Chem. Pharm. Bull. 30(4)1204—1209(1982)

Studies on the Constituents of *Picrasma quassioides* Bennet. I. On the Alkaloidal Constituents

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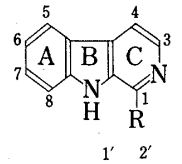
(Received September 3, 1981)

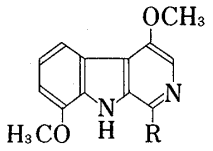
1-Acetyl-β-carboline (I), 4,8-dimethoxy-1-ethyl-β-carboline (II), and three new alkaloids, 4,8-dimethoxy-1-(2-methoxyethyl)-β-carboline (III), β-carbolin-1-yl 4,8-dimethoxy-β-carbolin-1-yl-ethyl ketone (IV) and 3-methylcanthin-2,6-dione (V), were isolated from the root of *Picrasma quassioides* Bennet (Simaroubaceae). The structures were elucidated on the basis of spectroscopic studies and chemical evidence.

Keywords——*Picrasma quassioides*; Simaroubaceae; alkaloid; β-carboline; canthinone; 1-acetyl-β-carboline; 4,8-dimethoxy-1-ethyl-β-carboline; 4,8-dimethoxy-1-(2-methoxyethyl)-β-carboline; β-carbolin-1-yl 4,8-dimethoxy-β-carbolin-1-yl-ethyl ketone; 3-methylcanthin-2,6-dione

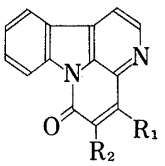
The wood of *Picrasma quassioides* Bennet (Simaroubaceae; Japanese name: Nigaki) has been used extensively as a bitter stomachic. *Picrasma quassioides* Bennet and *Ailanthus altissima* Swingle (Japanese name: Shinju) are the only two species belonging to Simaroubaceae grown in Japan. We have reported the isolation of alkaloids from the root-bark and wood of *A. altissima*.[1,2] Four alkaloids, methyl β-carboline-1-carboxylate (VI), 1-hydroxymethyl-β-carboline (XI), 4,5-dimethoxycanthin-6-one (XII) and 5-hydroxy-4-methoxycanthin-6-one (XIII), have been isolated from the wood of *P. quassioides*.[3-5]



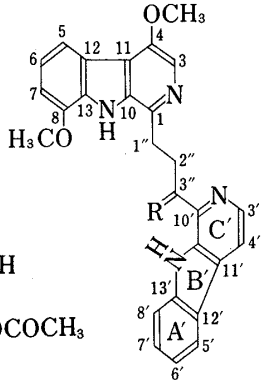
I : R=COCH$_3$
VI : R=COOCH$_3$
XI : R=CH$_2$OH



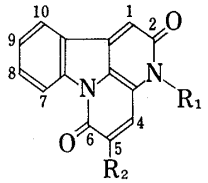
II : R=CH$_2$CH$_3$
III : R=CH$_2$CH$_2$OCH$_3$



XII : R$_1$=OCH$_3$, R$_2$=OCH$_3$
XIII : R$_1$=OCH$_3$, R$_2$=OH



IV : R=O
VII : R=<OH H
VIII : R=<OCOCH$_3$ H



V : R$_1$=CH$_3$, R$_2$=H
IX : R$_1$=OCH$_3$, R$_2$=H
X : R$_1$=H, R$_2$=OCH$_3$

Chart 1

From a chemotaxonomical point of view, we have examined the alkaloidal constituents of the root of *P. quassioides* and isolated two known compounds (I and II), and three new compounds (III, IV and V). In this paper, we wish to report the determination of the structures of these compounds (I—V).

Compound I was obtained as pale yellow needles with mp 204—205°C. High-resolution mass spectral examination of I gave the formula $C_{13}H_{10}N_2O$. The infrared absorption (IR) spectrum of I showed a conjugated carbonyl group at 1675 cm^{-1} and the ultraviolet absorption (UV) spectrum of I was compatible with those reported for β-carbolines.[2,6] The proton magnetic resonance (^{1}H-NMR) spectrum (see "Experimental") of I suggested the presence of an acetyl group at 2.89 ppm as a 3H singlet, which was also supported by the mass spectrum showing, besides the molecular ion peak at *m/z* 210, a prominent ion peak at *m/z* 167 corresponding to the elimination of an acetyl group.

