STUDIES IN THE CHEMICAL CONSTITUENTS OF THE SEEDS OF PEGANUM HARMALA:
ISOLATION AND STRUCTURE ELUCIDATION OF TWO β-CARBOLINE LACTAMS —
HARMALANINE AND HARMALACIDINE

Salimuzzaman Siddiqui,[*] Obaid Yusuf Khan, Shaheen Faizi and Bina Shaheen
Siddiqui

H.E.J.Research Institute of Chemistry
University of Karachi, Karachi-32, Pakistan

Abstract — Two β-carboline lactams harmalanine and harmalacidine have
been isolated from the seeds of Peganum harmala and their structures were
elucidated as I and II respectively through chemical and spectroscopic
studies. In the case of I, which is the simplest tetracyclic β-carboline
lactam from any source, detailed nmr (^{1}H and ^{13}C) investigations including
nOe difference, 2D nmr (cosy-45, noesy, J-resolved) double resonance ex-
periments and ^{1}H-^{13}C-hetero-COSY measurements have been used.

Peganum harmala (Zygophylaceae) known in vernacular as harmala, is commonly found

in Indo-Pakistan subcontinent and other parts of Asia.[1-4] Its seeds are narcotic,

anthelmintic, antispasmodic and employed in cases of asthma and rheumatism in the

indigenous system of medicine.[1,5] Recent investigations have revealed that its

main alkaloids, harmine, harmaline and tetrahydroharmine which belong to the category

of hallucinogenics, are also potent reversible inhibitors of monoamine oxidase.[6]

Further, alkaloids of Peganum harmala were found to possess antimicrobial activity.[2,3]

More recent studies undertaken by Siddiqui et al. in the seed of Peganum harmala

resulted in the isolation and structure elucidation of two new simple β-carboline

alkaloids harmalidine[7] and harmalicine.[8] The present paper deals with the isolation

of two β-carboline lactams harmalanine and harmalacidine from the seed and with

their structure elucidation through chemical and spectral studies. Harmalanine (I)
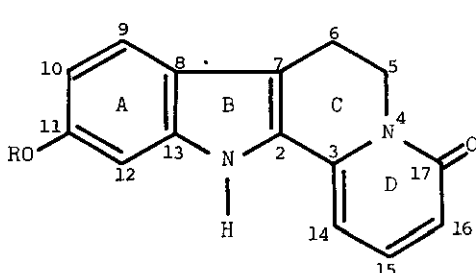
(1) R=CH₃
(3) R=H
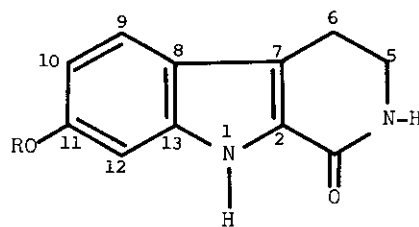
(2) R=CH₃
(4) R=H

is the first instance of the isolation of a tetracyclic β-carboline lactam without any substitution in ring D from any source, although a similar lactam with an aldehyde function at C-14 (nauclefidine) has been isolated earlier.[9] The second constituent, harmalacidine (II) which is a simple β-carboline has previously been reported from Banisteriopsis caapi.[10] Prior to these studies only one tetracyclic β-carboline base namely harmalidine[7] has been reported from Peganum harmala, in which the fourth ring was located at C-3 and N_a, while the others reported earlier from this source were simple tricyclic β-carbolines[1-5,11,12] and quinazoline[3-5,13,14] type of alkaloids. Harmalanine may be classified among the non-isoprenoid tryptophane (indoloquinolizidine group) alkaloids, biosynthesized through the condensation of five-carbon unit with tryptophan moiety.[15] On the other hand, its formation in nature may also be considered from a corynanthé skeleton through the removal of three carbons from C-15 and two carbons from C-16. A biomimetic transformation of 4,21-dehydrogeissoschizine into 5,6-dihydroflavopereirine, through elimination of three-carbons from C-15, has been demonstrated earlier.[16] It is noteworthy in this context that harmalanine can serve as an important intermediate in the synthesis of pentacyclic Corynanthe – Yohimbe type of alkaloids.[17,18]

The pharmacological significance of the <u>Peganum harmala</u> alkaloids stated at the out set and the fact that various other β-carbolines possess diverse biological activity[2,3,19-23] ranging from analgesic,[21] antimicrobial[2,3] and antitumour[22] properties suggest that harmalanine and harmalacidine might share these effects.

