6-Hydroxymanzamine A and 3,4-Dihydromanzamine A, New Alkaloids from the Okinawan Marine Sponge Amphimedon sp.

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6-HYDROXYMANZAMINE A AND 3,4-DIHYDROMANZAMINE A, NEW ALKALOIDS FROM THE OKINAWAN MARINE SPONGE *AMPHIMEDON* SP.

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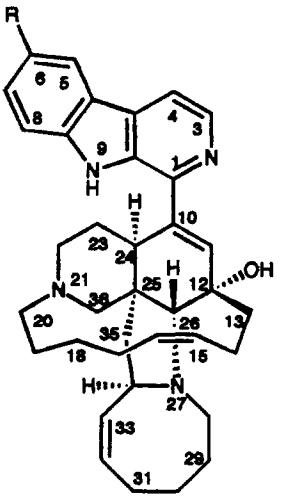
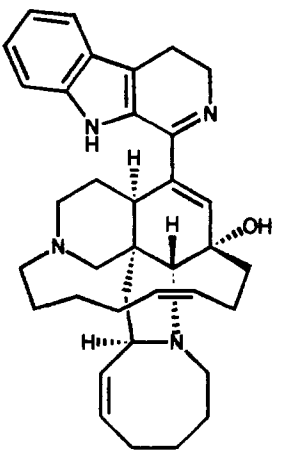
ABSTRACT.—Two new β-carboline alkaloids, 6-hydroxymanzamine A [**1**] and 3,4-dihydromanzamine A [**2**], have been isolated from the Okinawan marine sponge *Amphimedon* sp. and their structures elucidated on the basis of nmr spectral data.

Manzamine alkaloids are a series of β-carboline compounds with unique polycyclic systems from marine sponges in the genera *Haliclona* (1,2), *Pellina* (3) *Xestospongia* (4), *Ircinia* (5), and *Pachipellina* (6). In our search for bioactive substances from marine organisms, two new manzamine congeners, 6-hydroxymanzamine A [**1**] and 3,4-dihydromanzamine A [**2**], have been isolated from the Okinawan marine sponge *Amphimedon* sp. Here we describe the isolation and structure elucidation of **1** and **2**.

A sponge of the genus *Amphimedon* was collected off the Kerama Islands, Okinawa, and kept frozen until used. The MeOH extract of this sponge was partitioned between EtOAc and H_2O, and the aqueous layer was subsequently extracted with *n*-BuOH. The *n*-BuOH-soluble material was subjected to Si gel cc ($CHCl_3$-MeOH, 95:5 and then cyclohexane-Me_2CO-Et_2NH, 70:30:2) to afford 6-hydroxymanzamine A (**1**, 0.005%, wet wt). The EtOAc-soluble material was chromatographed over a Si gel column ($CHCl_3$-MeOH, 98:2 and then C_6H_6-Me_2CO-Et_2NH, 95:5:2) to yield 3,4-



1 R=OH
3 R=H



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dihydromanzamine A (**2**, 0.002%) together with a known compound, manzamine A [**3**] (1,3).[1]

The ^{1}H- and ^{13}C-nmr data (Table 1) of compound **1** were reminiscent of those of manzamine A [**3**] except for a downfield sp^2 carbon (δ_C 153.4). The molecular formula, $C_{36}H_{44}N_4O_2$, of **1** was established by hreims, suggesting that **1** had an additional oxygen atom as compared with **3**. Substitution for a hydroxy group at C-6 in **1** was revealed by comparison of the ^{13}C-nmr chemical shifts of C-5 through C-8 (Table 1) of **1** with those of a 6-hydroxy-β-carboline such as 10-hydroxylyalosidic acid (7) (C-5, δ_C 106.6; C-6, δ_C 153.0; C-7, δ_C 121.6; C-8, δ_C 114.1). Compound **1** was thus concluded to be 6-hydroxymanzamine A. The absolute stereochemistry of **1** is considered to be the same as that of manzamine A [**3**] since both compounds were dextrorotary and isolated from the same organism.

The ^{1}H- and ^{13}C-nmr data (Table 1) of **2** were similar to those of **3** except for the chemical shifts at C-3 and C-4, and the eims spectrum of compound **2** showed the molecular ion peak at *m/z* 550, which was larger than that of manzamine A [**3**] by two daltons. The presence of a 3,4-dihydro-β-carboline ring was elucidated by comparison of the ^{13}C-nmr chemical shifts at C-1, C-3, and C-4 of **2** with those of a 3,4-dihydro-β-carboline such as harmalane (8) (C-1, δ_C 158.4; C-3, δ_C 49.5; C-4, δ_C 20.2). Thus, compound **2** was elucidated as 3,4-dihydromanzamine A. The same sign of the optical rotations of **2** and **3**, which were isolated simultaneously, indicated that the absolute stereochemistry of **2** is the same as that of **3**.

