Three New Manzamine Congeners from *Amphimedon* Sponge

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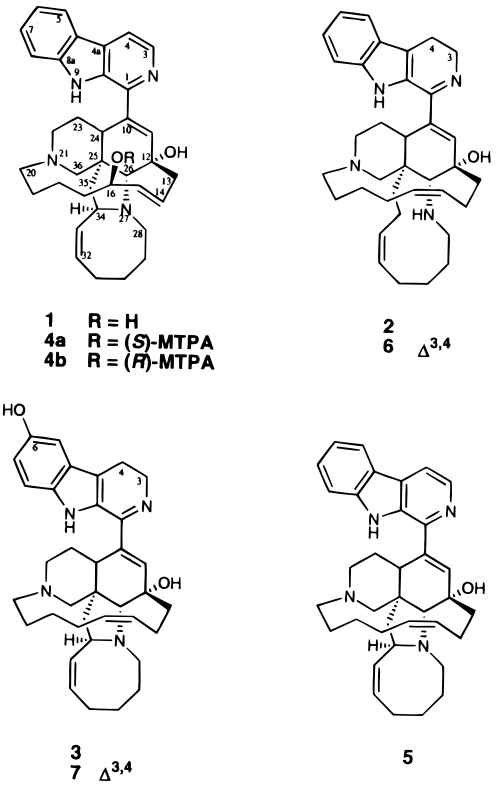
Three new manzamine congeners (**1**−**3**) have been isolated from the Okinawan marine sponge *Amphimedon* sp., and the structures, including relative stereochemistries, were elucidated on the basis of spectroscopic data. Absolute configuration at C-16 of **1** was determined by modified Mosher's method.

Manzamines are unique marine alkaloids possessing an intricate nitrogen-containing ring system at C-1 of the β-carboline ring. Related alkaloids have been isolated from eight genera of marine sponges: *Haliclona*, *Pellina*, *Xestospongia*, *Pachypellina*, *Petrosia*, *Cribochalina*, *Ircinia*, and *Amphimedon*.[1] In our continuing search for new manzamine-related alkaloids from Okinawan marine sponges we have isolated several novel alkaloids.[2−7] Further investigation of extracts from an *Amphimedon* sponge led to the isolation of three new manzamine congeners, manzamine M (**1**), 3,4-dihydromanzamine J (**2**), and 3,4-dihydro-6-hydroxymanzamine A (**3**). In this paper we describe the isolation and structure elucidation of compounds **1**−**3**.

The sponge *Amphimedon* sp. (SS-264), collected off the Kerama Islands, Okinawa, was extracted with MeOH. The EtOAc-soluble material of the extract was purified by Si gel chromatography to afford compounds **1** (1.5×10^{-3} %, wet wt), **2** (4×10^{-4} %), and **3** (1.5×10^{-3} %), together with several known manzamine alkaloids such as manzamine A (**5**).[7,8]

Manzamine M {**1**, $[\alpha]^{25}_D$ +16° (*c* 0.48, MeOH)} was revealed to possess the molecular formula $C_{36}H_{44}N_4O_2$ by HREIMS, suggesting the presence of an additional oxygen atom, as compared with manzamine A[7] (**5**). ^{1}H and ^{13}C NMR data (Table 1) of **1** corresponded well to those of **5**, except for the presence of an oxymethine signal [δ_H 4.06 (br t); δ_C 74.4 (d)] and a *trans*-disubstituted double bond [δ_H 5.73 (ddd, J = 14.8, 10.0, 4.3 Hz), δ_C 128.3 (d); δ_H 5.65 (dd, J = 14.8, 8.3 Hz), δ_C 140.6 (d)] in **1**. The hydroxyl group at C-16 and the $\Delta^{14,15}$ double bond were assigned on the basis of HOHAHA correlations from H_2-13 (δ_H 2.95 and 2.73) to H-14, H-15, and H-16. Thus, the structure of manzamine M was assigned as **1**. Relative configurations at C-12, C-24, C-25, C-26, and C-34 of **1** were elucidated to be the same as those of manzamine A (**5**) by NOESY data of **1** as well as by the similarity of the ^{13}C chemical shifts (C-12, δ_C 70.7; C-24, δ_C 42.2; C-25, δ_C 49.1; C-26, δ_C 74.7; C-34, δ_C 57.3) of **1** to those of **5** (δ_C 71.3, 41.0, 46.9, 78.0, and 57.0, respectively).[8] The *R*-configuration at C-16 was deduced from ^{1}H chemical shift differences [$\Delta\delta$ (ppm) = $\delta_S - \delta_R$] at H-14 (δ_S 5.45, δ_R 5.55; $\Delta\delta$ −0.10), H-15 (δ_S 5.46, δ_R 6.03; $\Delta\delta$ −0.63), H_2-17 (δ_S 2.37, δ_R 1.75; $\Delta\delta$ +0.62 and δ_S 2.20, δ_R 1.80; $\Delta\delta$ +0.60), and H_2-18 [δ_S 2.12 (2H), δ_R 1.81 and 1.79; $\Delta\delta$ +0.31 and +0.33] of (*S*)- and (*R*)-MTPA esters[9] (**4a** and **4b**, respectively) of **1**. The absolute stereochemistries at the remaining five chiral centers of **1** also seem to be the same as those of manzamine A (**5**) inasmuch as both compounds were isolated from the same sponge and possessed dextrorotation.