The crude base fraction obtained from the ethanolic extract of the seed of <u>Peganum harmala</u> was divided into acid (5% acetic acid – 5% hydrochloric acid; 1:1) soluble and insoluble fractions. The latter on column chromatography (silica gel) followed by thick layer chromatography on alumina yielded harmalanine (I) and harmalacidine (II) as uniform constituents. I has molecular formula $C_{16}H_{14}N_2O_2$ (high resolution mass spectroscopy). Its uv spectrum showed maxima at 205, 220, 270, 377, 395 nm, while the ir spectrum displayed peaks at 3450 (N-H), 2910, 2850 (C-H), 1670 (amide carbonyl), 1650 (C=C), 1400-1600 (4 peaks, aromatic ring), 1020 (C-O), 860, 840 cm^{-1} (aromatic ring). The ^{1}H-nmr spectrum of I showed a double doublet at δ 6.82 (J=8.6, 2.2) and two doublets at δ 6.85 (J=2.2) and 7.45 (J=8.6) attributable to H-10, H-12 and H-9, respectively. It further showed two, two-proton triplets at δ 3.05 (J=7.1) and 4.42 (J=7.1) which were ascribed to H-6 and H-5 respectively. A three-proton singlet at δ 3.86 and a one-proton singlet at δ 8.03 which is exchangeable with D_2O were due to a methoxy group and indolic NH respectively. These

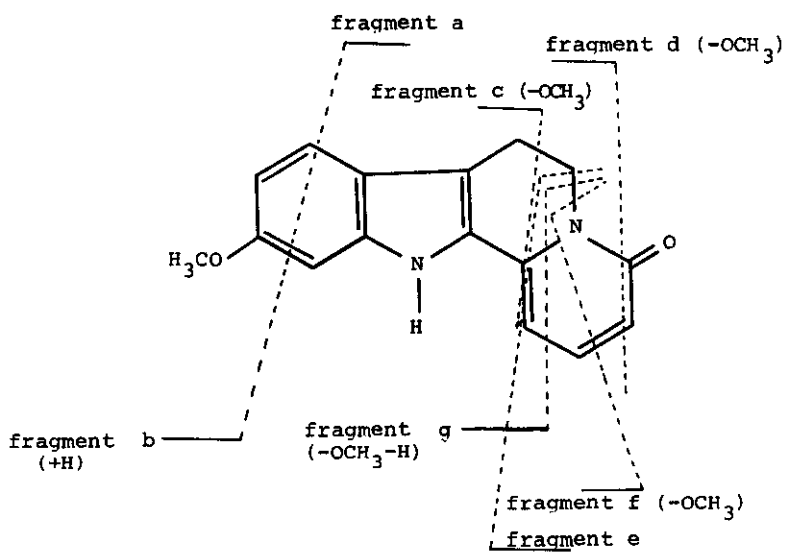Table-1. ^{1}H-NMR Spectral Data (δ_H ppm and J/Hz) of β-Carbolines

Pro-tons	Harmaline[7]	Tetrahydro-harmine[8]	Harmalanine (**I**)	Harmalacidine (**II**)[*]	**III**	**IV**[*]
1	8.17brs	9.67brs	8.03brs	9.09brs	8.80brs	8.61brs
3	-	4.07q(6.6)	-	-	-	-
4	-	-	-	5.59br.s	-	7.10brs
5	3.81t(8.5)	3.20m	4.42t(7.1)	3.01t(7.4)	4.22m	4.20t(6.0)
6	2.93t(8.5)	2.61m	3.05t(7.1)	2.29t(7.4)	3.64m	3.51t(6.0)
9	7.45d(8.9)	7.25d(8.5)	7.45d(8.6)	7.44d(8.8)	7.45m	7.40d(7.0)
10	6.80dd(8.9,2.1)	6.63dd(8.5,2.2)	6.82dd(8.6,2.2)	6.80dd(8.8,2.1)	6.82m	6.92dd(7.0,2.1)
12	6.93d(2.1)	6.83d(2.2)	6.85d(2.2)	6.85d(2.1)	6.89m	6.99d(2.1)
14	2.57s	1.38d(6.6)	6.21dd(6.8,1.7)	-	6.15m	-
15	-	-	7.32dd(8.8,6.8)	-	7.30m	-
16	-	-	6.47dd(8.8,1.7)	-	6.38m	-
OCH$_3$	3.84s	3.75s	3.86s	3.85s	-	-
OH	-	-	-	-	7.20	4.45

[*]Spectra recorded on a 400MHz instrument.

