8-Hydroxymanzamine A, a #-Carboline Alkaloid from a Sponge, Pachypellina sp.

Toshio Ichiba, Jay M. Corgiat, Paul J. Scheuer, and Michelle Kelly-Borges

J. Nat. Prod., **1994**, 57 (1), 168-170• DOI:
10.1021/np50103a027 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 1, pp. 168-170, January 1994

8-HYDROXYMANZAMINE A, A β-CARBOLINE ALKALOID FROM A SPONGE, *PACHYPELLINA* SP.[1]

TOSHIO ICHIBA, JAY M. CORGIAT, PAUL J. SCHEUER,*

Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822

and MICHELLE KELLY-BORGES

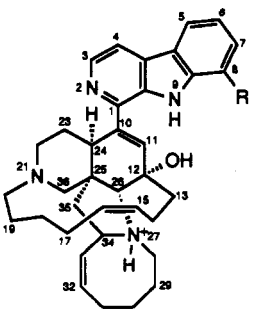
Harbor Branch Oceanographic Institution, 5600 North U.S. 1, Fort Pierce, Florida 34936[2]

ABSTRACT.—The previously described manzamine A [**1**] and the new 8-hydroxymanzamine A [**2**] were isolated from an undescribed sponge, *Pachypellina* sp. Both compounds exhibit moderate antitumor and anti-HSV-II activity.

The manzamines are β-carboline alkaloids isolated from marine sponges of the genera *Haliclona* (1,2), *Pellina* (3), and *Xestospongia* (4). They are substituted at C-1 by complex polycyclic isoquinoline substituents, or in the case of manzamine C, by an azacycloundecenyl moiety linked to β-carboline by a two-carbon chain.

A blackish-brown sponge reminiscent to the collector (T.I.) of an Okinawan *Xestospongia* sp. (4), and subsequently identified as an undescribed species of *Pachypellina* (Porifera, Demospongia, Petrosida, Oceanapiidae), was collected at Manado Bay, Sulawesi, Indonesia, in October 1992, at −10 m.[3] The sponge was preserved frozen and was freeze-dried prior to extraction with EtOH and partitioning between H_2O and CH_2Cl_2. The CH_2Cl_2-soluble mixture was separated by high-speed counter-current chromatography with a solvent system of hexane-MeCN-CH_2Cl_2 (10:7:3, lower mobile phase) into the known manzamine A [**1**] (1) and its 8-hydroxy derivative [**2**].[4] Both compounds were recrystallized from CH_2Cl_2/MeOH.[5]

^{13}C-Nmr data (Table 1) revealed that **1** was manzamine A (1) and that the new compound, **2**, obtained as pale yellow crystals, mp >230° (dec), $[\alpha]_D$ +118.5° ($CHCl_3$, c=1.94), differed from **1** only in the carbocyclic ring of the β-carboline moiety (C-5 to C-8a, Table 1). An additional oxygen atom in the molecular formula of **2** ($C_{36}H_{44}N_4O_2$ vs. $C_{36}H_{44}N_4O$ for **1**) pointed toward a phenolic hydroxyl. This was confirmed by preparing the methyl ether **3**, $C_{37}H_{46}N_4O_2$, as a pale



1 R=H
2 R=OH
3 R=OMe

[1]Presented in part at the 34th Annual Meeting of The American Society of Pharmacognosy, July 18–22, 1993, San Diego, CA, Abstract No. P. 46.

[2]Harbor Branch Oceanographic Institution Contribution No. 987.

[3]The sponge forms a spherical to thickly encrusting mass, with a smooth surface in areas where the dermal spicule layer remains intact. The sponge is compressible in life, but firm in the preserved condition. The interior of the sponge is fibrous in appearance and riddled with holes. The sponge had a blackish-brown exterior and a yellowish-brown interior in life. A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (Catalog No. 003:850).

[4]In the preliminary report[1] compound **2** is referred to as manzamine K.

[5]The manzamines crystallize readily. The structures of manzamines A (1), B, and C (2) were secured by X-ray diffraction crystallography. In our experience, the manzamines also have a tendency to co-crystallize.

TABLE 1. ^{13}C-Nmr Data Comparison of Manzamine A, Compounds **1**, **2**, and Manzamine F.

