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Chiral Resolution of (±)-Keramaphidin B and Isolation of Manzamine L, a New β-Carboline Alkaloid from a Sponge *Amphimedon* sp.

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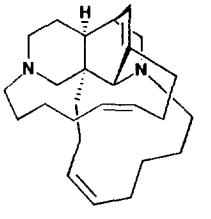
Abstract: Both enantiomers of keramaphidin B (**1**) were separated by using chiral HPLC, of which one may be a plausible biogenetic precursor of ircinals A (**4**) and B (**5**) and manzamines A (**2**) and B (**3**), while the other may be associated with antipodes of manzamine alkaloids such as ircinols A (**6**) and B (**7**). Isolation and structure elucidation of a new manzamine congener, manzamine L (**8**), and absolute configurations at C-1 of **8** and manzamines D (**10**) and H (**9**) deduced from the Cotton effects are also described.

Keramaphidin B[1] (**1**) is a unique pentacyclic alkaloid with an unprecedented skeleton isolated from an Okinawan marine sponge *Amphimedon* sp., while several alkaloids with similar skeletons to that of **1** such as ingenamine,[2] ingenamines B ~ F,[3] ingamines A and B,[4] and xestocyclamines A[5] and B[6] from sponges of genus *Xestospongia* have been recently reported. These sturctures are very close to those of the corresponding biogenetic intermediate of manzamines A[7] (**2**) and B[8] (**3**) proposed by Baldwin and Whitehead,[9] in which a *bis*-3-alkyldihydropyridine macrocycle (**a**) may be converted through a Diels-Alder-type [4+2] intramolecular cycloaddition into a pentacyclic intermediate like **1**, which turns out manzamines A (**2**) and B (**3**) via a tetracyclic intermediate such as ircinals A (**4**) and B (**5**).[10] This might be followed by that manzamines A (**2**) and B (**3**) and keramaphidin B (**1**) possess the same absolute configurations, and indeed, ircinals A (**4**) and B (**5**) were found to have the same absolute configurations as those of manzamines A (**2**) and B (**3**). However, a small amount of crystals of keramaphidin B (**1**) employed for X-ray analysis was revealed to be racemic[1] though it possessed four asymmetric centers, and ircinols A (**6**) and B (**7**) were found to have opposite configurations concerning the four asymmetric centers of manzamines and ircinals.[11] On the other hand, ingenamine, ingamine A, and ingenamine E were reported to be antipodal to manzamines and ircinals.[3]



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In this paper we describe the separation of (+)- and (-)-keramaphidin B (**1**) using chiral HPLC, the isolation and structure elucidation of a new manzamine congener, manzamine L (**8**), from a sponge *Amphimedon* sp., and a plausible biogenetic path of manzamines A (**1**) and B (**2**) on the basis of the structures and ratios of all the manzamine-related alkaloids isolated from this sponge.

EtOAc and *n*-BuOH soluble materials of MeOH extracts of the sponge *Amphimedon* sp. collected off Kerama Islands, Okinawa were purified by silica gel columns to give keramaphidins B (**1**, 0.0054 %, wet weight, $[\alpha]_D^{20}$ +22.2°) and C^{12} (0.0022 %), manzamines A (**2**, 0.081 % $[\alpha]_D^{20}$ +46°), B (**3**, 0.0072 %, $[\alpha]_D^{20}$ +93°), C^{8} (0.002%), and $D^{10,13}$ (**10**, 0.012 %, $[\alpha]_D^{24}$ +44°), ircinals A (**4**, 0.0009 %, $[\alpha]_D^{15}$ +42°) and B (**5**, 0.0006 %, $[\alpha]_D^{15}$ +15°), keramamine C^{12} (0.0026 %), ircinols A (**6**, 0.006 %, $[\alpha]_D^{18}$ -19°) and B (**7**, 0.0003 %, $[\alpha]_D^{18}$ -2.8°), 6-hydroxymanzamine A^{14} (= manzamine Y^{15}, 0.005 %, $[\alpha]_D^{20}$ +139°), 3,4-dihydromanzamine A^{14} (0.002 %, $[\alpha]_D^{20}$ +86°), and 8-hydroxymanzamine A^{16} (= manzamine G^{17}, 0.002%, $[\alpha]_D^{20}$ +118°). Further separation of *n*-BuOH soluble materials with silica gel columns ($CHCl_3$/MeOH and cyclohexane/acetone/Et_2NH) and then silica gel HPLC (hexane/*i*-PrOH/Et_3N) afforded manzamine L (**8**, 0.0056 %, $[\alpha]_D^{24}$ -15°) together with manzamine H^{10} (**9**, 0.0028 %, $[\alpha]_D^{24}$ +21°).