Table-2. ^{13}C-NMR Shifts (δ_C/ppm) of β-Carbolines

Carbons	Harmaline[30]	Tetrahydroharmine[8]	Harmalanine (**I**)	Harmalacidine (**II**)
2	126.6	137.7[a]	139.4[b]	125.1
3	162.1	49.0	154.5	163.2
5	41.6	43.5	40.3	42.1
6	17.3	23.5	19.6	21.0
7	114.4	108.2	115.0	119.7
8	126.6	123.1	126.0[c]	120.6
9	121.7	118.8	120.3[c]	121.1
10	109.3	108.9	111.0	111.7
11	157.7	156.8	158.5	158.8
12	90.7	95.6[a]	94.7[b]	94.4
13	136.8	138.0[a]	138.0[b]	138.5
14	19.1	21.0	99.1	-
15	-	-	133.6[c]	-
16	-	-	117.6	-
17	-	-	163.0	-
OCH$_3$	54.6	55.6	55.6	55.5

a,b,c = Assignments may be reversed.

fragment a

fragment d (-OCH$_3$)

fragment c (-OCH$_3$)

H$_3$CO

N

O

N

H

fragment b
(+H)

fragment g
(-OCH$_3$-H)

fragment f (-OCH$_3$)

fragment e

Harmalanine (I)

fragment d

fragment c (-H)

fragment b(-H)

H$_3$CO

N

H

N-H

O

fragment a

Harmalacidine (II)

— 1404 —
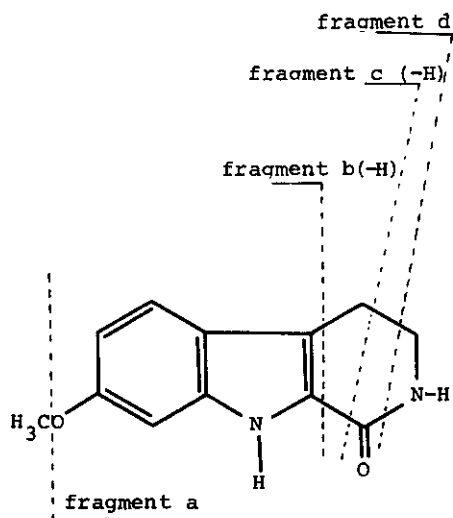
assignments were confirmed by [1]H-^{1}H homonuclear decoupling experiments. Thus, irradiation at δ 6.82 collapsed each of the two doublets at δ 6.85 and δ 7.45 into a singlet, while on irradiation at δ 6.85 and 7.45 the double doublet at δ 6.82 was converted into a doublet with $J_{9,10}$ = 8.6 and $J_{10,12}$ = 2.2 respectively. On the other hand, irradiation at δ 3.05 resulted in the collapse of the triplet at δ 4.42 to a singlet and vice versa. The above [1]H-nmr spectral data are comparable with those of harmaline[7] (table 1). However, the signal for C_3 methyl group was missing in I, and the presence of an additional ring (unsaturated lactam) was indicated instead by the molecular formula ($C_{16}H_{14}N_2O_2$; eleven double bond equivalance), ir (vide infra) and [1]H-nmr spectrum. Thus, three mutually coupled one-proton double doublets were further observed at δ 7.32 (J=8.8, 6.8), δ 6.21 (J=6.8, 1.7) and δ 6.47 (J=8.8, 1.7). Irradiation at δ 7.32 (H-15) collapsed each of the two double doublets at δ 6.21 (H-14) and 6.47 (H-16) into a doublet with $J_{14,16}$=1.7, while irradiation at δ 6.47 and 6.21 collapsed the double doublet at δ 7.32 into a doublet in each case, with $J_{14,15}$=6.8, and $J_{15,16}$ =8.8 respectively. These observations indicated the partial structure -N-C=CH-CH=CH-CO-N, also supported by the ^{13}C-nmr spectrum (broad band and DEPT) which showed an amide carbonyl (δ_{16} 163.0) and three tertiary olefinic carbons (δ_{14} 99.1, δ_{15} 133.6 and δ_{16} 117.6) apart from the β-carboline carbons (Table 2). These data which are comparable with those of 2-pyridone[24,25] led to the assignment of the fourth ring as depicted in the structure I. Finally, the ^{1}H and ^{13}C-nmr assignments were conclusively established through two-dimensional [1]H-^{13}C chemical shift correlation experiment (hetero-COSY which showed connectivities of C-5 (δ 40.3) with H-5 (δ 4.42), C-6 (δ 19.6) with H-6 (δ 3.05), C-9 (δ 120.3) with H-9 (δ 7.45), C-10 (δ 111.0) with H-10 (δ 6.82), C-12 (δ 94.7) with H-12 (δ 6.85), C-15 (δ 133.6) with H-15 (δ 7.32) and O$\underline{C}$H$_3$ (δ 55.6) with OC$\underline{H}_3$ (δ 3.86). In the light of the above discussion structure I has been ascribed to harmalanine which was corroborated by the significant fragments at m/z 265.0942, 251.0772, 223.0857, 222.0788, 203.0855 and 194.0895 corresponding to the loss of H, CH_3, CH_3+CO, C_2H_4O, C_5H_3 and OCH_3+C_2HO respectively. Demethylation of I with hydriodic acid yielded III (M^+ m/z 252), the [1]H-nmr spectrum of which showed a signal at δ 7.20 for the hydroxyl function while the singlet of the methoxy group disappeared (Table 1).