# C	Manzamine A[a]	Compound **1**	Compound **2**	Manzamine F[b]
1	143.60	143.50	143.33	142.5
3	137.54	137.44	137.89	137.8
4	113.76	113.94	114.72	113.7
4a	129.27	129.51	129.76	130.2
4b	121.13	121.15	123.15	122.8
5	120.90	121.00	112.60	112.0
6	119.22	119.37	120.72	120.9
7	127.89	128.12	114.32	113.0
8	112.78	112.89	143.82	143.4
8a	141.37	141.54	130.55	129.9
9a	133.25	133.37	132.93	133.2
10	141.18	141.14	141.85	140.9
11	135.12	135.33	134.55	137.3
12	71.25	71.27	71.20	69.0
13	39.05	39.12	39.22	39.9
14	20.62	20.70	20.69	21.4
15	126.76	126.88	126.69	127.9
16	132.82	132.89	132.99	130.1
17	24.86	24.95	24.69	25.5
18	26.36	26.44	26.52	26.6
19	24.45	24.55	24.54	25.0
20	53.31	53.46	53.39	52.8
22	49.12	49.24	49.12	49.6
23	33.51	33.59	33.39	34.0
24	41.00	41.07	41.25	42.3
25	46.91	47.07	47.06	47.3
26	77.98	78.08	78.26	81.7
28	53.32	53.46	53.67	53.0
29	26.23	26.32	26.42	32.7
30	24.16	24.30	24.15	45.1
31	28.31	28.41	28.41	216.2
32	142.27	142.44	142.78	38.8
33	123.50	123.68	123.34	24.4
34	57.02	57.13	57.44	63.6
35	44.65	44.75	44.79	46.6
36	70.26	70.46	70.19	69.0

[a]Taken from Sakai *et al.* (1).
[b]Taken from Ichiba *et al.* (4).

yellow oil by reacting **2** with trimethylsilyldiazomethane ($TMSCHN_2$). That the new manzamine **2** was 8-hydroxymanzamine A could be deduced by comparing the ^{13}C-nmr data of manzamine F (4), which is 8-hydroxymanzamine E, with those of **2** (Table 1). The ^{1}H-nmr spectrum of **2** includes four exchangeable protons, which indicates that **2** is a cation. The nature of the counter ion is unknown. The absolute stereochemistry of **2** should be the same as that of manzamine A [**1**], as both compounds are dextrorotatory.

All three compounds, manzamine A [**1**], 8-hydroxymanzamine A [**2**], and 8-methoxymanzamine A [**3**] are moderately active in the KB, LoVo, and HSV-II assays. Only manzamine A [**1**] exhibits activity in the P-388 assay (Table 2).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra (chemical shifts in ppm) were measured on a General Electric QE-300 instrument, and ir spectra on a Perkin Elmer 1420 spectrometer. Uv spectra were determined on an HP 8452A spectrophotometer. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. A Fisher-Johns melting point apparatus was used.

TABLE 2. Bioactivities of Manzamine A [**1**], 8-Hydroxymanzamine A [**2**], and 8-Methoxymanzamine A [**3**].

Assay	**1**	**2**	**3**
KB (IC_{50} μg/ml)	0.05	0.30	0.33
LoVo (IC_{50} μg/ml)	0.15	0.26	0.10
P388 (IC_{50} μg/ml)	0.07[a]	inactive	not tested
HSV-II (MIC μg/ml)	0.05	0.1	0.1

[a]Sakai *et al.* (1).

Solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements. Analytical tlc separations were performed on precoated hp-tlc plates with Si gel 60 F_{254} (Merck, Darmstadt). Counter-current chromatography was carried out using the Ito Multi-Layer Coil Separator-Extractor (PC Inc., Potomac, MD).

ISOLATION.—A freeze-dried specimen of *Pachypellina* sp. (10 g) was extracted with EtOH (3×300 ml). The CH_2Cl_2-soluble portion (320 mg) of the EtOH extract was separated by high-speed countercurrent chromatography into four fractions with the solvent system hexane-MeCN-CH_2Cl_2 (10:7:3, lower mobile phase) to yield semi-pure manzamine A [**1**] (Fraction 3) and compound **2** (Fraction 2). Both compounds were further purified by recrystallization from CH_2Cl_2-MeOH to furnish 40.3 mg (0.4%, based on dry weight) of manzamine A [**1**] and 30.5 mg (0.3%) of compound **2**.