6-Hydroxymanzamine A [**1**] and 3,4-dihydromanzamine A [**2**] are new manzamine A congeners from the marine sponge *Amphimedon* sp. Although manzamines with an OH-8 group such as manzamine F (4) [keramamine B (3)] and 8-hydroxymanzamine A (6) [manzamine G (9)] have been reported previously, compound **1** is the first manzamine alkaloid with a hydroxy group at C-6. Compound **2** is the first 3,4-dihydro analogue of the manzamine alkaloids and may be a direct precursor that generates manzamine A [**3**] through dehydrogenation. 6-Hydroxymanzamine A [**1**] and 3,4-dihydromanzamine A [**2**] showed antibacterial activity against a Gram-positive bacterium, *Sarcina lutea* (MIC value, 1.25 and 4 μg/ml, respectively). Compounds **1** and **2** were cytotoxic against L-1210 (IC_{50} values, 1.5 and 0.48 μg/ml, respectively) and KB cells (IC_{50} 2.5 and 0.61 μg/ml, respectively) in vitro.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were determined on a Jasco DIP-370 digital polarimeter. Uv and ir spectra were taken on Shimadzu uv-220 and Jasco ir Report-100 spectrometers, respectively. ^{1}H- and ^{13}C-nmr spectra were conducted with a Bruker ARX-500 and a JEOL GX-270 spectrometer, respectively. Eims spectra were recorded employing a JEOL DX-303 spectrometer.

ANIMAL MATERIAL.—The sponge *Amphimedon* sp. (order Haploscerida, family Niphatidae) was collected off the Kerama Islands, Okinawa, Japan, and kept frozen until used. The sponge was brown throughout in color, and firm and compressible in texture. The mesohyl consisted of a fibrous reticulation with dense plumoreticulate fiber centrally. Primary fibers were 18 to 60 μm wide and cored by 1–3 oxeas, while others were fasciculate and were 80 to 90 μm wide and cored by 6–8 oxeas. Choanosomal meshes were 100 to 200 μm wide. At the surface, spicules formed fans but did not penetrate beyond the surface mesohyl. Spicules were small oxeas, of mean size 94×4 μm. No microscleres were evident. The voucher specimen (SS-326) was deposited at James Cook University of North Queensland, Townsville, Queensland, Australia (Dr. J. Fromont).

EXTRACTION AND ISOLATION.—The sponge *Amphimedon* sp. (1.5 kg) was extracted with MeOH (1 liter×2) and then evaporated under reduced pressure to give a residue (68.4 g). The residue was partitioned between EtOAc (300 ml×3) and H_2O (500 ml) and subsequently the aqueous layer was extracted with *n*-BuOH (300 ml×3). The *n*-

[1]Manzamine A is referred to as keramamine A by Nakamura *et al.* (3).

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of 6-Hydroxymanzamine A [**1**] and 3,4-Dihydromanzamine A [**2**].

Position	Compound			
	1[a]		**2**[b]	
	δ_H	δ_C	δ_H	δ_C
1		145.7		158.9
3	8.23, d (5.2)	139.0	3.96, m 3.84, m	48.8
4	7.83, d (5.2)	115.9	2.82, m (2H)	19.1
4a		124.3		117.1
4b		131.9		125.5
5	7.49, d (1.7)	107.4	7.60, d (7.9)	119.7
6		153.4	7.13, d (7.9)	120.2
7	7.09, dd (1.7, 8.8)	120.5	7.28, d (7.9)	124.3
8	7.50, d (8.8)	114.9	7.41, d (7.9)	112.1
8a		136.7		136.1
9a		138.1		127.6
10		142.0		139.0
11	6.30, s	139.0	6.32, s	140.8
12		72.7		69.9
13	2.10, m 1.86, m	42.4	1.93, m 1.67, m	40.5
14	2.38, m 2.07, m	23.7	2.35, m 2.13, m	21.6
15	5.60, m	130.4	5.63, m	128.4
16	5.51, dt (10.3, 4.7)	134.2	5.53, dt (10.6, 4.7)	132.3
17	2.56, m 1.74, m	28.1	2.53, m 1.70, m	25.9
18	1.75, m 1.33, m	29.1	1.70, m 1.33, m	25.8
19	1.72, m 1.37, m	27.8	1.73, m 1.42, m	25.6
20	2.62, dt (12.0, 4.6) 2.37, m	55.7	2.60, dt (13.2, 5.2) 2.44, m	53.4
22	2.82, br d (10.2) 1.90, m	51.9	2.76, br d (10.8) 1.95, m	49.5
23	1.98, m 1.66, m	33.0	1.97, m 1.45, m	32.5
24	2.94, dd (6.3, 11.9)	42.8	2.00, m	40.8
25		48.6		46.9
26	3.59, s	77.6	3.44, s	75.1
28	3.18, m (2H)	53.3	3.17, m (2H)	50.9
29	1.93, m 1.64, m	34.8	1.96, m 1.62, m	31.3
30	1.88, m 1.42, m	27.7	1.87, m 1.34, m	25.6
31	2.31, m 2.15, m	30.0	2.35, m 2.13, m	28.1
32	5.99, m	138.7	5.94, m	134.9
33	5.33, t (9.4)	131.2	5.26, br d (10.6)	129.6
34	4.26, m	57.7	4.19, m	55.0
35	2.29, m 1.77, m	46.9	2.14, m 1.69, m	44.5
36	2.88, d (11.2) 2.28, d (11.2)	70.9	2.78, d (11.6) 2.27, d (11.6)	68.6