From the above results, compound I was identified as 1-acetyl-β-carboline, previously isolated from *Ailanthus malabarica* DC. (Simaroubaceae).[6]

Compound II was obtained as colorless prisms with mp 155—156°C. The high resolution mass spectral examination of II gave the formula $C_{15}H_{16}N_2O_2$. The UV spectrum of II exhibited typical absorption of a β-carboline. The ^{1}H-NMR spectrum (Table I) of II showed a typical quartet-triplet pattern at 3.10 and 1.37 ppm (J=8 Hz) which was assignable to the ethyl group, and showed two methoxyl groups at 3.85 and 4.02 ppm each as a 3H singlet. The upfield shift of the chemical shift value of C_3–H from the normal value of 8.45 ppm to 7.95 ppm indicated that one methoxyl group is located at the C-4 position,[1] and the other methoxyl group could only be attached to the A-ring, and must be located at the C-8 position since, besides two doublets at 7.90 and 6.83 ppm (J=8 Hz) attributable to C_5–H and C_7–H, one triplet at 7.14 ppm (J=8 Hz) assigned to C_6–H was observed.

The above results suggested the structure II, and the alkaloid was finally identified as 4,8-dimethoxy-1-ethyl-β-carboline by mixed melting point determination and comparisons of TLC behavior and IR spectra with those of an authentic sample isolated from *Aeschrion crenata* Vell (Simaroubaceae).[7]

Table I. ^{1}H-NMR Spectral Data for Compounds II, III, IV, and VI[a]

Position	II[b]	III[b]	IV[c]	VI[c]
3	7.95 (s)	7.87 (s)	7.87 (s)	8.46 (d, J=5)
4				8.37 (d, J=5)
5	7.90 (d, J=8)	7.84 (d, J=8)	7.78 (d, J=8)	8.27 (d, J=8)
6	7.14 (t, J=8)	7.14 (t, J=8)	7.18 (t, J=8)	7.62 (t, J=8)
7	6.83 (d, J=8)	6.87 (d, J=8)	7.11 (d, J=8)	7.29 (t, J=8)
8				7.82 (d, J=8)
N_9–H	9.64 (br s)	9.80 (br s)	11.88 (br s)	11.81 (br s)
1′ (1″)	3.10 (q, J=8)	3.40 (t, J=8)	3.60 (t, J=8)	
2′ (2″)	1.37 (t, J=8)	3.83 (t, J=8)	3.92 (t, J=8)	
3′			8.54 (d, J=5)	
4′			8.46 (d, J=5)	
5′			8.31 (d, J=8)	
6′			7.59 (t, J=8)	
7′			7.31 (t, J=8)	
8′			7.78 (d, J=8)	
$N_{9'}$–H			12.06 (br s)	
C_1-$COOCH_3$				4.03 (s)
C_4-OCH_3	4.02 (s)	4.06 (s)	4.04 (s)	
C_8-OCH_3	3.85 (s)	3.96 (s)	4.03 (s)	
$C_{2'}$-OCH_3		3.38 (s)		

a) Chemical shifts in ppm units; s=singlet, d=doublet, t=triplet, q=quartet, br s=broad singlet.
b) In $CDCl_3$ solution.
c) In DMSO-d_6 solution.

Compound III was obtained as colorless prisms with mp 201—202°C. The high resolution mass spectral examination of III gave the formula $C_{16}H_{18}N_2O_3$. The UV spectrum of III was similar to that of II, suggesting that it has same chromophoric system as ll. The ^{1}H-NMR spectrum (Table I) of III showed three methoxyl groups at 3.38, 3.96 and 4.06 ppm each as a 3H singlet, and two methylene protons at 3.40 and 3.83 ppm (J=8 Hz) each as a 2H triplet, which suggested the presence of a methoxyethyl group. The methoxyl group at 3.38 ppm was clearly linked to an aliphatic system in view of its appearance in a high field region. By comparing the ^{1}H-NMR spectrum of III with that of II, it was concluded that the methoxyethyl and two methoxyl groups were located at the C-1, C-4 and C-8 positions, respectively. This was also supported by the mass spectrum, which showed peaks at m/z 286 (M^+), 255 ($M^+ - OCH_3$) and 227 ($M^+ - CH_2CH_2OCH_3$); the mass fragmentation pattern of III was also in good agreement with that of II.

Based on these results, compound III was identified as 4,8-dimethoxy-1-(2-methoxyethyl)-β-carboline.