8-Hydroxymanzamine A [**2**].—Pale yellow crystals: mp >230° (dec); $[\alpha]_D$ +118.5° ($CHCl_3$, c=1.94); fabms *m/z* $[M+K]^+$ 603, $[M+H]^+$ 565, $[M+H-H_2O]^+$ 547; hrfabms *m/z* $[M+H]^+$ 565.3525 (calcd for $C_{36}H_{45}N_4O_2$, 565.3507, Δ 1.8 mmu); uv (EtOH) λ max 206 ε (sh, 22700), 222 (32300), 245 (30600), 268 (14000), 360 nm (8100); ir (neat on NaCl plate) ν max 3280, 2900, 1570, 1540, 1420, 1410, 1330, 1260, 1230, 1220, 1200, 1055, 1035, 1010, 970, 940, 745, 720 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$): δ 11.50 (1H, s, exchangeable), 9.80 (1H, br s, exchangeable), 8.33 (1H, d, J=5.1, Hz, H-3), 7.83 (1H, d, J=5.1 Hz, H-4), 7.79 (1H, br s, exchangeable), 7.62 (1H, br d, J=7.2 Hz, H-5), 7.15 (1H, t, J=7.5 Hz, H-6), 7.09 (1H, dd, J=7.5, 0.9 Hz, H-7), 6.46 (1H, s, H-11), 6.34 (1H, dt, J=10.8, 6.8 Hz, H-32), 5.59 (1H, m, H-15), 5.55 (1H, m, H-16), 5.42 (1H, t, J=9.8 Hz, H-33), 5.23 (1H, s, exchangeable, H-32), 4.98 (1H, br q, J=6.9 Hz, H-34), 4.03 (1H, dd, J=12.0, 6.9 Hz, H-28), 3.77 (1H, d, J=6.9 Hz, H-26), 3.32 (1H, q, J=10.1 Hz, H-28), 2.97 (1H, H-23), 2.97 (1H, H-22), 2.92 (1H, H-36), 2.62 (1H, H-20), 2.53 (1H, H-24), 2.50 (1H, H-29), 2.49 (1H, H-17), 2.43 (1H, H-20), 2.40 (1H, H-36), 2.39 (1H, H-35), 2.35 (2H, H-31), 2.30 (1H, H-29), 2.23 (2H, H-14), 2.06 (1H, H-13), 2.03 (1H, H-30), 1.92 (1H, H-35), 1.88 (1H, H-22), 1.86 (1H, H-23), 1.85 (1H, H-19), 1.80 (1H, H-13), 1.64 (1H, H-17), 1.54 (1H, H-30), 1.54 (1H, H-18), 1.50 (1H, H-19), 1.23 (1H, H-18).

8-Methoxymanzamine A [**3**].—8-Hydroxymanzamine A (12.2 mg) was dissolved in 250 μl MeOH-CH_2Cl_2 (5:1) and 500 μl $TMSCHN_2$ (Aldrich) was added. The solution was allowed to stand at room temperature overnight. Solvent was removed over N_2, yielding a pale yellow oil (12.4 mg): hreims *m/z* 578.362091 (calcd 578.36238 for $C_{37}H_{46}N_4O_2$, Δ 0.3 mmu); ^{13}C nmr (75 MHz, $CDCl_3$) δ 146.14, 143.55, 139.78, 138.55, 137.59, 134.43, 133.28, 132.33, 130.57, 129.99, 129.45, 128.56, 122.76, 120.47, 113.60 (× 2), 107.57, 75.10, 70.05, 68.67, 55.49, 54.84, 53.33, 50.76, 49.49, 47.09, 40.55, 40.87, 39.95, 32.34, 31.65, 28.11, 26.73, 25.87, 25.74, 25.70, 21.78.

ACKNOWLEDGMENTS

We thank Mark Hamann and Mike Severns for specimen collection; Wesley Yoshida and Mike Burger for assistance with ms measurements; and Faith Caplan, Linda K. Larsen, and PharmaMar biologists for bioassays. Financial support by NSF, the Sea Grant College Program, and PharmaMar, S.A. is gratefully acknowledged.

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Received 17 June 1993