The molecular formula, $C_{12}H_{12}N_2O_2$ of harmalacidine II was derived through exact mass measurement of molecular ion at m/z 216.0916. It has maxima at 220, 250, and

Table-3. Connectivities Observed In the 2-dimensional ^{1}H-^{1}H Correlation Spectrum (Cosy-45 plot of **I**)

Protons (δ)	Connected with (δ)
H-5 (4.42)	H-6 (3.05)
H-9 (7.45)	H-10 (6.82)
H-14 (6.21)	H-16 (6.47)
	H-15 (7.32)
H-15 (7.32)	H-14 (6.21)
	H-16 (6.47)

Table-4. nOe Difference Spectral Data of Harmalanine (**I**)

Protons irradiated (δ)	Protons affected (δ)
H-6 (3.05)	H-5 (4.42)
	H-1 (8.50)
H-5 (4.42)	H-1 (8.50)
H-16 (6.47)	H-1 (8.50)
	H-5 (4.42)
	H-14 (6.21)
H-10 (6.82)	OCH$_3$ (3.86)
H-12 (6.85)	OCH$_3$ (3.86)
	H-1 (8.50)
H-1 (8.50)	H-5 (4.42)

315 nm in the uv spectrum while its ir spectrum displayed peaks at 3400 (NH), 1560 (amide II), 1680 (amide I), 1400-1610 (4 peaks aromatic ring) and 1130 cm^{-1}(C-O). The [1]H-nmr spectrum exhibited a double doublet at δ 6.80 (J=8.8, 2.1), two doublets at δ 6.85 (J=2.1) and δ 7.44 (J=8.8) attributable to H-10, H-12 and H-9 respectively. Two triplets (J=7.4) at δ 3.01 and δ 2.29 have been assigned to two sets of ethylenic protons, H-5 and H-6 respectively and a three-proton singlet at δ 3.85 to a methoxy group. Indolic and amide NH resonating at δ 9.09 and δ 5.59 were exchangeable with D_2O. These assignments were confirmed through double resonance experiments. Thus, irradiation at δ 6.80 collapsed each of the two doublets at δ 6.85 and 7.44 into a singlet, while on irradiation at δ 6.85 and 7.44 the double doublet at δ 6.80 was converted into a doublet with $J_{9,10}$ = 8.8 and $J_{10,12}$ = 2.1 respectively. On the other hand, irradiation at δ 3.01 resulted in the collapse of the triplet at δ 2.29 to a singlet and vice versa. These assignments are comparable with those reported for tetrahydroharmine[8] and harmaline[7] (Table 1). However, the signal of C-3 methyl was missing in II, and the absence of any other proton signal, calculation of double bond equivalents, and presence of one more oxygen in the molecule, led to the placement of a carbonyl function at C-3. In the light of these spectral data structure of harmalacidine has been deduced as II which was substantiated by the mass spectrum which showed significant fragments at m/z 201.0663, 187.0633, 158.0605 and 144.0449 corresponding to the loss of CH_3, CH_2-NH, C_2H_4NO and C_3H_6NO respectively from the molecular ion. In conformity to this structure, it yielded (IV; M^+ 202) on demethylation, the [1]H-nmr spectrum of which showed a signal at δ 4.45 due to OH while the singlet of the methoxy group disappeared. Final evidence of the structure was provided by the ^{13}C-nmr chemical shifts (broad band and DEPT) (Table 2) which was not reported earlier.