Compound IV was obtained as pale yellow crystals with mp 263—264°C (dec.). The high resolution mass spectral examination of IV gave the formula $C_{27}H_{22}N_4O_3$. The IR spectrum of IV showed a conjugated carbonyl group at 1670 cm^{-1} and the UV spectrum of IV exhibited typical absorption of a β-carboline. The ^{1}H-NMR spectrum (Table I) of IV showed two methoxyl groups at 4.03 nad 4.04 ppm each as a 3H singlet and two methylene protons at 3.60 and 3.92 ppm (J=8 Hz) each as a 2H triplet, which suggested the presence of a 1,2-disubstituted ethane function, and two indolic NH protons at 11.88 and 12.06 ppm each as a 1H broad singlet.

The above finding suggested that compound IV has a dimeric structure of two β-carboline moieties. By comparing the ^{1}H-NMR spectrum (Table I) of IV with those of II, III and VI, it was concluded that one methoxyl group is attached to C-4 and the other methoxyl group to C-8. Four aromatic protons at 8.31 (doublet), 7.59 (triplet), 7.31 (triplet) and 7.78 ppm (doublet) (1H each, J=8 Hz) assigned to C_5'-H, C_6'-H, C_7'-H and C_8'-H, respectively, and AB splitting aromatic protons at 8.54 and 8.46 ppm (1H each, J=5 Hz) assigned to C_3'-H and C_4'-H, respectively, indicated that the A′ and C′ rings were unsubstituted.

TABLE II. ^{13}C-NMR Spectral Data for Compounds III, IV, and VI[a)]

Position	III[b)]	IV[c)] (III−IV)	VI[c)]	Position	IV (VI−IV)
C-1	137.64 s	138.49 s (−0.85)	141.50 s	C-1′	141.79 s (−0.29)
C-3	120.42 d	120.07 d (0.35)	137.95 d	C-3′	137.30 d (0.65)
C-4	151.01 s	149.91 s (1.10)	118.91 d	C-4′	119.14 d (−0.55)
C-5	120.04 d	120.07 d (−0.03)	121.81 d	C-5′	121.63 d (0.17)
C-6	116.30 d	115.46 d (0.84)	129.09 d	C-6′	128.73 d (0.36)
C-7	106.94 d	107.28 d (−0.34)	120.11 d	C-7′	119.36 d (0.75)
C-8	146.00 s	146.01 s (−0.01)	112.91 d	C-8′	112.97 d (−0.06)
C-10	130.25 s	130.90 s (−0.65)	130.94 s	C-10′	133.99 s (−3.05)
C-11	118.50 s	117.03 s (1.47)	120.11 s	C-11′	119.85 s (0.85)
C-12	122.24 s	121.53 s (0.71)	129.78 s	C-12′	129.93 s (−0.05)
C-13	135.79 s	134.80 s (0.79)	136.10 s	C-13′	135.83 s (0.27)
C-1″(C-1′)	35.67 t	27.09 t			
C-2″(C-2′)	72.46 t	35.01 t			
C-3″		202.20 s			
C_1–$COOCH_3$			56.16 q		
C_4–OCH_3	55.93 q	55.91 q			
C_8–OCH_3	55.51 q	55.34 q			
$C_{2'}$–OCH_3	58.74 q				

a) Chemical shifts in ppm units, s=singlet, d=doublet, t=triplet, q=quartet.
b) In $CDCl_3$ solution.
c) In DMSO-d_6 solution.

From the above findings, the 1,2-disubstituted ethane function (*i.e.*, carboethyl function) was deduced to involve two β-carbolines at their C-1 positions. The structure of IV was further supported by the mass fragmentation pattern (Chart 2), which showed peaks at m/z 450 (M^+), 255 (fragment ion a), 196 (b) and 168 (c), as well as by comparison of the ^{13}C-NMR spectrum (Table II) of IV with those of III and VI.

