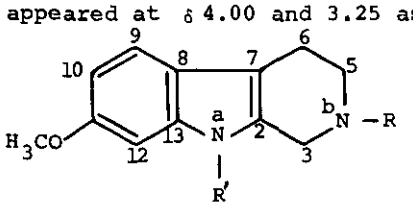
STUDIES ON THE CHEMICAL CONSTITUENTS OF THE SEEDS OF PEGANUM HARMALA ——— ISOLATION AND STRUCTURE OF A NEW β-CARBOLINE ALKALOID — HARMALICINE

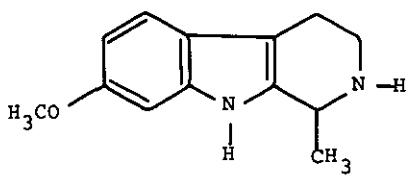
Salimuzzaman Siddiqui*, Obaid Yusuf Khan, Shaheen Faizi, and Bina S.Siddiqui
H.E.J.Research Institute of Chemistry
University of Karachi, Karachi-32, Pakistan

Abstract — A new β-carboline alkaloid named as harmalicine has been isolated from the aqueous fraction of the methanolic extract of the uncrushed seeds of Peganum harmala and its structure established as I through chemical and spectral studies.

Extended studies in the chemical constituents of Peganum harmala,[1] have yielded a new alkaloid harmalicine (I) from the uncrushed seeds, the structure of which has been elucidated through chemical and spectral studies. The mass spectrum of I showed a molecular ion peak at m/z 230.1059 corresponding to formula $C_{13}H_{14}N_2O_2$ (calc. 230.1055). The ir spectrum showed bands at 3400 (NH), 3050 (aromatic C-H), 2750 (CHO), 1710 (N-C(=O)-H), 2830, 2950 (aliphatic C-H), 1450 (CH_2), 1100 (C-O), 1250 (=C-O-C-) and 800 cm^{-1} (aromatic substituted ring). The uv spectrum showed maxima at 210, 265, 285 (sh) and 300 nm (sh), characteristic of β-carboline type of alkaloids.

The molecular formula indicated 8 double bond equivalents in the molecule, 3 of which have been accounted for by three rings, 1 by one carbonyl function while 4 by 4 double bonds. The ^{1}H-nmr spectrum showed three one-proton aromatic signals at δ 7.60 (d, J=8.9Hz, H-9), δ 6.86 (dd, J=8.9, 1.8Hz, H-10) and δ 6.95 (d, J=1.8Hz, H-12). One two-proton narrow multiplet at δ 3.30 has been ascribed to H-3 while H-5 and H-6 appeared at δ 4.00 and 3.25 as triplets (J=8.8Hz). A three-proton





I R=H, R'= CHO

II R=H, R'=CH_2OH

III R=$COCH_3$, R'=CHO

Tetrahydroharmine

singlet at δ 3.60 has been assigned to methoxy group, and a one-proton singlet at δ 8.01 unexchangeable by D_2O to aldehydic proton. ^{1}H-NMR spectrum also showed a broad signal at δ 2.87 due to NH group which is exchangeable by D_2O. These assignments were confirmed through double resonance experiments. Thus, irradiation at δ 3.25 collapsed the triplet at δ 4.00 into a singlet and vice versa, while irradiation of the signal at δ 6.86, converted the doublets of H-9 and H-12 into each singlet. Irradiation at δ 7.60 and 6.95 collapsed the double doublet of H-10 into doublets, J=1.8 and J=8.9 respectively. The mass spectrum of I showed significant fragments at m/z 200.0941 (M^+-CH_2O), 199.0868 (M^+-OCH_3), 187.0865 (M^+-CO-CH_3) and 185.0710 (M^+-CH_3-CH_2O) resulting from the loss of CH_2O, OCH_3, CO+CH_3 and CH_3+CH_2O,respectively along with an ion at m/z 133.0527 corresponding to formula C_8H_7NO. On the basis of these spectral data structure I has been assigned to harmalicine. In conformity to this structure, it yielded II (M^+ 232.1210) on catalytic reduction, the ^{1}H-nmr spectrum (Table I) of which showed a two-proton singlet at δ 3.80 for CH_2OH while the singlet of the aldehydic proton disappeared, while on acetylation it yielded the monoacetyl derivative III (M^+ 272, $NCOCH_3$ δ 1.97, Table 1). The multiplicities observed for aromatic protons, indicate that the 10-OCH_3 regioisomer is also possible. However, a comparison of the chemical shifts of aromatic protons of I with those of other β-carbolines with a OCH_3 group at C-10 or C-11 suggests that the latter is more plausible.[1-4]

