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Ircinols A and B, First Antipodes of Manzamine-Related Alkaloids from an Okinawan Marine Sponge

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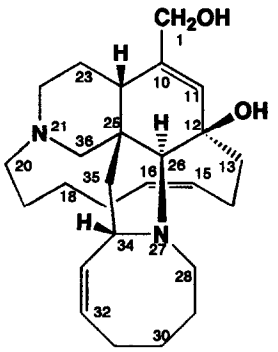
Abstract: Two new manzamine-related alkaloids, ircinols A (**1**) and B (**2**), have been isolated from the Okinawan marine sponge *Amphimedon* sp. and the structures elucidated to be enantiomers of alcoholic forms at C-1 of ircinals A and B, respectively, on the basis of extensive 2D NMR data and chemical correlations. Compounds **1** and **2** are the first examples possessing opposite absolute configurations to those of manzamine alkaloids.

The manzamines[1~5] are a series of β-carboline alkaloids substituted at C-1 by unusual heterocycles from marine sponges. Among the manzamine alkaloids the most intricate manzamine A[1,2] has a pyrrolo[2,3-*i*]isoquinoline framework associated with 8- and 13-membered rings. In our search for biogenetic precursors of the manzamines, two novel manzamine-related alkaloids, ircinals A and B,[6] have been isolated from an Okinawan marine sponge of the genus *Ircinia*. Biosynthetically manzamines A and B may be derived from coupling of tryptamine with aldehydes such as ircinals A and B, respectively. Further search for biogenetic precursors of the manzamines resulted in the isolation of two new alkaloids, designated ircinols A (**1**) and B (**2**), having opposite absolute configurations to those of ircinals A and B, respectively, from the Okinawan marine sponge *Amphimedon* sp. Here we describe the isolation and structure elucidation of **1** and **2**.

The sponge *Amphimedon* sp. was collected off the Kerama Islands, Okinawa, and kept frozen until used. The methanolic extract of the sponge was partitioned between ethyl acetate and water. The ethyl acetate soluble material was subjected to silica gel column chromatography. A fraction eluted with



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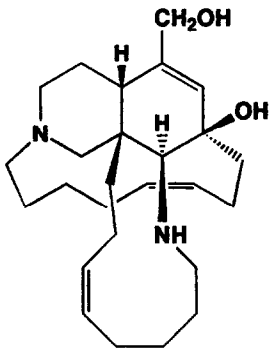

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Table. 1 The ^1H and ^{13}C NMR Data of Ircinols A (**1**)[a] and B (**2**)[b].

Positn.	1 δ_H	δ_C	HMBC (H)	2 δ_H	δ_C
1	4.00[c], s	66.0	11, 24	3.88[c], s	65.2
10		144.3	1, 24		143.8
11	5.68, s	130.4	1, 24, 26	5.70, s	126.8
12		71.6	26		70.6
13	1.80, m	42.1	26	2.18, brt (10.8)	41.2
	1.61, m			1.64, m	
14	2.26, m	23.0	13, 15, 16	2.15, m	22.6
	1.96, m			1.91, m	
15	5.49, m	129.1	14, 17	5.42, m	130.5
16	5.52, m	134.6	14, 17	5.29, m	128.9
17	2.52, m	27.1	15	2.98, brt (12.7)	30.0
	1.63, m			1.74, m	
18	1.42, m	28.6	17, 20	1.65, m	29.6
	1.22, dt (9.2, 4.3)			1.19, m	
19	1.77, m	26.9		1.36, m	25.5
	1.46, m			1.34, m	
20	2.67, dt (5.2, 12.0)	55.0		2.49, dt (12.1, 1.3)	59.4
	2.34, m			2.25, ddd (4.6, 11.6, 16.9)	
22	2.87, dd (5.5, 11.3)	51.2	20, 23, 36	2.77, dd (4.7, 9.4)	49.9
	1.83, m			1.74, m	
23	1.90, m	33.6	24	1.68, m	30.2
	1.41, m			1.59, m	
24	2.05, dd (6.8, 12.0)	39.9	1, 11, 22, 35	1.71, m	44.1
25		48.7	23, 24, 35, 36		43.9
26	3.59, s	78.8	11, 13, 24, 36	3.66, s	60.3
28	3.56, dt (12.7, 7.0)	54.5	26	3.00, m	52.4
	3.32[d], m			2.90, m	
29	1.98, m	30.1	31	1.61, m	29.4
	1.58, m			1.47, m	
30	1.77, m	26.5	31	1.65, m	29.7
	1.45, m			1.34, m	
31	2.36, m	29.7	30, 33	2.55, m	23.0
	2.24, m			1.94, m	
32	6.18, dt (10.7, 7.1)	141.5		5.32, brt (10.1)	130.9
33	5.40, dd (10.7, 9.4)	127.6	31, 35	5.54, brt (10.8)	132.0
34	4.52, brt (7.9)	58.8	33, 35	1.99, m	29.7
				1.96, m	
35	2.11, dd (8.0, 13.6)	46.3	26	2.23	37.5
	1.71, m			1.08, brt (12.7)	
36	2.94, brd (11.5)	71.3	22, 35	3.30, brd (11.9)	65.3
	2.29, d (11.5)			1.84, d (11.9)	

