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NEW ORGANIC BASES FROM AMAZONIAN *BANISTERIOPSIS CAAPI*

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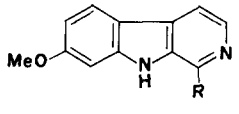
Key Word Index—*Banisteriopsis caapi*; Malpighiaceae; alkaloids; harmine *N*-oxide; harmic acid methyl ester; harmalinic acid.

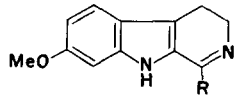
Abstract—Three new bases were isolated from *Banisteriopsis caapi*; they are harmine *N*-oxide, harmic acid methyl ester (methyl 7-methoxy-β-carboline 1-carboxylate) and harmalinic acid (7-methoxy-3,4-dihydro-β-carboline 1-carboxylic acid).

INTRODUCTION

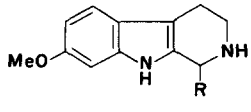
Certain Amazonian tribes prepare fanciful and telepathic drinks from *Banisteriopsis caapi* (Malpighiacea) [1], a vine called "Caapi, Yage or Ayahusca" by natives. The plant contains β-carboline-type alkaloids, namely harmine (**1a**), harmaline (dihydrohamine) (**2a**), and tetrahydroharmine (**3a**) [2]. Amongst them harmine and harmaline are reported to be hallucinogenic [3]. We have now separated three new alkaloids, all of which possess the β- carboline skeleton.



(**1a**) R = Me (harmine)
(**1b**) R = Me Nb-oxide (harmine *N*-oxide)
(**1c**) R = CO_2H
(**1d**) R = CO_2Me (harmic acid methyl ester)



(**2a**) R = Me (harmaline)
(**2b**) R = CO_2H (harmalinic acid)
(**2c**) R = CO_2Me



(**3a**) R = Me (tetrahydroharmine)
(**3b**) R = CO_2H

RESULTS AND DISCUSSION

The three new alkaloids were isolated and purified by solvent fractionation, TLC and electrophoresis (see Experimental). High resolution MS spectral examination of the first one (**1b**) gave the formula as $C_{13}H_{12}O_2N_2$, which indicated the presence of an additional oxygen to that of harmine. Comparison of the UV spectrum of (**1b**) (λ_{max} 213, 248 and 330 nm) with that of harmine revealed a bathochromic shift [4]. In the NMR spectrum the 1-methyl proton signal of (**1b**) is at δ2·64, while that of harmine is at δ2·73. Its coupling constant (J 7·2 Hz) for the AB splitting signals of H-3 and H-4 was larger than that of harmine (J 5·7 Hz). The NMR spectrum indicated shielding by the *N*-oxide group [5]. In the MS spectrum M-16 and M-17 ion peak is at *m/e* 212 and 211 respectively, suggesting the presence of *N*-oxide [6]. Confirmation came from reduction of (**1b**) with ferrous sulfate and ammonia, and identification of the reduction product (by UV, IR and TLC comparison) as harmine. Oxygenation of harmine with H_2O_2 gave the *N*-oxide, which was identical to (**1b**) in every respect.

The second compound (**1d**) has the typical UV spectrum of β-carboline (λ_{max} 256, 282 and 323 nm). High resolution MS spectral examination gave the formula as $C_{14}H_{12}O_3N_2$. The NMR spectrum presented 3 aromatic proton signals at H-5: δ8·18, H-6: δ6·93 and H-8: δ7·31 ($J_{5,6}$ 8·7 Hz, $J_{5,8}$ 0·2 Hz and $J_{6,8}$ 2·2 Hz), AB splitting aromatic proton signals at H-3: δ8·43 and H-4: δ8·26 ($J_{3,4}$ 4·5 Hz), indolic NH proton signal at δ11·46, methoxy proton signal at δ4·04, and

methyl ester proton signal at δ3·89 in place of methyl proton signal of harmine. The presence of an ester bond in (**1d**) was suggested by IR spectral analysis (ν 1693 cm^{-1}). On treatment with benzaldehyde harmine gave benzalharmine, which was oxidized with $KMnO_4$ to 7-methoxy-β-carboline 1-carboxylic acid (**1c**) [7], which was then methylated with diazomethane. On the basis of UV, IR and MS spectra and TLC comparison, this methyl ester was found to be identical with the natural product; thus, (**1d**) is harmic acid methyl ester.