Final evidence of structure I was provided by the ^{13}C-nmr chemical shifts (Table 2) observed in the broad band and DEPT spectra. These assignments are based on chemical shift rules and comparison with those of tetrahydroharmine. It is also the first report of ^{13}C-nmr of tetrahydroharmine, the assignments of which have been made through comparison with similar compounds.[5,6] The downfield appearance of C-2 and C-13 (δ 145.0, 138.9) in I as compared to that of tetrahydroharmine (δ 137.7, 138.0) may be explained due to the electron withdrawing substituent at N_a,[7] whereas the upfield resonance of C-3, is due to the γ-gauche effect[8] of the carbonyl function. This observation and the inspection of the Dreiding model suggested that the lone pair electrons of N_a in I have β-disposition.

It is interesting to note that naturally occurring N_a and N_b substituted derivatives of simple β-carbolines are rare[7,9,10] and harmalicine is the first report of a simple β-carboline with a formyl group on indolic nitrogen (N_a).

Preliminary experiments carried out on tetrahydroharmine revealed that it produced significant reduction of E.P.G. (egg/gram) count in the faeces in goat suffering from natural gastrointestinal nematodes when treated orally with 5, 10 and 20 mg/body wt. doses. It was also found to be effective against coccidia (eimeria species). It is interesting to note that tetrahydroharmine did not produce any adverse effect including tremor in the doses mentioned above, as was observed earlier.[11] Moreover β-carbolines have diverse biological activity[9,12,13] and some of the bases have shown high affinity for the benzodiazepine (Valium) receptor.[14] Hallucinogenic effects have also been noted in some South American drugs containing tetrahydroharmine.[15] These properties suggest potential biological significance of harmalicine.

EXPERIMENTAL

Melting points were recorded in glass capillary tubes and are uncorrected. Ms spectra were recorded on double focussing mass spectrometers connected to PDP 11/34 computer system. Ir (KBr disc) and uv spectra (in methanol) were measured on JASCO IRA-1 and Shimadzu UV 240 spectrometers. ^{1}H and ^{13}C-nmr broad band and DEPT spectra were recorded on a 300 MHz instrument, model Bruker Aspect 3000. The purity of samples was checked on tlc (silica gel SIF-254 precoated aluminium cards).

Extraction and Isolation of I: 4 Kg of uncrushed seeds of Peganum harmala were repeatedly percolated with methanol. The dark reddish brown thick residue obtained on removal of the solvent in vacuo from the combined percolates, was basified and partitioned between ethyl acetate and water. The aqueous phase showing three major spots on tlc corresponding to harmine, harmaline and harmalicine was freez dried. The residue was taken in methanol and subjected to preparative thick layer chromatography (silica gel; ethyl acetate-methanol-ammonia (50%), 9:1:1) yielding harmalicine which on crystallization from methanol formed fine needles, mp 251-252°C.

Catalytic Reduction of I: I (7mg) was hydrogenated in ethanol over platinum black at room temperature for 48h. After conventional work-up and crystallization from chloroform II was obtained in the form of short prismatic rods 262-264°C. UV λ_{max} (MeOH) (nm) 205, 235 (sh), 300 and 330. IR ν_{max} $CHCl_3$ (cm^{-1}): 3400, 3000, 2810, 1620, 1580, 1590-1430, 1310, 990. EIMS m/z (rel.int.) 232.1209 M^+ (3), 201.1025 $(M-31)^+$ (4).