a) in CD_3OD. b) in $CDCl_3$. c) 2H. d) This signal was overlapped in MeOH signal.

$CHCl_3$/MeOH (85:15) was further purified by a silica gel column (hexane/acetone/Et_2NH, 90:10:2) to afford ircinol A (**1**, 0.004 %, wet weight). The fraction eluted with $CHCl_3$/MeOH (95:5) from the first silica gel column was separated by a silica gel column (cyclohexane/acetone/Et_2NH, 80:20:2 and then hexane/EtOAc/Et_2NH, 80:20:2) to yield ircinol B (**2**, 0.0003 %) together with known compounds,[7] manzamines A,[1,2] B,[3] and C,[3] and ircinals A and B.[6]

The molecular formula, $C_{26}H_{40}N_2O_2$, of ircinol A (**1**)[8] was established by the HREIMS (*m/z* 412.3090, M^+, Δ +1.7 mmu). The ^1H and ^{13}C NMR data (Table 1) of **1** were similar to those of ircinal A except for the presence of an oxymethylene (δ_H 4.00, 2H; δ_C 66.0) for **1** in place of a formyl group (δ_H 9.45; δ_C 193.3) for ircinal A. From the molecular weight, which was larger than that of ircinal A by two

Daltons, in combination with the 2D NMR data {^{1}H-^{1}H COSY, HOHAHA, HMQC, HMBC (Table 1), and NOESY} of **1**, the structure of ircinol A was concluded to be **1**, corresponding to the alcoholic form at C-1 of ircinal A. Treatment of ircinal A, which was isolated from this sponge, with DIBALH afforded a reductive product, the spectral data of which were identical with those of ircinol A (**1**) except for the optical rotation [reductive product of ircinal A, $[\alpha]_D^{18}$ +20° (*c* 0.2, MeOH); **1**, $[\alpha]_D^{18}$ -19° (*c* 0.5, MeOH)]. This result revealed that ircinol A (**1**) was an enantiomer of the alcoholic derivative of ircinal A which has been shown to have the same absolute configuration as that of manzamine A.[6]

HREIMS data of ircinol B (**2**)[8] provided the molecular formula, $C_{26}H_{42}N_2O_2$, which corresponded to that of **1** plus two hydrogen atoms. Although the ^{1}H and ^{13}C NMR data (Table 1) of **2** were similar to those of **1**, **2** lacked the C–N bond between C-34 and N-27 as shown by the presence of a methylene signal (δ_C 29.7) at C-34. The structure of ircinol B (**2**) was confirmed by the following chemical correlation with ircinal B from this sponge. Reduction of ircinal B with DIBALH yielded a reductive product, the spectral data of which were identical with those of ircinol B (**2**) except for the optical rotation [reductive product of ircinal B, $[\alpha]_D^{18}$ +4.2° (*c* 0.2, MeOH); **2**, $[\alpha]_D^{18}$ -2.8° (*c* 0.12, MeOH)]. Thus the absolute stereochemistry of ircinol B was concluded as shown in structure **2**.

Ircinols A (**1**) and B (**2**) are antipodes of the alcoholic forms of ircinals A and B, respectively, and are the first alkaloids having opposite absolute configurations to those of the manzamines reported so far. Ircinols A (**1**) and B (**2**) are considered to be compounds parallel to ircinals A and B, respectively, in the biogenetic path of manzamine alkaloids proposed by Baldwin *et al.*[9] Although there is an example of isolation of antipodal alkaloids from marine sponges of different genera,[10,11] it is very rare that both enantiomeric forms have been simultaneously isolated from the same organism.[12] Ircinols A (**1**) and B (**2**) were cytotoxic against L1210 cells (IC_{50} values: 2.4 and 7.7 μg/mL, respectively) and KB cells (IC_{50} values: 6.1 and 9.4 μg/mL, respectively). Compound **1** showed inhibitory activity against endothelin converting enzyme (IC_{50}: 55 μg/mL).