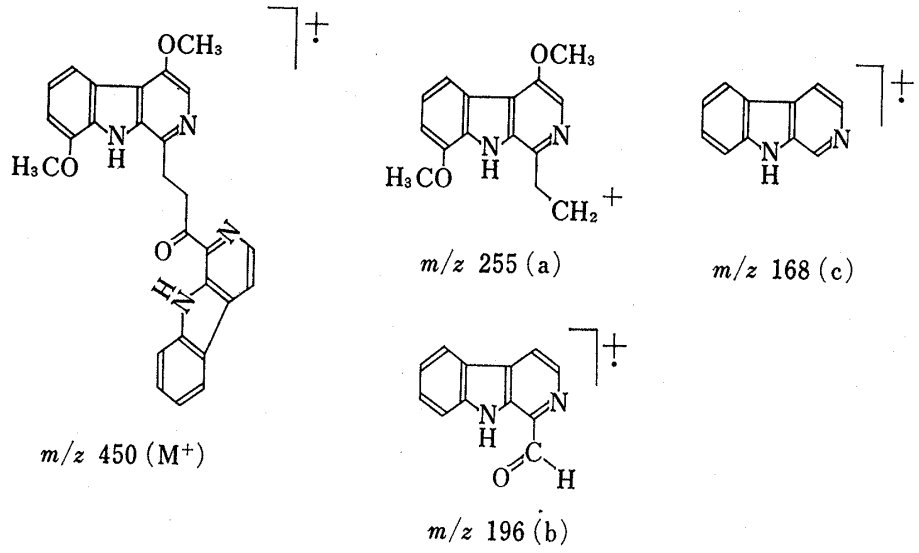


Chart 2

Based on these results, the structure of compound IV was deduced to be β-carbolin-1-yl 4,8-dimethoxy-β-carbolin-1-ylethyl ketone.

The assigned structure (IV) was consistent with the fact that the reduction of IV with $LiAlH_4$ in tetrahydrofuran gave VII, which afforded the monoacetate (VIII) on treatment with acetic anhydride.

Compound V was obtained as orange-red needles, mp higher than 330°, which displayed strong yellowish-green fluorescence in organic solvents. The high resolution mass spectral examination of V gave the formula $C_{15}H_{10}N_2O_2$. The IR spectrum of V showed carbonyl groups at 1655 and 1695 cm^{-1}, which indicated that at least one was a lactam carbonyl group,

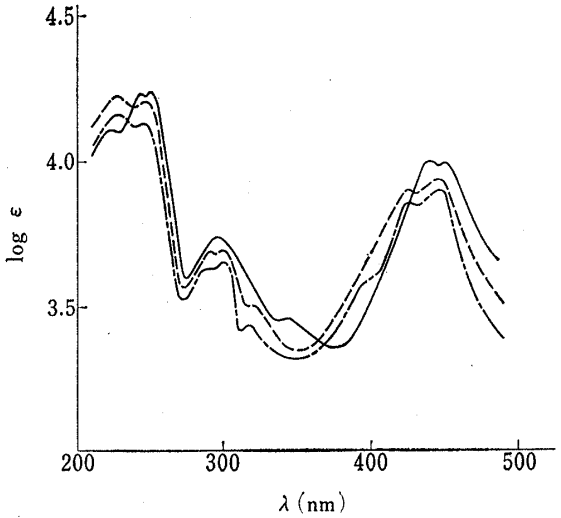


Fig. 1. Ultraviolet Spectra of Compounds V, IX, and X in Ethanol

———: V, –·–·–: IX, – – – –: X.

Table III. ^1H-NMR Spectral Data for Compounds V and IX[a]

Position	V[b]	IX[c]
1	7.29 (s)	7.27 (s)
4	7.67 (d, J=8)	7.72 (d, J=10)
5	7.22 (d, J=8)	6.93 (d, J=10)
7	8.47 (d, J=8)	8.60 (d, J=8)
8	7.58 (t, J=8)	7.68 (t, J=8)
9	7.48 (t, J=8)	7.49 (t, J=8)
10	7.87 (d, J=8)	7.95 (d, J=8)
N_3–CH_3	3.93 (s)	
N_3–OCH_3		4.20 (s)

a) Chemical shifts in ppm units; s=singlet, d=doublet, t=triplet.
b) In $CDCl_3$–CD_3OD solution.
c) In $CDCl_3$ solution.

and there was no evidence for the presence of a hydroxyl group. The UV spectrum (Fig. 1) of V was in good agreement with that of canthinone and was particularly similar to those of 3-methoxycanthin-2,6-dione (IX) obtained from *Simaba cuspidata* Spruce ex Engl., var *typica* Cronquist (Simaroubaceae),[8] and 5-methoxycanthin-2,6-dione (X) from *Samadera indica* Baill (Simaroubaceae).[9] The ^{1}H-NMR spectrum (Table III) of V was almost identical with that of canthin-6-one as regards the signals of six protons from C_4–H to C_{10}–H,[1,2] and the signal due to C_1–H (1H singlet at 7.29 ppm) was shifted upfield, suggesting the presence of a carbonyl group at C-2 position. Though the signal seen at 3.92 ppm as a 3H singlet can be assigned to either a methoxyl group or an *N*-methyl group, the molecular formula of V and comparison of the ^{1}H-NMR spectrum (Table III) of V with that of IX, clearly ruled out the presence of a methoxyl group in V. Furthermore, the mass fragmentation pattern of V was very similar to that of IX.