Table-1: ^{1}H-NMR Spectral Data OF β-Carbolines (300 MHz)

Protons	Tetrahydroharmine	I	II	III
1	9.67s	-	-	-
3	4.07q(6.6)	3.30s br	3.31s br	4.07s br
5	3.2m	4.00t(8.8)	3.21m	4.20m
6	2.60m	3.25t(8.8)	3.10m	3.07m
9	7.25d(8.5)	7.60d(8.9)	7.70d(8.8)	7.65d(8.2)
10	6.63dd(8.5,2.2)	6.86dd(8.9,1.8)	6.88dd(8.8,2.0)	6.84dd(8.2,2.1)
12	6.83d(2.2)	6.95d(1.8)	7.08d(2.0)	7.02d(2.1)
CHO	-	8.01s	-	7.90s
CH_2OH	-	-	3.80s	-
OCH_3	3.75s	3.60s	3.61s	3.60s
OH	-	-	2.89s br	-
OAc	-	-	-	1.97
C_3-Me	1.38d(6.6)	-	-	-
NH	2.19	2.87	-	-

All values are in δ (ppm) and the coupling constants (in parenthesis) are in Hz.
Solvent used: Acetone-d_6.

Table-2: ^{13}C-NMR Spectral Data

Carbons	Tetrahydroharmine	Harmalicine (I)
2	137.7[a]	145.0[b]
3	49.0	32.5
5	43.5	42.7
6	23.5	22.4
7	108.2	97.5
8	123.1	115.2
9	118.8	123.1
10	108.9	115.3
11	156.8	153.0
12	95.6	94.7
13	138.0[a]	138.9[b]
CHO	-	172.3
C_3-Me	21.0	-
OCH_3	55.6	55.9

a,b: Assignments may be reversed
All values are in δ (ppm)
Solvent used: Acetone-d_6.

Acetylation of I: Acetic anhydride (5 ml) was added to a solution of I (10 mg) in pyridine (5 ml) and kept at room temperature overnight. After usual work-up III was obtained as fine needles, mp 235-236°C. UV λ_{max} (MeOH) (nm) 210, 253 (sh), 260, 267 (sh). IR ν_{max} $CHCl_3$ (cm^{-1}): 1640, 1590-1500, 1260, 1100, 890. EIMS m/z (rel.int.): 272 M^+ (4) and 230 $(M-42)^+$ (6).

ACKNOWLEDGEMENT

For the biological results our thanks are due to Dr.M.S.Akhtar and coworkers, Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan.

REFERENCES

1. S.Siddiqui, O.Y.Khan, B.S.Siddigui, and S.Faizi, Phytochemistry, 26, 1987 (in press).
2. P.V.R.Shannon, and W.M.Leyshon, J.Chem.Soc.(C), 1971, 2837.
3. Y.Hashimoto, and K.Kawanishi, Phytochemistry, 1976, 15, 1559.
4. Y.Hashimoto, and K.Kawanishi, Phytochemistry, 1975, 14, 1633.
5. F.Ungemach, D.Soerens, R.Weber, M.DiPierro, O.Campos, P.Mokry, J.M.Cook and J.V.Silverton, J.Am.Chem.Soc., 1980, 102, 6976.
6. A.Coune, L.J.G.Angenot, and J.Denoël, Phytochemistry, 1980, 19, 2009.
7. M.P.Jain, S.K.Koul, K.L.Dhar, and C.K.Atal, Phytochemistry, 1980, 19, 1880.
8. F.W.Wehrli and T.Wirthlin (1976), Interpretation of Carbon-13 NMR Spectra p.38 Heyden, London.
9. J.R.F.Allen and Bo.R.Holmstedt, Phytochemistry, 1980, 19, 1573.
10. T.Ohmoto and K.Koike, Chem.Pharm.Bull., 1983, 31, 3198.
11. J.A.Gunn, Quart.J.Pharm.Pharmacol., 1983, 3, 1.
12. R.Plate, R.J.F.Nivard, H.C.J.Ottenheijm, J.Kardos, and M.Simonyi, Heterocycles, 1986, 24, 3105.
13. D.W.Shoemaker, T.G.Bidder, H.G.Boettger, J.T.Cummins, and M.Evans, J. Chromatog., 1979, 174, 159.
14. F.Ungemach, M.DiPierro, R.Weber, and J.M.Cook, J.Org.Chem., 1981, 46, 164.
15. Z.Koblicová, and J.Trojánek, Chem.and Indus., 1966, 1342.

Received, 13th January, 1987