In both the β-carbolines I and II, the 10-OCH_3 regioisomer could also be considered in the light of the observed multiplicities in the [1]H-nmr spectra (Table 1). Inspection of the Dreiding model also showed that nOe's observed in I (Table 4) are possible in both the isomers. However, a comparison of the chemical shifts of the aromatic protons particularly the downfield doublets at δ 7.45 (J=8.6) and 7.44 (J=8.8) in I and II respectively with those reported for 10 and 11-OCH_3 isomers[7,8,10,26-28] suggests that the placement of methoxy group at C-11 is more plausible in these cases. In the 10-OCH_3 instances the downfield doublet resonates comparatively upfield ranging from 7.12-7.27.[26,28]

It has to be noted in this context that harmalanine and harmalacidine were initially obtained through column chromatography, showing a single spot on tlc (silica gel, $CHCl_3$, MeOH, 9:1). However, the nmr (^{1}H and ^{13}C) spectral data indicated that it was a mixture of two compounds. After trying a number of solvent systems on plates coated with silica gel, these were ultimately separated into two bands on alumina ($CHCl_3$-MeOH 9.5:0.5) and characterized as **I** and **II**.

EXPERIMENTAL

Melting points were recorded on an airbath type melting point apparatus and are un-corrected. 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 Shimadzu UV 240 spectrometers respectively. [1]H-nmr spectra were recorded in $CDCl_3$ on 300MHz and 400MHz instruments, Model Bruker Aspect 3000 AM 300 and Bruker Aspect 3000 AM 400 spectrometers, while ^{13}C-nmr (broad band and DEPT) spectra were recorded at 75MHz. The chemical shifts are in δ (ppm) and the coupling constants (J) are in Hz. The [1]H-nmr assignments are based on the multiplicities, homonuclear decoupling experiments, 2D-J resolved and 2D homonuclear correlation (cosy 45) (Table 3) spec-tral data. Assignments of ^{13}C-nmr chemical shifts are based on chemical shift rules,[29] hetero-COSY and comparison with those of similar compounds.[7,8,24,30] The purity of samples was checked on tlc (silica gel SIF-254 precoated aluminium cards, and aluminium oxide 60 PF_{254} coated on glass plates).

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% hydro-chloric 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[31] (silica gel, $CHCl_3$ and $CHCl_3$-MeOH in order of increasing polarity). The $CHCl_3$ eluate afforded a fraction showing single spot on tlc (silica gel, $CHCl_3$, MeOH, 9:1) which was ultimately separated into **I** and **II** on plates coated with Al_2O_3 ($CHCl_3$ - MeOH, 9.5:0.5). Compound **I** crystallized from $CHCl_3$ as rods (45 mg), mp 108-110°C.

High resolution mass spectroscopy m/z 266.0997 M^+ (calcd. for $C_{16}H_{14}N_2O_2$, 266.1055)

(12) 265.0942 (M^+-1) (100), 251.0772 (M^+-15) (15), 223.0857 ($C_{14}H_{11}N_2O$) (3),

222.0788 ($C_{14}H_{10}N_2O$) (4), 206.0768 ($C_{15}H_{10}O$) (7), 205.0836 ($C_{15}H_{11}N$) (1),

203.0855 ($C_{11}H_{11}N_2O_2$), (1), 197.0754 ($C_{12}H_9N_2O$, fragment a), (5), 196.0674.

