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Ma'eganedin A, a New Manzamine Alkaloid from *Amphimedon* Sponge

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Abstract: A new manzamine-related tetrahydro-β-carboline alkaloid with a methylene carbon bridge between N-2 and N-27, ma'eganedin A (**1**), has been isolated from an Okinawan marine sponge *Amphimedon* sp., and the structure including absolute stereochemistry was elucidated on the basis of spectroscopic data. The most stable conformation of **1** was deduced from molecular mechanics calculation.

During our search for new manzamine-related alkaloids from Okinawan marine sponges,[1] we have investigated extracts of the Okinawan marine sponge *Amphimedon* sp., which resulted in isolation of ma'eganedin A (**1**), a unique tetrahydro-β-carboline alkaloid with a methylene carbon bridge between N-2 and N-27 in addition to the same backbone ring system as that of manzamine B.[2] In this paper we describe the isolation, structure elucidation, and stable conformation analysis of **1**.

The sponge *Amphimedon* sp.[3] collected off Kerama Islands, Okinawa, was extracted with MeOH. EtOAc-soluble materials of the MeOH extract were purified by silica gel and alumina column chromatographies to afford ma'eganedin A (**1**, 9 x 10^{-4} %, wet weight) together with several known manzamine alkaloids such as manzamines A[4,5] and B.[2]

EIMS data of ma'eganedin A[6] {**1**, $[\alpha]_D^{25}$ +47° (*c* 0.40, MeOH)} showed only an ion peak at *m/z* 566 [$(M-H_2O)^+$], and the molecular ion peak was not observed in FABMS, FDMS, or ESIMS as well as EIMS. HRFABMS [*m/z* 567.4067, $(M-H_2O+H)^+$, Δ + 0.4 mmu] data of **1** indicated the molecular formula to be $C_{37}H_{52}N_4O_2$. ^1H NMR data (Table 1) including aromatic proton resonances [δ_H 7.49 (H-5), 7.08 (H-6),

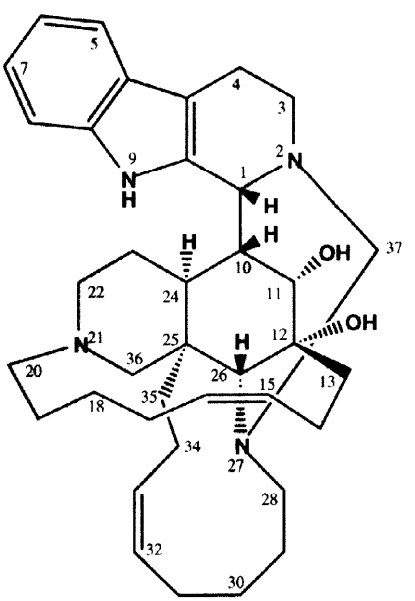

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Table 1. ^1H and ^{13}C NMR Data of Ma'eganedin A (1) in CD_3OD.

positn	δ_H[a]	m;J (Hz)	δ_H[a]	m;J (Hz)	δ_C[b]	m	J_{CH}(Hz)[c]	HMBC (H)
1	4.68	brs			57.9	d	143	3α, 37
3	3.45[d]	brdd; 2.7, 13.7	3.28[e]	ddd; 3.4, 8.1, 13.7	54.7	t	137	37β
4	3.03[d]	m	2.69[e]	brdd; 3.4, 16.2	19.4	t	127	
4a					112.2	s		3β, 4, 5
4b					129.2	s		6, 8
5	7.49	d; 7.8			119.5	d	158	7
6	7.08	dd; 7.2, 7.8			121.0	d	163	8
7	7.18	dd; 7.2, 8.1			123.6	d	158	5
8	7.43	d; 8.1			112.8	d	163	6
8a					138.5	s		5, 7
9	11.7[f]	brs						
9a					132.1	s		1, 4β
10	3.31	m			40.0	d	127	1, 23α
11	4.40	d; 5.5			68.1	d	158	26
12					75.7	s		11[f], OH-12[f]
OH-12	10.9[f]	brs						
13	2.52[e]	m	1.73[d]	m	45.5	t		14, 26
14	2.54	m	2.51	m	22.5	t		
15	5.56	m			131.2	d	158	
16	5.69	m			131.8	d	153	
17	2.48	m	2.27	m	29.1	t		
18	1.34	m	1.29	m	24.7	t		
19	2.25	m	2.07	m	27.3	t		20β
20	3.96[e]	brt; 13.2	2.97[d]	m	60.4	t	142	36β
22	4.08[e]	brdd; 11.5, 9.0	3.24[d]	dd; 3.5, 11.5	57.3	t	148	20β, 24, 36α
23	2.13[e]	m	1.98[d]	m	24.9	t		
24	1.84	brd; 4.3			34.1	d		11
25					43.0	s		26, 35b[g]
26	3.44	s			73.5	d	137	11, 36α, 37β
28	3.57	dd; 5.6, 14.0	2.99	m	53.5	t	132	26, 37
29	1.93	m	1.44	m	26.6	t		
30	1.62	m	1.24	m	27.3	t		31a[g]
31	2.30	m	1.63	m	25.2	t		33
32	5.62	m			133.7	d	157	34a[g]
33	5.62	m			130.4	d	163	34a[g], 35b[g]
34	2.33	m	1.59	m	21.0	t		32, 33, 35
35	1.57	m	1.36	m	33.5	t		36β
36	3.39[e]	brd; 13.0	3.13[d]	d; 13.0	64.7	t	132	22β, 24, 26, 35b[g]
37	4.02[d]	d; 15.5	3.65[e]	d; 15.5	60.2	t	143	3β, 26, 28