Keramaphidin B (**1**) was optically active as mentioned above, while a small amount of crystals of **1** obtained from CH_3CN was racemic.[1] Chiral resolution of this racemate was performed by HPLC using a column [Chiralpak OP(+)] packed with (+)-poly(diphenyl-2-pryridylmethyl methacrylate) eluted with MeOH/H_2O (80:20) (flow rate, 0.3 mL/min; RI and $[\alpha]_D$ detection) and resulted in separation of (+)-kerampahidin B ((+)-**1**, t_R 11.5 min) and (-)-keramaphidin B ((-)-**1**, t_R 13.9 min) shown in Chart 1. Chiral HPLC analyses of the crystals and the mother liquid of keramaphidin B (**1**) revealed that the ratio of (+)- and (-)-forms of keramaphidin B (**1**) was ca. 1:1 and 20:1, respectively.

Manzamine L (**8**) showed the molecular ion peak at *m/z* 554 (M^+) in the EIMS spectrum, and

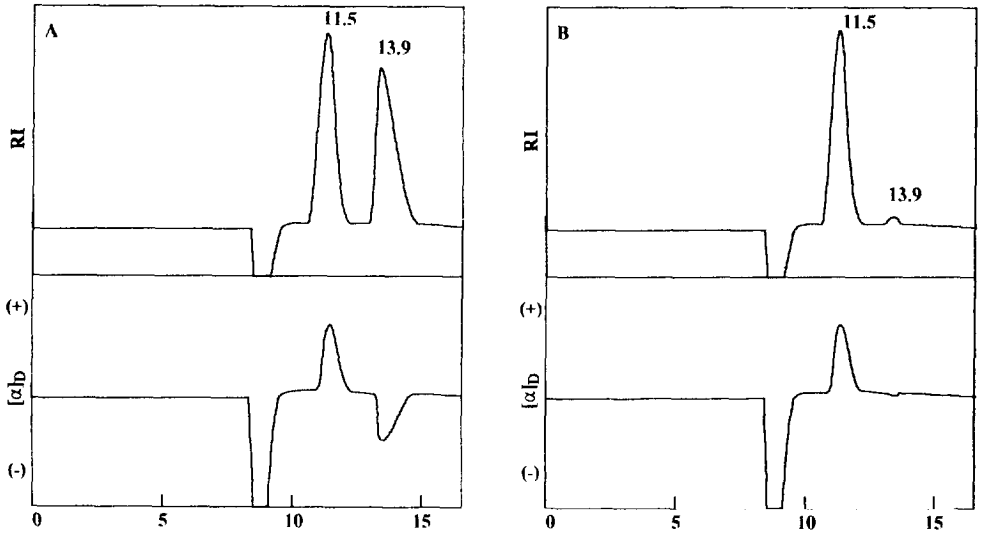
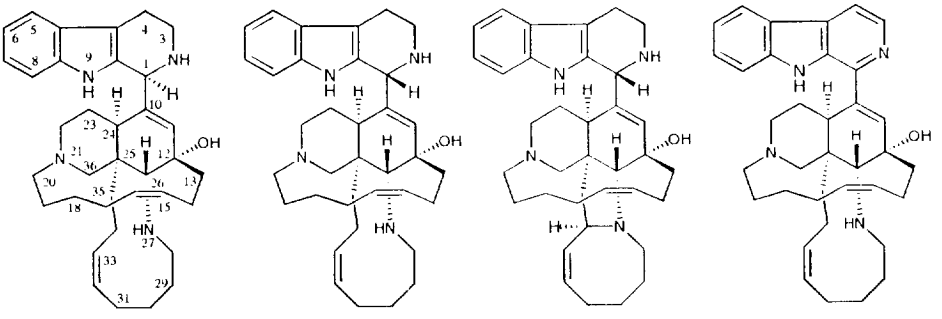


