ^{1}H and ^{13}C nmr chemical shifts showed some deviations from the reported values,[18] although the same solvent (DMSO-d_6) was used. It has been reported in other β-carbolines[20-22,10,17] also that H-5 resonates at downfield for instance in canthin-6-one,[19] 1-ethyl-β-carboline,[20] (S)-1-(1'-hydroxyethyl)-β-carboline,[21] nauclefidine,[22] and 9-acetamido-3,4-dihydropyrido (3,4-6) indole,[10] and the same has been confirmed in the case of harmalacidine (IV) through NOE difference spectroscopy as described above.

Table 1. ^{1}H- nmr Spectral Data (δ_H ppm and J/Hz) of β-Carbolines

Protons	Harmalacinine (I)[a]	Harmalanine[3] (III)	Norharmine (II)[b]	Harmine[c]	Harmol[e]	Harmalacidine[d] (IV)
1	7.60s	8.03br s	N.O.	N.O.	9.86br s	9.09br s
3	-	-	8.50s	-	-	-
4	-	-	-	-	-	5.59br s
5	4.44m	4.42t(7.1)	8.48d(4.9)	8.19d(6.0)	8.13d(5.3)	3.61td(7.4,1.5)
6	3.28m	3.05t(7.1)	8.01d(4.9)	7.92d(6.0)	7.73d(5.3)	2.92t(7.4)
9	7.48d(8.5)	7.45d(8.6)	7.99(8.3)	7.97d(8.8)	7.93d(8.6)	7.44d(8.8)
10	6.79dd(8.5, 2.0)	6.82dd(8.6, 2.2)	6.91dd(8.3, 2.0)	6.95dd(8.8, 2.0)	6.73dd(8.6, 1.9)	6.80dd(8.8, 2.1)
12	6.81d(2.0)	6.85d(2.2)	6.98d(2.0)	7.26d(2.0)	6.95d(1.9)	6.85d(2.1)
14	6.05d(2.5)	6.21dd(6.8, 1.7)	-	-	-	-
15	-	7.32dd(8.8, 6.8)	-	-	-	-
16	6.71d(2.5)	6.47dd(8.8, 1.7)	-	-	-	-
17	2.48s	-	-	-	-	-
OCH_3	3.86s	3.86	3.93s	3.93s	-	3.90s
C_3CH_3	-	-	-	3.20s	2.73s	-
OH	-	-	-	-	11.3s	-

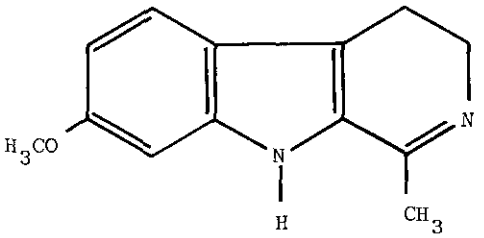
Solvent used a-d $CDCl_3$, e DMSO-d_6

N.O. = Not observed



Harmine R=CH_3

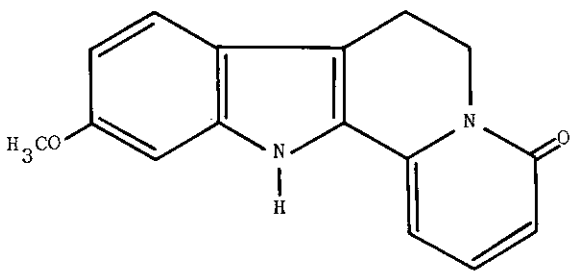
Harmol R=H



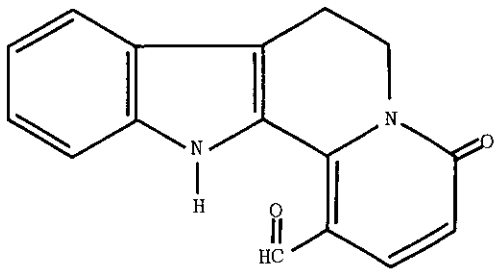
Harmaline

Table 2. 2D ^{1}H-^{13}C Heteronuclear Correlation Spectral Data of Harmine and Harmaline