From these results, compound V was identified as 3-methylcanthin-2,6-dione.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR and UV spectra were taken on Hitachi 295 and Hitachi 340 machines, respectively. ^{1}H-NMR spectra were measured with Hitachi R-900, JEOL FX-400 or JEOL 4H-100 spectrometer, ^{13}C-NMR spectra with Hitachi R-900 or JEOL FX-90Q machine, and low resolution mass spectra and high resolution mass spectra with JEOL JMS-01-SG-2 spectrometer.

Column chromatography was performed on silica gel (Wako, C-200). TLC and preparative TLC was carried out on precoated silica gel (Merck silica gel 60) and silica gel (Wako, B-5) plates with $CHCl_3$–MeOH–AcOH (1: 1: 1) and $CHCl_3$–MeOH (9: 1), respectively, as developing solvents. The alkaloids were detected by spraying with Dragendorff reagent or by the use of a UV lamp.

Extraction and Fractionation——Dried root chips (60 kg) of *P. quassioides* collected in December, 1980, at Yachiyo city, Chiba prefecture, were extracted with methanol under reflux for 48 h. The extract was concentrated to dryness under reduced pressure and the residue was dissolved in water and extracted with $CHCl_3$. The $CHCl_3$ solution was shaken with 5% H_2SO_4. The acidic layer was made alkaline with 5% NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ layer, after being washed with water and dried over Na_2SO_4, was evaporated to dryness under reduced pressure. The basic fraction (56 g) was chromatographed on silica gel, and eluted with the following solvents (each fraction, 200 ml). Fractions 1—10, benzene; frs. 11—60, $CHCl_3$; frs. 61—75, $CHCl_3$–MeOH (19: 1); frs. 76—99, $CHCl_3$–MeOH (9: 1); and frs. 100—130, MeOH.

Fractions 16 (22 mg), 55—63 (325 mg), 67—80 (1580 mg) and 102 (254 mg) were subjected to preparative TLC to yield compound I (4 mg), compound II (56 mg) and compound III (28 mg), compound IV (35 mg), and compound V (78 mg), respectively.

1-Acetyl-β-carboline (I)——Compound I was recrystallized from benzene to yield pale yellow needles (2 mg): mp 204—205°C. UV λ_{max}^{EtOH} nm (log ε): 220 (4.62), 252 (4.47), 262 (4.48), 286 (4.52), 308 (4.53), 380 (4.37). IR ν_{max}^{KBr} cm^{-1}: 3330, 2918, 1490, 1380, 1320, 1205, 745. MS *m/z*: 210 (M^+, 78%), 182 (53), 168 (100), 167 (78), 140 (40). High resolution MS *m/z*: Calcd for $C_{13}H_{10}N_2O$: 210.0793. Found: 210.0781. ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.89 (3H, s, C_1-$COCH_3$), 7.20—7.60 (2H, m, C_6, C_7–H), 7.93 (1H, d, J=8 Hz, C_8–H), 8.11 (1H, d, J=8 Hz, C_5–H), 8.36 (1H, d, J=5 Hz, C_4–H), 8.52 (1H, d, J=5 Hz, C_3–H), 10.23 (1H, br s, NH).

4,8-Dimethoxy-1-ethyl-β-carboline (II)——Compound II was recrystallized from acetone to yield colorless prisms (47 mg): mp 155—156°C. UV λ_{max}^{EtOH} nm (log ε): 245 (4.71), 270 (4.47), 286 (4.43), 336 (4.42), 350 (4.42). IR ν_{max}^{KBr} cm^{-1}: 3140, 2940, 1575, 1290, 1270, 1250, 1160, 1070, 730. MS *m/z*: 256 (M^+, 100%), 255 (68), 241 (45), 226 (20), 198 (13), 128 (12). High resolution MS *m/z*: Calcd for $C_{15}H_{16}N_2O_2$: 256.1152. Found: 256.1166. ^{1}H-NMR data are shown in Table I. All spectral data were identical with those of an authentic sample, and the mixed mp showed no depression.