[a] Recorded at 600 MHz. [b] Recorded at 125 MHz. [c] $^1J_{CH}$ values were determined from HMQC experiment without CPD decoupling during acquisition, and the resolution for F1 was 5.09 Hz. [d] αH. [e] βH. [f] Recorded in $CDCl_3$. In the ^1H NMR spectrum in $CDCl_3$, OH proton at C-11 was not observed. [g] a and b denote upfield and downfield resonances of geminal protons, respectively.

7.18 (H-7), and 7.43 (H-8)] of **1** were similar to those of manzamine alkaloids with a tetrahydro-β-carboline ring such as manzamine H.[1,7] In the ^{13}C NMR (Table 1) spectrum of **1**, totally 37 carbon signals including six quaternary carbons (four sp^2 and two sp^3), thirteen methines (eight sp^2 and five sp^3), and eighteen methylenes were observed, although manzamine H has 36 carbons. Detailed analyses of 2D NMR data [^1H-^1H COSY, HOHAHA, HMQC, and HMBC (Table 1)] of **1** revealed the presence of a decahydroisoquinoline moiety connected to a tetrahydro-β-carboline and 11- and 13-membered rings, consisting of 36 carbon

atoms. The remaining methylene carbon signal at δ_C 60.2 was assigned to be located between N-2 and N-27 on the basis of HMBC correlations for H_2-37/C-1, H-37β/C-3, H-37β/C-26, and H_2-37/C-28. The presence of the two hydroxy groups at C-11 and C-12 was inferred by the upfield chemical shifts of C-11 (δ_H 4.40; δ_C 68.1) and C-12 (δ_C 75.7) as well as the ^{13}C NMR deuterium-induced shift experiments using CD_3OH, which was also supported by positive coloring test to lead tetracetate. Thus the gross structure of ma'eganedin A was elucidated to be **1**.

The relative stereochemistry of ma'eganedin A (**1**) as well as both boat conformations of the cyclohexane (C-10–C-11,12,26,25,24) and piperidine (N-21–C-22,23,24,25,36) rings were deduced from NOESY data and ^1H-^1H coupling constants as shown in Fig. 1. The chemical shift of C-13 (δ_C 45.5) was close to the chemical shifts of C-13 (aprproximatly δ_C 40) of manzmaines possessing an α-hydroxy group at C-12,[2,7,8] thus indicating that **1** had an α-hydroxy group at C-12. The small ^1H-^1H coupling constant (< 1 Hz) for H-1/H-10 and NOESY correlations for H-1/H-10 and H-1/H-11 suggested that H-1 was β-oriented. *Cis*-ring junction between the cyclohexene and piperidine was indicated by a NOESY correlation for H-24/H-35b. Compound **1** showed a positive CD cotton effect (Δε +19.3) at 222 nm, implying *R*-configuration at C-1 of the tetrahydro-β-carboline ring.[1,9] Thus the absolute stereochemistry of **1** was elucidated to be 1*R*, 10*R*, 11*S*, 12*R*, 24*S*, 25*R*, and 26*R*.

