A NEW MANZAMINE CONGENER FROM MARINE SPONGE *AMPHIMEDON* SP.

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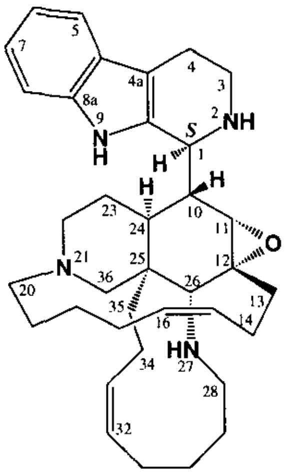
Abstract—A new manzamine congener, 1,2,3,4-tetrahydromanzamine B (**1**), has been isolated from a Okinawan marine sponge *Amphimedon* sp. and the structure including absolute stereochemistry was elucidated from spectroscopic data.

During our continuing search for manzamine-related alkaloids from marine sponges,[1] a new manzamine congener, 1,2,3,4-tetrahydromanzamine B (**1**), has been isolated from the Okinawan marine sponge *Amphimedon* sp. In this paper we describe the isolation and structure elucidation of **1**.

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



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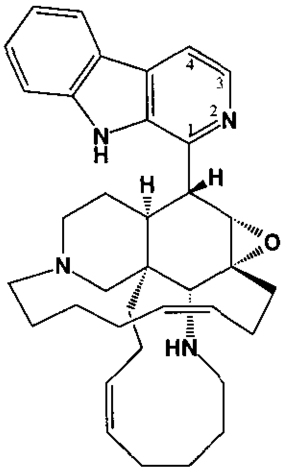

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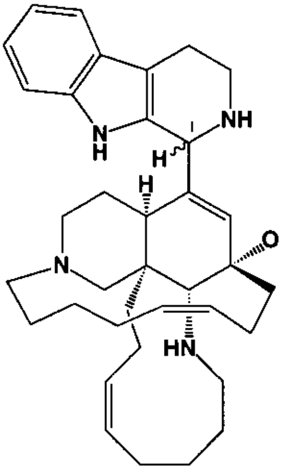
Table 1. ^{1}H and ^{13}C NMR Data of 1,2,3,4-Tetrahydromanzamine B (1) in CD_3OD.

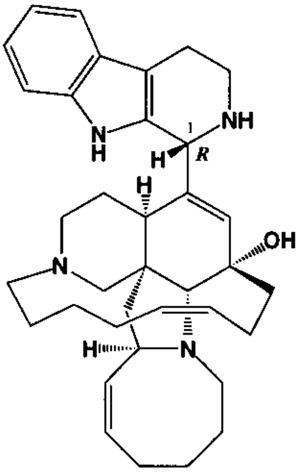
positn.	δ_H	m	J (Hz)	δ_C	m	positn.	δ_H	m	J (Hz)	δ_C	m
1	4.57	brs		55.3	d	19	1.65	m		28.9	t
3	2.91	m		37.5	t		1.55	m			
	2.72	m				20	2.76[a]	m		49.4	t
4	1.74	m		23.4	t	22	3.52[a]	m		49.0	t
	1.68	m				23	2.95	m		23.7	t
4a				109.1	s		2.85	m			
4b				125.9	s	24	1.74	m		35.5	d
5	7.50	d	7.7	118.8	d	25				40.9	s
6	7.07	dd	7.7, 7.1	121.0	d	26	2.72	s		59.8	d
7	7.17	dd	7.1, 8.2	124.2	d	28	2.77	m		48.8	t
8	7.40	d	8.2	122.6	d		2.85	m			
8a				137.2	s	29	1.72	m		29.5	t
9a				134.1	s		1.62	m			
10	2.88	brs		44.2	d	30	1.70	m		28.8	t
11	3.00	brs		61.5	d		1.58	m			
12				57.5	s	31	2.38	m		27.2	t
13	2.45	m		39.7	t		2.23	m			
	1.35	m				32	5.64	dd	10.9, 7.1	131.7	d
14	2.45	m		25.0	t	33	5.74	brt	8.8	131.4	d
	2.35	m				34	1.78	m		20.9	d
15	5.32	dd	14.8, 8.3	129.9	d		1.74	m			
16	5.37	dd	14.8,	132.3	d	35	2.19	m		34.1	t
17	2.93	m		29.1	t		1.68	m			
	2.90	m				36	3.66	m		49.1	t
18	2.37[a]	m		25.1	t		2.95	m			

[a] 2H.

The molecular formula of compound (1) {$[\alpha]_D^{25}$ -16° (c 0.14, MeOH)} was established as $C_{36}H_{50}N_4O$ by HREIMS (m/z 554.3998, M^+, Δ +1.4 mmu). The ^{1}H and ^{13}C NMR data (Table 1) were similar to those of manzamine B[4] (2), indicating that **1** had the same pentacyclic decahydroisoquinoline moiety as **2**. The presence of an epoxide ring on the C-11–C-12 bond was suggested by the ^{13}C chemical shifts at C-11 (δ_C 61.5) and C-12 (δ_C 57.5) as well as the $J_{C\text{-}H}$ value (182 Hz) at C-11. The ^{13}C chemical shifts (δ_C 55.3, C-1; δ_C 37.5, C-3; δ_C 23.4, C-4; δ_C 109.1, C-4a; δ_C 125.9, C-4b; δ_C 118.8, C-5; δ_C 121.0, C-6; δ_C 124.2, C-7; δ_C 122.6, C-8; δ_C 137.2, C-8a; δ_C 134.1, C-9a) for the 1,2,3,4-tetrahydro-β-carboline moiety corresponded to those of manzamine H[5] (3) and L[6] (4). DDQ oxidation of **1** gave manzamine B (**2**), indicating that the absolute configurations at C-10, C-11, C-12, C-24, C-25, and C-26 of **1** were the same as those of **2**. Compound (**1**) showed a negative CD Cotton effect ($\Delta\varepsilon$ -19.0) at 222 nm, implying *S*-configuration at C-1 of the tetrahydro-β-carboline ring.[7] Thus compound (**1**) was elucidated to be (1*S*)-1,2,3,4-tetrahydromanzamine B.