($C_{12}H_8N_2O$) (14), 195.0898 ($C_{13}H_{11}N_2$) (4), 194.0895 ($C_{13}H_{10}N_2$, fragment d), (3),

193.0774 ($C_{13}H_9N_2$) (5), 191.0785 ($C_{14}H_9N$) (1), 190.0830 ($C_{11}H_{12}NO_2$), (1), 189.0732

($C_{11}H_{11}NO_2$) (3), 180.0855 ($C_{13}H_{10}N$, fragment f), (2), 166.0700 ($C_{12}H_8N$, fragment g)

(4), 140.0542 ($C_{10}H_6N$, fragment c), (12), 135.0632 (C_8H_9NO), (6), 116.0616 (C_9H_8)

(4), 104.0602 (C_8H_8) (3), 81.0211 (C_4H_3NO, fragment e) (2), and 70.0391 (C_4H_6O,

fragment b) (10).

Demethylation of Harmalanine I: Harmalanine **I** (20 mg) was refluxed with HI for 48 h.
The mixture was then poured into crushed ice and extracted with ethyl acetate. On
usual work up **III** was obtained showing a single spot on tlc mp 179°C; uv λ_{max}(MeOH)
nm: 205, 220, 285, 365. Ir ν_{max}CHCl$_3$ cm^{-1}: 3350 (OH, NH), 2850 (C-H), 1690 (amide
carbonyl) 1410-1640 (aromatic ring). Eims m/z (rel.int.) 252 M^+ (2), 235(2), 205(2),
and 185 (3).

Harmalacidine **II** crystallized from CHCl$_3$ as rods (30 mg), mp 200-201°C. EIMS m/z
(rel.int.) 216.0916 M^+ (calcd. for $C_{12}H_{12}N_2O_2$ 216.0898) (100), 201.0675 (M-15,
fragment a) (20), 187.0646 (M-29, fragment d) (42), 185(10), 173(12), 172(6),
158.0607 (M-58, fragment c), (95), 156(7), 144.0451 (M^+-72, fragment b) (22),
129(12), 116(20), 111(5), 101(10) and 89(22).

Demethylation of Harmalacidine II: Harmalacidine **II** (15 mg) was refluxed with HI for
24 h. The mixture was then poured into H$_2$O and extracted with ethyl acetate. On
usual work up pure **IV** of mp 138°C was obtained; uv λ_{max}(MeOH) (nm): 212, 220, 290,
360. Ir ν_{max}CHCl$_3$ cm^{-1}: 3200-3500 (OH and NH), 1680, 1620 (amide I and II bands),
1400-1600 (4 peaks, aromatic ring). Eims m/z (rel.int.) 202.0793 M^+ (calcd. for
$C_{11}H_{10}N_2O_2$ 202.0742), (10), 185 (M-17) (10), 161 (M-41) (5), 146 (M-56) (20) and
128 (M-74) (40).

REFERENCES

1. W.Dymock, C.J.H.Warden, D.Hooper, Pharmacographia Indica, The Institute of
    Health and Tibbi Research, republished under the auspices of Hamdard National
    Foundation of Pakistan, 1890, p.252.

2. S.A.Ross, S.E.Megalla, D.W.Bishay, and A.H.Awad, Fitoterapia, 1980, 51, 309.

3. A.Al-Shamma, S.Drake, D.L.Flynn, L.A.Mitscher, Y.H.Park, G.S.R.Rao, A.Simpson,
    J.K.Swayze, T.Veysoglu, and S.T.S.Wu, J.Nat.Prod., 1981, 44, 745.

4.   D.Batsuren, M.V.Telezhenetskaya, and S.Yu.Yunusov, <u>Khim.Prir.Soedin.</u>, 1980, 736.

5.   B.Mukerji, The Indian Pharmaceutical Codex, Indigenous drugs, Council of Scientific and Industrial Research, New Delhi, 1953, 200.