Chart 1. Chiral HPLC of Keramaphidin B (1) in Resolution of Crystals (A) and Its Mother Liquid (B).

Peaks: t_R 11.5 min, (+)-Keramaphidin B {(+)-**1**}; t_R 13.9 min, (-)-Keramaphidin B {(-)-**1**}; HPLC Condition: eluent, MeOH/H_2O (80:20); column, Chiralpak OP (+) (4.6 i.d. x 250mm); flow rate, 0.3 mL/min; RI and $[\alpha]_D$ detection; sample injection, 50μg/100μL in MeOH.

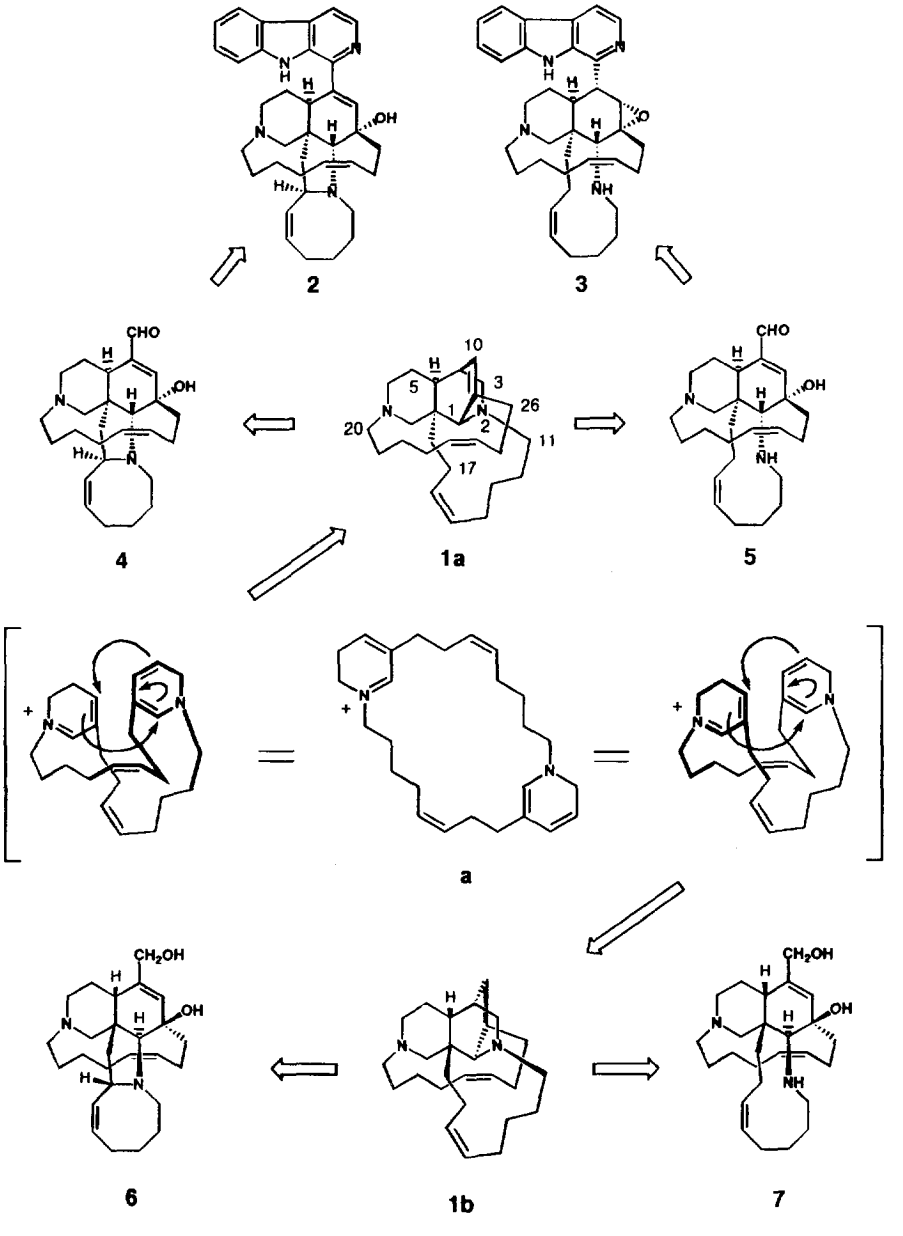


8 **9** **10** **11**

Table 1. ^{13}C NMR Data of Manzamines L (**8**) and H (**9**) in $CDCl_3$.

position	**8**		**9**		position	**8**		**9**	
1	56.1	d	59.9	d	17	28.1	t	29.1	t
3	41.3	t	43.2	t	18	27.5	t	28.6	t
4	21.7	t	22.4	t	19	28.2	t	29.2	t
4a	109.7	s	109.0	s	20	52.8	t	53.4	t
4b	126.6	s	127.8	s	22	48.6	t	49.6	t
5	117.1	d	118.0	d	23	31.7	t	32.3	t
6	118.6	d	119.3	d	24	46.2	d	44.6	d
7	120.6	d	121.4	d	25	42.9	s	43.5	s
8	110.0	d	111.0	d	26	58.1	d	59.2	d
8a	134.8	s	135.5	s	28	58.1	t	59.2	t
9a	132.6	s	134.2	s	29	28.2	t	29.2	t
10	143.3	s	143.9	s	30	28.2	t	29.2	t
11	128.6	d	130.1	d	31	24.1	t	25.0	t
12	68.9	s	70.1	s	32	130.6	d	131.6	d
13	39.6	t	40.6	t	33	130.0	d	131.1	d
14	21.0	t	21.9	t	34	25.1	t	26.2	t
15	128.4	d	129.4	d	35	37.0	t	37.3	t
16	127.92	d	129.1	d	36	64.9	t	65.7	t

HREIMS data (*m/z* 554.3975, M^+, Δ -0.9 mmu) provided the molecular formula, $C_{36}H_{50}N_4O$, which was the same as that of manzamine H (**9**). The ^1H NMR spectrum of **8** was similar to that of **9**, except