High resolution MS spectral examination of the third new alkaloid (**2b**) gave the formula as $C_{13}H_{12}O_3N_2$. The NMR spectrum revealed a remarkable similarity to that of harmaline, namely three aromatic proton signals at H-5: δ7·59, H-6: δ6·77 and H-8: δ6·92 ($J_{5,6}$ 8·8 Hz, $J_{5,8}$ 0·6 Hz and $J_{6,8}$ 2·1 Hz), an indolic NH proton signal at δ11·58, a methoxy proton signal at δ3·84 and ethylene proton signals at δ2·93–3·30 (multiplet) and δ3·38–3·76 (multiplet). Carboxylic acid proton signal was indicated at δ8·72 (broad triplet) in place of the methyl proton signal due to harmaline. The presence of a carboxylic acid in (**2b**) was suggested by the IR spectrum (ν 1690 cm^{-1}). On reduction with $NaBH_4$ in MeOH (**2b**) gave (**3b**), $C_{13}H_{14}O_3N_2$ with the molecular ion peak at *m/e* 246 in MS spectrum and a methine proton signal at δ5·25 (doublet) and Nb-H proton signal at δ5·50–5·75 (broad). Thus, (**2b**) must be a Schiff's base [8]. When treated with diazomethane in tetrahydrofuran, (**2b**) yielded a methyl ester (**2c**), m/e 258, showing a methyl ester proton signal at δ3·33 in the NMR spectrum. Thus **2b** is 7-methoxy-3,4-dihydro-β-carboline 1-carboxylic acid. Dehydrogenation of the methyl ester **2c** with chromium trioxide gave an oxidation product identical with harmic acid methyl ester (**1d**) by UV, IR, and MS spectral and TLC comparison. Thus, (**2b**) is harmalinic acid.

EXPERIMENTAL

Extraction of the alkaloids. 21 kg crushed leaves and stems were extracted with 70% aqueous MeOH, followed by removal of MeOH and centrifugation at 5000 rpm for 20 min. The ppt. (101·5 g) was dissolved successively in 3 l. Et_2O, $CHCl_3$, Me_2CO and MeOH. Supernatant was basified with conc. NH_4OH and the alkaloids taken into 4 × 1 litre $CHCl_3$. The organic phase was dried. Detection of the alkaloids was by spraying with Dragendorff's reagent, following TLC and PE (see Table 1). **2b** was crystallized from each soluble portion and the $CHCl_3$ layer. Each portion free from **2b** was chromatographed on Si gel and eluted with CCl_4:$CHCl_3$ 1:5, $CHCl_3$, $CHCl_3$:MeOH 20:1, 15:1, 10:1, 5:1, 1:1 and MeOH and then further purified by chromatography.

Identification of harmine N-*oxide* (**1b**). Needles from MeOH, yield 0·0005%, mp 226–7° (dec.) $C_{13}H_{12}O_2N_2$ (M^+ Found: 228·089, calc 228·090), UV: λ_{max}^{MeOH} 213 (log ϵ 4·45), 248 (4·72) and 330 (4·62) nm. IR: ν_{max}^{Nujol} 1635, 1625, 1565 and 1500 cm^{-1}. NMR: δ2·64 (3-H, *s*: C–Me), 3·87 (3-H, *s*: OMe), 6·86 (1-H, *q*: J 0·9 Hz; H-6), 6·98 (1-H, *d*: *J* 2·0 Hz; H-8), 7·83 (1-H, *q*: *J* 7·2 Hz; H-4), 7·99 (1-H, *q*: *J* 8·2 Hz; H-5), 8·06 (1-H, *q*: *J* 7·2 Hz; H-3) and 11·38 (1-H, *b*: indolic NH). MS: *m/e* 228 (52), 212 (39), 211 (100), 197 (9), 196 (14), 169 (22) and 168 (23).

Reduction of harmine N-*oxide*. Harmine *N*-oxide (8 mg) was suspended in 2 ml conc NH_4OH with excess $FeSO_4$ and heated on a steam bath for 30 min. Harmine was extracted with Et_2O and isolated by preparative electrophoresis (see Table 1).

Preparation of harmine N-*oxide*. Harmine (30 mg) was dissolved in 2 ml EtOH, boiled with 30% H_2O_2 (2 ml) for 30 hr, followed by removal of solvent. Oxide was separated by chromatography on alumina with AcOEt–MeOH (9:1).