^{1}H and ^{13}C NMR data (Table 2) of compound **2** {$[\alpha]^{30}_D$ +50° (*c* 0.10, MeOH)} were similar to those of known manzamine J[2] (**6**), except for the chemical shifts of C-3 (δ_C 48.8) and C-4 (δ_C 19.1), which were identical to the corresponding resonances in 3,4-dihydromanzamine A.[6] The molecular formula $C_{36}H_{48}N_4O$ of **2**, as established by HREIMS data, also supported the presence of two additional hydrogen atoms in manzamine J (**6**). Treatment of **2** with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) gave manzamine J (**6**). Thus, the structure of **2** was concluded to be 3,4-dihydromanzamine J.

HREIMS data of compound **3** {$[\alpha]^{25}_D$ +28° (*c* 1.2, MeOH)} indicated the molecular formula to be $C_{36}H_{46}N_4O_2$. The ^{13}C NMR data (Table 2) of **3** suggested the presence of a 3,4-dihydro-β-carboline ring (C-3, δ_C

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Table 1. ^{1}H and ^{13}C NMR Data of Manzamine M (**1**) in CD_3OD

position	δ_H	m	*J* (Hz)	δ_C	m
1				145.6	s
3	8.35	t	5.2	139.6	d
4	8.00	d	5.2	115.3	d
4a				123.5	s
4b				132.1	s
5	8.19	d	7.7	123.1	d
6	7.30	dd	7.7, 7.1	121.7	d
7	7.59	dd	7.1, 8.2	130.3	d
8	7.72	d	8.2	114.1	d
8a				135.7	s
9a				141.3	s
10				143.5	s
11	6.54	s		138.4	d
12				70.7	s
13	2.95	m		50.5	t
	2.73	dd	10.0, 14.4		
14	5.73	ddd	14.8, 10.0, 4.3	128.3	d
15	5.65	dd	14.8, 8.3	140.6	d
16	4.06	brt	7.5	74.4	d
17	1.72	m		37.2	t
	1.52	m			
18	1.68	m		27.4	t
	1.45	m			
19	1.66	m		22.5	t
	1.45	m			
20	2.60	tt	4.4, 8.6	55.9	t
	2.30	m			
22	2.81	m		55.3	t
	2.18	m			
23	1.98	m		33.3	t
	1.72	m			
24	2.93	m		42.2	d
25				49.1	s
26	4.20	s		74.7	d
28	2.95[a]	m		53.1	t
29	1.99	m		34.5	t
	1.67	m			
30	1.95	m		27.2	t
	1.45	m			
31	2.34	m		30.1	t
	2.22	m			
32	5.98	dt	10.9, 7.1	136.9	d
33	5.36	brt	8.8	131.4	d
34	4.32	brt	8.3	57.3	d
35	2.41	dd	8.6, 13.2	45.3	t
	1.68	m			
36	2.95	d	11.6	65.6	t
	1.95	d	11.6		

[a] 2H.