EXPERIMENTAL

Collection, Extraction, and Isolation. The sponge *Amphimedon* sp. was collected off the Kerama Islands, Okinawa, and stored frozen until used. The sponge (1.5 kg) was extracted with methanol (1 L x 2) and the extract was partitioned between ethyl acetate (300 mL x 3) and water (500 mL). A portion (0.92 g) of the ethyl acetate soluble material (3.98 g) was chromatographed on a silica gel column with $CHCl_3$/MeOH to afford two fractions a and b. Fraction a (56 mg) eluted with $CHCl_3$/MeOH (85:15) was further purified on a silica gel column with hexane/acetone/Et_2NH (90:10:2) to give ircinol A (**1**, 14.2 mg, 0.004 %, wet weight). Fraction b eluted with $CHCl_3$/MeOH (95:5) was purified by silica gel column chromatography (cyclohexane/acetone/Et_2NH, 80:20:2 and then hexane/EtOAc/Et_2NH, 80:20:2) to yield ircinol B (**2**, 1.2 mg, 0.0003 %) together with manzamines A (130 mg, 0.035 %), B (12 mg, 0.0032 %), and C (8 mg, 0.002 %), and ircinals A (3.4 mg, 0.0009 %) and B (0.6 mg, 0.0002 %).

Ircinol A (1). A colorless amorphous solid; mp 83 ~ 85 °C; $[\alpha]_D^{18}$ -19° (*c* 0.54, MeOH); IR (KBr) ν_{max} 3400 and 2940 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); EIMS *m/z* 412 (M^+), 394, and 162; HREIMS *m/z* 412.3107 (M^+, calcd for $C_{26}H_{40}N_2O_2$, 412.3090).

Ircinol B (2). A colorless amorphous solid; mp 78 ~ 79 °C; $[\alpha]_D^{18}$ -2.8° (*c* 0.12, MeOH); IR (KBr) ν_{max} 3400 and 2940 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* 414 (M^+), 397, and 164; HREIMS *m/z* 414.3248 (M^+, calcd for $C_{26}H_{42}N_2O_2$, 414.3246).

Reduction of Ircinal A. To a THF solution (1.5 mL) of ircinal A (5.2 mg,13 μmol), 150 mM toluene solution of DIBALH (200 μL, 30 μmol) was added at -78 °C, and stirring was continued for 3 h. After addition of MeOH (3 μL), ether (70 μL) and saturated aqueous potassium sodium tartrate (500 μL), the mixture was stirred at room temperature for 1 h. After extraction with EtOAc (3 mL x 3), the organic phase was washed with brine and concentrated under reduced pressure to afford a residue, which was purified on a silica gel column ($CHCl_3$/MeOH, 95:5) to give a reductive product (2.2 mg): a colorless amorphous solid; $[\alpha]_D^{18}$ +20° (*c* 0.2, MeOH); IR (KBr) ν_{max} 3400 and 2940 cm^{-1}; ^1H NMR (CD_3OD) δ 6.20 (1H, m), 5.69 (1H, s), 5.54 ~ 5.46 (2H, m), 5.41 (1H, m), 4.00 (2H, s), 3.60 (1H, s), 3.57 (1H, m), 3.27 (1H, m), and 3.0 ~ 1.1 (28H, m); EIMS *m/z* 412 (M^+), 394, and 162; HREIMS *m/z* 412.3112 (M^+, calcd for $C_{26}H_{40}N_2O_2$, 412.3090).

Reduction of Ircinal B. Ircinal B (4.1 mg, 10 μmol) was treated by the same procedure as described above to give a reductive product (1.9 mg): a colorless amorphous solid; $[\alpha]_D^{18}$ +4.2° (*c* 0.2, MeOH); IR (KBr) ν_{max} 3400 and 2940 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.70 (1H, s), 5.54 (1H, m), 5.42 (1H, m), 5.35 ~ 5.25 (2H, m), 3.86 (2H, s), 3.66 (1H, s), 3.32 (1H, d, *J* = 12.0 Hz), 3.00 (1H, m), 2.98 (1H, m) and 2.95 ~ 1.0 (29H, m); EIMS *m/z* 414 (M^+), 397, and 164; HREIMS *m/z* 414.3219 (M^+, calcd for $C_{26}H_{42}N_2O_2$, 414.3246).

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