6.   D.J.McKenna and G.H.N.Towers, <u>Phytochemistry</u>, 1981, <u>20</u>, 1001.

7.   S.Siddiqui, O.Y.Khan, B.S.Siddioui,and S.Faizi, <u>Phytochemistry,</u> 1987, <u>26</u>, 1548.

8.   S.Siddioui, O.Y.Khan, S.Faizi, and B.S.Siddiqui, <u>Heterocycles</u>, 1987, <u>26</u>, 1563.

9.   L.Mao, L.Xin and Y.Dequan, <u>Planta Medica,</u> 1984, <u>50</u>, 459.

10.   Y.Hashimoto and K.Kawanishi, <u>Phytochemistry</u>, 1976,<u>15</u>, 1559.

11.   J.S.Glasby, <u>Encyclopaedia of the Alkaloids</u>, Plenum Press, New York, 1975, 2, 658.

12.   E.McKenzie, L.Nettleship and M.Slaytor, <u>Phytochemistry,</u> 1975, <u>14</u>, 273, and references quoted therein.

13.   B.Kh.Zharekeev, M.V.Telezhenetskaya, and S.Yu.Yunusov, <u>Khim.Prir.Soedin.</u>, 1973, 272 and references cited therein.

14.   B.Kh.Zharekeev, K.N.Khashimov, M.W.Telezhenetskaya,and S.Yu.Yunusov, <u>Khim.Prir. Soedin.</u>, 1973, 264 and references cited therein.

15.   A.Rahman and A.Basha, Biosynthesis of Indole Alkaloids, International series of monographs on Chemistry, Clasendon Press, Oxford, 1983.

16.   C.K.Fan and H.P.Husson, <u>Tetrahedron Lett.</u>, 1980, <u>21</u>, 4265.

17.   F.V.Brutcher, Jr., W.D.Vanderwerff,and B.Dreikorn, <u>J.Org.Chem.</u>, 1972, <u>37</u>, 297.

18.   T.Kametani, T.Suzuki, S.Kamada,and K.Unno, <u>Heterocycles,</u> 1982, <u>19</u>, 815.

19.   J.R.F.Allen and Bo.R.Holmstedt, <u>Phytochemistry,</u> 1980, <u>19</u>, 1573.

20.   G.Lazurjevski and I.Terentjeva, <u>Heterocycles</u>, 1976, <u>4</u>, 1783.

21.   N.Bikova, E.Denkova, L.Daleva, S.Vankov, and S.Velichkova, Tr.Nauchnoizsled, <u>Khim.Farm.Inst.</u>, 1981, <u>11</u>, 30. <u>Chem.Abs.</u>, 1982, <u>96</u>, 181482.

22.   S.Matsumura, H.Enomoto, Y.Aoyagi, Y.Nomiyama, T.Kono, M.Matsuda,and H.Tanaka, <u>Ger.Offen.</u>, 2941449, 1980; <u>Chem.Abs.</u>, 93, 114495r.

23.   T.J.Hagen, F.Guzman, C.Schultz,and J.M.Cook, <u>Heterocycles</u>, 1986, <u>24</u>, 2845.

24.   L.F.Johnson and W.C.Jankowski, Carbon-13 NMR Spectra, John Wiley and Sons, New York, 1972,   107.

25.   D.H.Williams and L.Fleming, Spectroscopic Methods in Organic Chemistry, Third .   Edition, McGraw-Hile Book Comoany (UK) Limited, 1980,   136.

26.   P.V.R.Shannon and W.M.Leyshon, <u>J.Chem.Soc.(C)</u>, 1971, 2837.

27.   Y.Hashimoto and K.Kawanishi, <u>Phytochemistry,</u> 1975, <u>14</u>, 1633.

28.   D.S.Filho and B.Gilbert, <u>Phytochemistry,</u> 1975, <u>14</u>, 821.

29.   J.B.Stothers, Carbon-13 NMR Spectroscopy, A series of Monographs, Academic Press, 1972, 24.

30.   C.A.Coune, L.J.G.Angenot,and J.Denoël, <u>Phytochemistry,</u> 1980, <u>19</u>, 2009.

31.   W.J.Still, M.Kahn,and A.Mitra, <u>J.Org.Chem.</u>, 1978, <u>43</u>, 2923.