48.8; C-4, δ_C 19.2). The presence of a hydroxyl group at the C-6 position was deduced from similarity of the ^{13}C chemical shifts at C-4b (δ_C 126.0), C-5 (δ_C 103.4), C-6 (δ_C 149.8), C-7 (δ_C 114.5), C-8 (δ_C 112.9), and C-8a (δ_C 135.2) to those of 6-hydroxymanzamine A (**7**) (C-4b, δ_C 131.9; C-5, δ_C 107.4; C-6, δ_C 153.4; C-7, δ_C 120.5; C-8, δ_C 114.9; C-8a, δ_C 136.7).[6] DDQ oxidation of **3** gave 6-hydroxymanzamine A (**7**). Thus, the structure of compound **3** was elucidated to be the 3,4-dihydro form of 6-hydroxymanzamine A (**7**).

Manzamine M (**1**) is the first manzamine congener with a hydroxyl group on the C-13−C-20 chain. Manzamine M (**1**), 3,4-dihydromanzamine J (**2**), and 3,4-dihydro-6-hydroxymanzamine A (**3**) showed cytotoxicity against murine leukemia L1210 cells (IC_{50}, 1.4, 0.5, and 0.3 μg/mL, respectively). Compounds **1**, **2**, and **3** exhibited antibacterial activity against *Sarcina lutea* (MIC, 2.3, 12.5, and 6.3 μg/mL, respectively) and *Corynebacterium xerosis* (MIC, 5.7, 12.5, and 3.1 μg/mL, respectively).

Experimental Section

General Procedures. Optical rotations were recorded on a JASCO DIP-360 polarimeter. The IR and UV spectra were taken on JASCO FT/IR-5300 and JASCO Ubest-35 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-600 and ARX-500 spectrometers, respectively. EIMS were obtained on a JEOL DX-303 spectrometer at 70 eV.

Extraction and Isolation. The *Amphimedon* sponge[10] (1.0 kg, wet wt) collected off the Kerama Islands, Okinawa, was extracted with MeOH (1 L × 2). The MeOH extract (71 g) was partitioned between EtOAc (400 mL × 3) and 1N NaCl solution. Part (15 g) of the EtOAc-soluble material (51.1 g) was subjected to a Si gel column ($CHCl_3$−MeOH, 95:5 → 90:10 → 70:30). The fraction eluting with $CHCl_3$−MeOH (70:30) was separated on an alumina column ($CHCl_3$) and then a Si gel column (cyclohexane−Et_2NH, 100:2) to yield manzamine M (**1**, 4.8 mg, 1.5×10^{-3} % wet wt). The fraction eluting with $CHCl_3$−MeOH (90:10) of the first Si gel column was separated by Si gel columns (cyclohexane−acetone−Et_2NH, 95:5:2 and then hexane−acetone, 9:1) to afford 3,4-dihydromanzamine J (**2**, 1.2 mg, 4×10^{-4} %). The fraction eluting with $CHCl_3$−MeOH (95:5) in the first Si gel column was separated by Si gel columns (cyclohexane−acetone−Et_2NH, 90:10:2 and then $CHCl_3$−MeOH, 90:10) to give 3,4-dihydro-6-hydroxymanzamine A (**3**, 4.9 mg, 1.5×10^{-3} %).

Manzamine M (1): a colorless amorphous solid; $[\alpha]^{25}_D$ +16° (*c* 0.48, MeOH); UV (MeOH) λ_{max} 205 (ϵ 14 000), 233 (3000), 276 (1500), and 359 nm (1000); IR (KBr) ν_{max} 3410 (br), 2925, 1630, 1405, and 1070 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); HOHAHA cross peaks (H/H): 3/4, 5/6, 5/7, 5/8, 6/7, 6/8, 7/8, 13a/13b, 13a/14, 13a/15, 13a/16, 13b/14, 13b/15, 13b/16, 14/15, 15/16, 15/17b, 16/17a, 16/17b, 17a/19b, 19a/20a, 19a/20b, 19b/20a, 19b/20b, 20a/20b, 22a/22b, 22a/23a, 22a/23b, 22a/24, 22b/23a, 22b/23b, 22b/24, 23a/23b, 23b/24, 28(2H)/29a, 28(2H)/29b, 28(2H)/30b, 30a/32, 30b/31a, 30b/31b, 31a/32, 31a/33, 31b/32, 31b/33, 32/33, 32/34, 32/35b, 33/34, 33/35b, 34/35a, 34/35b, and 36a/36b; NOESY correlations (H/H) 3/4, 5/6, 6/7, 7/8, 11/13a, 11/13b, 13a/13b, 13a/14, 13b/15, 13b/17a, 14/16, 14/26, 15/16, 15/17b, 16/17a, 16/17b, 19a/20a, 19a/22a, 19b/22a, 20a/20b, 20a/22a, 22a/22b, 22b/23a, 22b/24, 24/35a, 24/35b, 26/28, 28/29a, 28/29b, 29a/29b, 30a/31b, 31a/31b, 31b/32, 32/33, 33/35b, 34/35a, 34/35b, 34/36a, and 36a/36b; EIMS *m/z* 564 (M^+) and 162; HREIMS *m/z* 564.3459 (M^+), calcd for $C_{36}H_{44}N_4O_2$, 564.3464.