4,8-Dimethoxy-1-(2-methoxyethyl)-β-carboline (III)——Compound III was recrystallized from acetone to yield colorless prisms (25 mg): mp 201—202°C. UV λ_{max}^{EtOH} nm (log ε): 245 (2.77), 250 (4.50), 286 (4.50), 336 (4.45), 350 (4.45). IR ν_{max}^{KBr} cm^{-1}: 3200, 2900, 1580, 1560, 1430, 1360, 1310, 1295, 1250, 1185, 1160, 735. MS *m/z*: 286 (M^+, 71%), 271 (100), 255 (30), 241 (57), 227 (21), 199 (21), 45 (23). High resolution MS *m/z*: Calcd for $C_{16}H_{18}N_2O_3$: 286.1317. Found: 286.1361. ^{1}H and ^{13}C-NMR data are shown in Tables I and II, respectively.

β-Carbolin-1-yl 4,8-Dimethoxy-β-carbolin-1-ylethyl Ketone (IV)——Compound IV was recrystallized from $CHCl_3$–MeOH to yield crystals (27 mg): mp 263—264°C (dec.). UV λ_{max}^{EtOH} nm (log ε): 221 (4.97), 245 (4.98), 286 (4.87) 322 (4.71), 336 (4.71), 382 (4.63). IR ν_{max}^{KBr} cm^{-1}: 3200, 2930, 1670, 1575, 1500, 1435, 1320, 1265, 1255, 1210, 1125, 745. MS *m/z*: 450 (M^+, 45%), 255 (100), 240 (16), 196 (2), 168 (18), 140 (12). High resolution MS *m/z*: Calcd for $C_{27}H_{22}N_4O_3$: 450.1692. Found: 450.1680. ^{1}H and ^{13}C-NMR data are shown

Conversion of Compound IV to Compound VIII——$LiAlH_4$ (80 mg) was added to solution of IV (24 mg) in anhydrous THF (10 ml). The mixture was stirred for 8 h at room temperature, then excess $LiAlH_4$ was destroyed by addition of moist ethyl acetate. The resulting solution was shaken with $CHCl_3$. The $CHCl_3$ layer was concentrated under reduced pressure. The residue (16 mg) was acetylated with acetic anhydride (0.3 ml) in pyridine. The product was recrystallized from acetone to give the monoacetate VIII (15 mg) as pale yellow crystals: mp 208—210°C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1730, 1625, 1575, 1500, 1430, 1290, 1240, 1145, 1040, 730. MS *m/z*: 494 (M^+, 2%), 434 (12), 255 (14), 197 (13), 43 (69). ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.07 (3H, s, C_3''–$OCOCH_3$), 4.12 (3H, s, C_8–OCH_3), 4.14 (3H, s, C_4–OCH_3), 6.97 (1H, d, J=8 Hz, C_7–H), 7.15 (1H, d, J=8 Hz, C_6'–H), 7.79 (1H, s, C_3–H), 7.87 (1H, d, J=8 Hz, C_8'–H), 7.96 (1H, d, J=8 Hz, C_5–H), 8.10 (1H, d, J=8 Hz, C_5'–H), 8.81 (1H, d, J=5 Hz, C_3'–H), 8.87 (1H, d, J=5 Hz, C_4'–H), 10.03 (1H, br s, N_9–H), 11.18 (1H, br s, N_9'–H).

3-Methylcanthin-2,6-dione (V)——Compound V was recrystallized from $CHCl_3$–MeOH to yield orange-red needles (55 mg): mp 330°C<. UV λ_{max}^{EtOH} nm (log ε): 227 (4.25), 242 (4.33), 250 (4.30), 287 (3.87), 345 (3.55), 444 (4.10), 462 (4.10). IR ν_{max}^{KBr} cm^{-1}: 1695, 1655, 1540, 1510, 1445, 1330, 1215. MS *m/z*: 250 (M^+, 69%), 222 (87), 193 (100), 179 (5), 168 (33), 152 (9), 140 (14). High resolution MS *m/z*: Calcd for $C_{15}H_{10}N_2O_2$: 250.0715. Found: 250.0715. ^{1}H-NMR data are shown in Table III.

Acknowledgement The authors are very grateful to Prof. Dr. J. Comin, Department de Química Orgánica, Fucultad de Ciencias Exacts y Naturales, Argentina, for his generous gift of authentic 4,8-dimethoxy-1-ethyl-β-carboline. Thanks are due to Mr. M. Takayama and Miss Y. Sakamoto of this school for the measurements of mass and NMR spectra.

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