[a]In CD_3OD.
[b]In $CDCl_3$.

BuOH-soluble portion was evaporated under reduced pressure to give a residue (5.16 g), a part (0.96 g) of which was subjected to cc over a Si gel column with $CHCl_3$/MeOH. The fraction eluting with $CHCl_3$/MeOH (95:5) was further purified with a Si gel column (cyclohexane-Me_2CO-Et_2NH, 70:30:2) to give 6-hydroxymanzamine A (**1**, 13.7 mg, 0.005%, wet wt). A part (1 g) of the EtOAc-soluble material (4.6 g) was subjected to a Si gel column with $CHCl_3$/MeOH as eluent. The fraction eluting with $CHCl_3$-MeOH (98:2) was separated over a Si gel column (C_6H_6-Me_2CO-Et_2NH, 95:5:2) to afford 3,4-dihydromanzamine A (**2**, 6.8 mg, 0.002%) together with manzamine A (**3**, 130 mg, 0.04%).

6-Hydroxymanzamine A [**1**].—Yellowish amorphous solids: mp 253°; $[\alpha]^{20}D$ +139° (c=1.1, MeOH); eims *m/z* 564 $[M]^+$, 546 $[M-H_2O]^+$, and 162; hreims *m/z* 564.3447 $[M]^+$ calcd for $C_{36}H_{44}N_4O_2$, 564.3465, Δ −1.8 mmu; uv (MeOH) λ max 210 (ϵ 32000), 293 sh (15000), 300 (16000), and 370 (5100) nm; ir (neat on NaCl plate) ν max 3300, 2920, and 1450 cm^{-1}.

3,4-Dihydromanzamine A [**2**].—Colorless amorphous solid: mp 237–241°; $[\alpha]^{20}D$ +86° (c=0.25, $CHCl_3$); eims *m/z* 550 $[M]^+$, 532 $[M-H_2O]^+$, and 162; hreims *m/z* 550.3653 $[M]^+$ calcd for $C_{36}H_{46}N_4O$, 550.3672, Δ −1.9 mmu; uv (MeOH) λ max 230 sh (ϵ 25000), 244 (21000), and 323 (10000) nm; ir (neat on NaCl plate) ν max 3280, 2940, 1470, and 1450 cm^{-1}.

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LITERATURE CITED

1. R. Sakai, T. Higa, C.W. Jefford, and G. Bernardinelli, *J. Am. Chem. Soc.*, **108**, 6404 (1986).
2. R. Sakai, T. Kohmoto, T. Higa, C.W. Jefford, and G. Bernardinelli, *Tetrahedron Lett.*, **28**, 5493 (1987).
3. H. Nakamura, S. Deng, J. Kobayashi, Y. Ohizumi, Y. Tomotake, T. Matsuzaki, and Y. Hirata, *Tetrahedron Lett.*, **28**, 621 (1987).
4. T. Ichiba, R. Sakai, S. Kohmoto, G. Saucy, and T. Higa, *Tetrahedron Lett.*, **29**, 3083(1988).
5. K. Kondo, H. Shigemori, Y. Kikuchi, M. Ishibashi, T. Sasaki, and J. Kobayashi, *J.Org. Chem.*, **57**, 2480 (1992).
6. T. Ichiba, J.M. Corgiat, P.J. Scheuer, and M. Kelly-Borges, *J. Nat. Prod.*, **57**, 168 (1994).
7. N. Aimi, H. Murakami, T. Tsuyuki, T. Nishimura, S. Sakai, and J. Haginiwa, *Chem. Pharm. Bull.*, **34**, 3064 (1986).
8. C.A. Coune, L.J.G. Angenot, and J. Denoël, *Phytochemistry*, **19**, 2009 (1980).
9. T. Higa, "Proceedings of the First Princess Chulabhorn Science Congress," Bangkok, Thailand, 1987, pp. 450–459.

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