3,4-Dihydromanzamine J (2): a colorless amorphous solid; $[\alpha]^{30}_D$ +50° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 209 (ϵ 13 000), 242 (9000), and 322 nm (4500); IR (KBr) ν_{max} 3420 (br), 2920, 1630, and 1090 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 2); EIMS *m/z* 552 (M^+); HREIMS *m/z* 552.3815 (M^+), calcd for $C_{36}H_{48}N_4O$, 552.3828.

3,4-Dihydro-6-hydroxymanzamine A (3): a colorless amorphous solid; $[\alpha]^{25}_D$ +28° (*c* 1.2, MeOH); UV (MeOH) λ_{max} 207 (ϵ 9000), 225 (6500), 250 (3500), and 337 nm (2500); IR (KBr) ν_{max} 3420 (br), 2920, 1630, 1400, and 1070 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 2); EIMS *m/z* 566 (M^+); HREIMS *m/z* 566.3604 (M^+), calcd for $C_{36}H_{46}N_4O_2$, 566.3621.

Table 2. ^{1}H and ^{13}C NMR Data of 3,4-Dihydromanzamine J (**2**) and 3,4-Dihydro-6-hydroxymanzamine A (**3**) in $CDCl_3$

	2					**3**				
positn	δ_H	m	*J* (Hz)	δ_C	m	δ_H	m	*J* (Hz)	δ_C	m
1				159.8	s				158.8	s
3	3.45	m		48.8	t	3.93	m		48.8	t
	3.35	m				3.89	m			
4	2.88	m		19.1	t	2.81	m		19.2	t
	2.86	m				2.79	m			
4a				117.1	s				116.2	s
4b				125.5	s				126.0	s
5	7.59	d	7.7	119.7	d	6.97	brs		103.4	d
6	7.14	dd	7.7, 7.1	120.1	d				149.8	s
7	7.26	dd	7.1, 8.2	124.2	d	6.86	brd	8.2	114.5	d
8	7.40	d	8.2	112.0	d	7.29	d	8.2	112.9	d
8a				136.1	s				135.2	s
9a				127.7	s				128.3	s
10				140.1	s				138.9	s
11	6.15	s		133.5	d	6.36	s		140.2	d
12				70.2	s				69.9	s
13	2.05	m		40.7	t	1.92	m		40.3	t
	1.48	m				1.72	m			
14	2.27	m		21.9	t	1.73	m		21.4	t
	1.98	m				1.43	m			
15	5.32	ddd	7.0, 8.3, 10.6	129.3	d	5.63	ddd	7.3, 8.3, 10.8	128.9	d
16	5.28	dt	10.6, 7.6	129.2	d	5.54	dt	10.8, 7.3	132.4	d
17	2.93	m		29.1	t	1.77	m		25.5	t
	2.90	m				1.43	m			
18	1.90	m		28.6	t	1.91	m		26.2	t
	1.55	m				1.43	m			
19	1.70	m		29.1	t	1.73	m		25.1	t
	1.20	m				1.43	m			
20	2.91	m		53.4	t	2.61	tt		53.4	t
	2.82	m				2.43	m			
22	2.95	m		49.5	t	2.78	m		49.4	t
	1.94	m				1.95	m			
23	2.17	m		32.3	t	2.01	m		32.6	t
	1.72	m				1.48	m			
24	2.45	m		45.0	d	2.78	m		38.2	d
25				43.2	s				46.8	s
26	3.78	s		59.2	d	3.47	s		75.2	d
28	2.62	m		59.2	t	3.19	m		51.2	t
	2.35	m				3.08	m			
29	1.78	m		29.1	t	1.93	m		29.6	t
	1.70	m				1.27	m			
30	1.78	m		29.1	t	2.29	m		28.2	t
	1.28	m				2.16	m			
31	1.74	m		25.0	t	1.74	m		25.8	t
	1.48	m				1.48	m			
32	5.61	dt	10.9, 7.1	131.0	d	5.97	t	10.4, 7.6	135.2	d
33	5.36	brt	8.8	131.4	d	5.25	brt	9.5	128.2	d
34	2.72	m		26.2	t	4.30	br		55.2	d
	2.19	m								
35	2.18	m		37.4	t	2.78	m		44.6	t
	1.45	m				1.72	m			
36	3.30	d	12.1	65.6	t	2.80	m		68.9	t
	2.08	d	12.1			2.37	m			