3 : 1*R*
4 : 1*S*



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Several manzamine congeners possessing a 1,2,3,4-tetrahydro-β-carboline ring have been reported.[5,6,8-11] Manzamine D[5] (**5**), tetrahydro form of manzamine A, and had 1*R*-configuration, while configurations at C-1 of manzamines H[5] (**3**) and L[6] (**4**), possessed *R* and *S*, respectively. From this *Amphimedon* sponge, only 1*S*-form (**1**) of tetrahydromanzamine B was isolated. 1,2,3,4-Tetrahydromanzamine B (**1**) exhibited cytotoxicity against L1210 murine leukemia cells and KB human epidermoid carcinoma cells *in vitro* with IC_{50} values of 0.3 and 1.2 μg/mL, respectively.

EXPERIMENTAL SECTION

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

Collection, Extraction, and Isolation. The medium brown color sponge *Amphimedon* sp. (order Haplosclerida; family Niphatidae) was collected off Okinawa Island, and kept frozen until used. The voucher specimen (SS-932) was deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University. The sponge (1.3 kg, wet weight) was extracted with MeOH (1.3 L x 2) for 30 min at rt. The methanolic extract (89 g) was partitioned between ethyl acetate (500 mL x 3) and H_2O. Part (3.2 g) of the EtOAc soluble material (5.05 g) was subjected to a SiO_2 column ($CHCl_3$ → $CHCl_3$/MeOH 70:30). The fraction eluting with $CHCl_3$/MeOH (70:30) was separated by an alumina column chromatography ($CHCl_3$ and then MeOH). The $CHCl3$ eluate (18 mg) was purified by silica gel column chromatographies (cyclohexane/acetone/Et_2NH, 30:70:2 and then $CHCl_3$/MeOH, 1:1) to yield (1*S*)-1,2,3,4-tetrahydromanzamine B (**1**, 3.8 mg, 5 x 10^{-4} % wet weight).

(1*S*)-1,2,3,4-Tetrahydromanzamine B (1). A colorless amorphous solid; $[\alpha]_D^{25}$ -16° (*c* 0.14, MeOH); UV (MeOH) λ_{max} 225 (ε 9700), 273 (2500), 280 (2600), and 289 nm (2100); CD (MeOH) λ_{ext} 222 ($\Delta\varepsilon$ -19.0) and 269 nm (+3.8); IR (KBr) ν_{max} 2920, 2850, 1640, and 1060 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); EIMS *m/z* 554 (M$^+$); HREIMS *m/z* 554.3998 (M$^+$), calcd for $C_{36}H_{50}N_4O$, 554.3984.

DDQ Oxidation of (1*S*)-1,2,3,4-Tetrahydromanzamine B (1). (1*S*)-1,2,3,4-Tetrahydromanzamine B (**1**, 0.5 mg) in $CHCl_3$ (0.8 mL) and EtOH (0.3 mL) was treated with DDQ (0.5 mg) at rt for 1 h. The reaction mixture was subjected to a Sep-Pak NH_2 cartridge (hexane/EtOH, 8:2) to afford manzamine B (**2**, 0.2 mg, 40 %), of which physicochemical data were identical with those of natural manzamine B (**2**).

ACKOWLEDGMENTS

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REFERENCES

1. D. Watanabe, M. Tsuda, and J. Kobayashi, *J. Nat. Prod.*, 1998, **61**, 689 and references cited therein.
2. R. Sakai, T. Higa, C. W. Jefford, and G. Bernardinelli, *J. Am. Chem. Soc.*, 1986, **108**, 5404.
3. H. Nakamura, S. Deng, J. Kobayashi, Y. Ohizumi, Y. Tomotake, T. Matsuzaki, and Y. Hirata, *Tetrahedron Lett.*, 1987, **28**, 621.
4. R. Sakai, S. Kohmoto, T. Higa, C. W. Jefford, and G. Bernardinelli, *Tetrahedron Lett.*, 1987, **28**, 5493.
5. K. Kondo, H. Shigemori, Y. Kikuchi, M. Ishibashi, T. Sasaki, and J. Kobayashi, *J. Org. Chem.*, 1992, **57**, 2480.
6. M. Tsuda, K. Inaba, N. Kawasaki, K. Honma, and J. Kobayashi, *Tetrahedron*, 1996, **52**, 2319.
7. J. Stöckigt and M. H. Zenk, *J. Chem. Soc., Chem. Commun.*, 1977, 646.
8. M. Tsuda, N. Kawasaki, and J. Kobayashi, *Tetrahedron Lett.*, 1994, **35**, 4387.
9. P. Crews, X.-C. Cheng, M. Adamczeski, J. Rodrîguez, M. Jaspers, F. J. Schmitz, S. C. Traeger, and E. O. Pordesimo, *Tetrahedron*, 1994, **50**, 13567.
10. I. I. Ohtani, T. Ichiba, M. Isobe, M. Kelly-Borges, and P. J. Scheuer, *J. Am. Chem. Soc.*, 1995, **117**, 10743.
11. M. Tsuda, D. Watanabe, and J. Kobayashi, *Tetrahedron Lett.*, 1998, **39**, 1207.

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