The most stable conformation (total energy; 61.3 kcal/mol) of ma'eganedin A (**1**) (Fig. 2) was obtained by systematic conformational searching using pseudo Monte Carlo simulation[10,11] in MacroModel ver. 5.0 program.[12] The distances of OH-12/N-2 (2.29 Å) and OH-12/N-27 (3.15 Å) in the conformation suggested the presence of two hydrogen bonds between OH-12 and N-2 and between OH-12 and N-27, which might be associated with the low-field chemical shift (δ_H 10.9) of the hydroxy proton at C-12 observed for the ^1H NMR spectrum of **1** in $CDCl_3$. Comparison of the stable conformation of **1** with that of manzamine B (total energy; 107.9 kcal/mol) revealed that the tosion angle (-170°) between C-9a–C-1 and C-10–C-11 bonds in **1** was quite different from that (52°) of manzamine B as shown in Fig. 2. This difference may be mainly derived from the fact that orientation of the tetrahydro-β-carboline moiety in **1** was fixed by the C-37 carbon

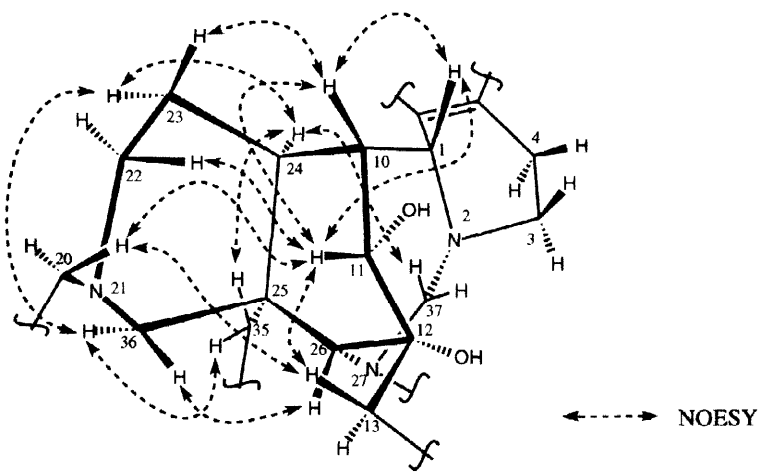

Fig. 1 Relative Stereochemistry of Tetracyclic System of Ma'eganedin A (**1**) Elucidated by NOESY Data and Proton Couplings.[a]

The coupling constants for this moiety (H/H in Hz) are as follows: 1/10 (<1), 3α/4α (2.7), 3α/4β (<1), 3β/4α (8.1), 3β/4β (3.4), 10/11 (5.5), 10/24 (<1), 23α/24 (4.3), 23β/-24 (<1), 22α/23α (3.5) 22α/23β (<1), 22β/23α (9.0), and 22β/23β (<1).

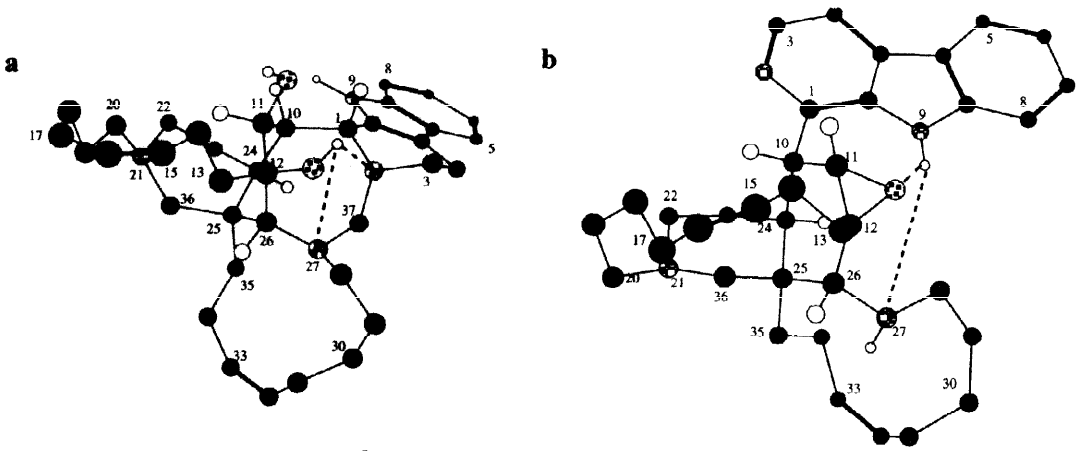

Fig. 2 Most Stable Conformation[a] of Ma'eganedin A (**1**) (**a**) and Manzamine B (**b**) Calculated by MacroModel Program.

[a]Methylene and olefin protons were omitted and dotted lines suggested the presence of hydrogen bonds.

bridge between N-2 and N-27.

Ma'eganedin A (**1**) is the first manzamine alkaloid with a methylene carbon bridge between N-2 and N-27. Compound **1** exhibited antibacterial activity against *Sarcina lutea* (MIC; 2.8 μg/mL), *Bacillus subtilis* (2.8 μg/mL), and *Corynebacterium xerosis* (5.7 μg/mL), and showed cytotoxicity against murine leukemia L1210 cells (IC_{50}, 4.4 μg/mL).

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