Identification of harmic acid methyl ester (**1d**). Light yellow needles from $CHCl_3$, yield 0·0002%, mp 118° (dec.), $C_{14}H_{12}O_3N_2$ (M^+ found: 256·087, calc 256·085), UV: $\lambda_{max}^{CHCl_3}$ 256 (log ϵ 4·29), 282 (4·25) and 323 (4·12) nm. IR: ν_{max}^{Nujol} 3398, 1693, 1635, 1625, 1600 and 1570 cm^{-1}. NMR: δ3·89 (3-H, *s*: OMe), 4·04 (3-H, *s*: COOMe), 6·93 (1-H, *q*: *J* 0·2 Hz; H-6), 7·31 (1-H, *d*: *J* 2·2 Hz; H-8), 8·18 (1-H, *q*: *J* 8·7 Hz; H-5), 8·26 (1-H, *q*: *J* 4·5 Hz; H-4), 8·43 (1-H, *q*: *J* 4·5 Hz; H-3) and 11·46 (1-H, *b*: indolic NH). MS: *m/e* 256 (68), 224 (10), 198 (100), 196 (73) and 153 (19).

Preparation of methyl 7-methoxy-β-carboline 1-*carboxylate*. Harmine (97 mg) was treated with 0·5 ml benzaldehyde at 180–220° for 4 hr. After cooling, the brown mixture was solidified. Benzalharmine was separated from benzoic acid by recrystallization from $CHCl_3$. The product (97 mg) was dissolved in 0·5 ml pyridine, treated with 1·5 ml saturated aqueous $KMnO_4$ at 0°. The acid **1c** was purified [7] and 10 mg was suspended in 3 ml THF, treated with excess CH_2N_2 at r.t. for 48 hr and isolated by preparative TLC.

Identification of harmalinic acid (**2b**). Yellow plates from MeOH, yield 0·005%, mp 224–5° (dec.), $C_{13}H_{12}O_3N_2$ (M^+

Table 1. Electrophoresis and TLC of alkaloids from *Banisteriopsis caapi*

Alkaloid*	**1a**	**2a**	**1d**	**4**	**5**	**6**	**1b**	**2b**	**7**
Migration distance (mm)†	151	150	146	144	141	123	115	53	50
R_f (× 100) on TLC‡	17	04	79	09	85	60	22	25	42

* For alkaloid structures **1–3**, see formulae. Alkaloids **4–7** are unidentified.
† On Toyo Roshi No. 51 paper in 5 N HOAc for 2 hr at 800 V and 0·38 mA/cm current.
‡ On Si gel G in $CHCl_3$–MeOH (15:1).

found: 224·085, calc 244·085). UV: λ_{max}^{MeOH} 250 (log ϵ 3·90) and 372 (4·36) nm. IR: ν_{max}^{Nujol} 3300, 1690, 1635, 1620, 1570 and 1525 cm^{-1}. NMR: δ2·93–3·30 (2-H: *m*: H-3 or H-4), 3·38–3·76 (2-H: *m*: H-4 or H-3), 3·84 (3-H: *s*; OMe), 6·77 (1-H: *q*: *J* 0·6 Hz; H-6), 6·92 (1-H: *d*: *J* 2·1 Hz; H-8), 7·59 (1-H: *q*: *J* 8·8 Hz; H-5), 8·72 (1-H: *t*: COOH) and 11·58 (1-H: *b*: indolic NH). MS: *m/e* 244 (82), 216 (93), 201 (29), 187 (39) and 159 (100).

Reduction of harmalinic acid. Harmalinic acid (22 mg) was dissolved in 10 ml MeOH, added a solution of $NaBH_4$ (20 mg) in MeOH (5 ml) at 0° and reacted at 50° for 2 hr. The MeOH was evaporated. The product was crystallized.

Methylation of harmalinic acid. Harmalinic acid (60 mg) was suspended in 13·5 ml THF, treated with excess CH_2N_2, evaporated and isolated by preparative TLC. *Methyl 7-methoxy β-carboline 1-carboxylate from* (**2b**). (**2b**) was methylated as described above. The product (**2c**) (19 mg) was dissolved in 4·5 ml 1 N H_2SO_4, treated with a soln of CrO_3 (130 mg) in H_2O (3 ml) and boiled for 10 min. The product was pptd, filtered and recrystallized from $CHCl_3$.

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