	Harmine		Harmaline	
No. H/C	δ_H	δ_C	δ_H	δ_C
5	8.19	137.5	3.65	48.0
6	7.80	112.0	2.70	19.1
9	8.01	122.5	7.38	120.5
10	6.85	109.0	6.70	110.5
12	7.00	94.5	6.85	94.8
14	2.72	20.0	2.25	21.8
OCH_3	3.85	55.0	3.80	55.0



Harmalanine (III)



Nauclefidine

EXPERIMENTAL

Melting points were recorded on an air bath type melting point apparatus and are uncorrected. Mass spectra were run on double focussing mass spectrometer connected to PDP 11/34 computer system. Exact mass measurements were carried out through peak matching. Ir in ($CHCl_3$) and uv spectra (in MeOH) were measured on JASCO IRA-1 and uv 240 spectrophotometers respectively. ^{1}H 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 the comparison of the chemical shifts with those of harmalanine.[3] Delay time for heterocosy was 1.5 ms. The purity of samples was checked on silica gel SiF-254 precoated aluminium cards.

Extraction and Isolation of **I** and **II**: Uncrushed seeds of Peganum harmala (5 Kg) were repeatedly percolated with methanol and the dark reddish brown residue obtained from the combined percolates on removal of the solvent in vacuo, was basified and filtered. The liberated base fraction thus obtained was repeatedly treated with a 1:1 mixture of 5% acetic acid and 5% hydrochloric acid. The acid insoluble portion was partitioned between ethyl acetate and a mixture of 5% acetic acid and 5% hydrochloric acid (1:1). The residue obtained on removal of the solvent, from the ethyl acetate layer after usual work up, was divided into petroleum ether soluble and insoluble portions, and the latter was subjected to flash column chromatography (silica gel, $CHCl_3$ and $CHCl_3$-MeOH in order of increasing polarity). The initial $CHCl_3$ eluate afforded a fraction which furnished **I** and **II** through thin layer chromatography (silica gel, $CHCl_3$).

Harmalacinine (**I**) (amorphous), Eims m/z (rel.int.) 281 (M + 1) (7) 280.1213 M^+ (calcd for $C_{17}H_{16}N_2O_2$ 280.1212) (56), 265.0945 (M^+-15) (14), 250(6), 249(2), 237(20), 215(14), 207(13), 199(8), 173.0839 ($C_{11}H_{11}NO$,fragment a) (80), 159(14), 158(13), 145(18), 142.0724 ($C_{11}H_{10}$) (10), 119.0859 (C_9H_{11}) (26), 107(30), 106(20), 92.0611 (C_7H_8), 55(100). ^{13}C Nmr (δ), 167.0 (C-17), 157.5 (C-11), 155.0 (C-3), 138.0 (C-15), 137.2 (C-13), 137.0 (C-2), 127.8 (C-8), 121.4 (C-9), 118.0 (C-16), 115.1 (C-7), 111.9 (C-10), 101.1 (C-14), 98.0 (C-12), 55.6 (OCH_3), 48.2 (C-5), 22.7 (C-6) and 14.1 (C_3-CH_3).

Norharmine (**II**) (mp 213-215^{o}C) Eims m/z (rel.int.) 199 (M + 1) (6%), 198.0793 M^+ (calcd for $C_{12}H_{10}N_2O$ 198.0793) (46), 197(14), 183(12), 171(2), 170(5), 167(6), 158(2), 155.0603 (M^+-C_2H_3O) (19), 146(8), 134(10), 127.0425 (M^+-OCH_3-C_2H_2N) (14),

123(14), 122(10), 107(12), 106(6), 105(14), 97.0651 (C_6H_9O) (28), 91(22), 83(98), 77(18), 69(64), 67(40), and 55 (100).

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