MTPA Esters of Manzamine M (1). Manzamine M (**1**, 0.5 mg) in pyridine (500 μL) was treated with (*R*)-(−)-MTPA chloride (5 μL) at room temperature for 24 h. After addition of MeOH (500 μL), the solvent was evaporated *in vacuo*, and the residue was purified on SiO_2 TLC ($CHCl_3$−MeOH, 90:10) to afford the (*S*)-MTPA ester (**4a**, 0.1 mg) of **1**. Compound **4a**: ^{1}H NMR (CD_3OD) δ 1.4−2.9 (25H, m), 3.51 (3H, s, OMe), 4.10 (1H, s, H-25), 4.31 (1H, m, H-34), 5.41 (1H, m, H-33), 5.45 (1H, m, H-14), 5.46 (1H, m, H-15), 6.01(1H, m, H-16), 6.03 (1H, m, H-32), 6.53 (1H, s, H-12), 7.64−7.25 (8H, m, Ph and H-6, H-7, and H-8), 8.01 (1H, d, J = 5.2 Hz, H-4), 8.22 (1H, d, J = 7.2 Hz, H-5), and 8.35 (1H, d, J = 5.2 Hz, H-3); EIMS *m/z* 780 (M^+) and 547; HREIMS *m/z* 780.3865 (M^+), calcd for $C_{46}H_{51}F_3N_4O_4$, 780.3862.

Compound **1** (0.5 mg) was treated with (*S*)-(+)-MTPA chloride (5 μL) by a procedure similar to that described above to afford the (*R*)-MTPA ester (**4b**, 0.5 mg) of **1**. Compound **4b**: ^{1}H NMR (CD_3OD) δ 1.4−2.9 (25H, m), 3.51 (3H, s, OMe), 4.10 (1H, s, H-25), 4.19 (1H, m, H-34), 5.40 (1H, m, H-16), 5.47 (1H, m, H-33), 5.55 (1H, m, H-14), 6.03 (1H, m, H-15), 6.30 (1H, br t, J = 9.8 Hz, H-32), 6.53 (1H, s, H-12), 7.25−7.64 (8H, m, Ph and H-6, H-7, and H-8), 8.01 (1H, d, J = 5.2 Hz, H-4), 8.22 (1H, d, J = 7.2 Hz, H-5), and 8.35 (1H, d, J = 5.2 Hz, H-3); EIMS *m/z* 780 (M^+) and 547; HREIMS *m/z* 780.3846 (M^+), calcd for $C_{46}H_{51}F_3N_4O_4$, 780.3862.

DDQ Oxidation of 3,4-Dihydromanzamine J (2). 3,4-Dihydromanzamine J (**2**, 0.3 mg) in $CHCl_3$ (0.3 mL) and EtOH (0.3 mL) was treated with DDQ (0.5 mg) at room temperature for 1 h. The reaction mixture was subjected to a Sep-pak NH_2 cartridge (hexane−EtOH, 9:1) to afford manzamine J (**6**, 0.2 mg), of which

physicochemical data were identical to those of authenthic manzamine J in our laboratory.

DDQ Oxidation of 3,4-Dihydro-6-hydroxymanzamine A (3). Compound **3** (0.3 mg) was treated with DDQ (0.5 mg) in the same way as described above to give 6-hydroxymanzamine A (**7**, 0.2 mg), of which physicochemical data were identical to those of authenthic 6-hydroxymanzamine A in our laboratory.

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