for the signals due to H-1 (δ 4.57 for **8**; δ 4.65 for **9**) and H-11 (δ 5.36 for **8**; δ 5.64 for **9**). Comparison of the ^{13}C NMR data (Table 1) of **8** with those of **9** indicated that manzamine L (**8**) was stereoisomer at C-1 of manzamine H (**9**); the carbon resonance at C-1 (δ 56.1) on the tetrahydro-β-carboline ring in **8** slightly differed from that of **9** (δ 59.9). 1*S*-Configuration for manzamine L (**8**) was deduced from a negative Cotton effect (Δε -10.8) at 221 nm,[18] while manzamines H (**9**) and D[19] (**10**) isolated from this sponge showed the opposite sign (Δε +15.0 and +26.3, respectively) at 224 nm, implying 1*R*-configuration for **9** and **10**. The stereochemistry of manzamine L (**8**) was established by chemical correlation with manzamine J[10] (**11**) as follows. Treatment of **8** with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) yielded the corresponding β-carboline (**11**), which was identical with manzamine J (**11**) in comparison with all spectral data of **11**, indicating that the isoquinoline moiety of manzamine L (**8**) possessed the same absolute stereochemistry as those of manzamines A (**2**), B (**3**), and J (**11**).



Scheme 1. Plausible Biogenetic Path of Manzamines A (2) and B (3).

One enantiomer of keramaphidin B (**1**) is considered to possess the same configuration as most of manzamine-related alkaloids with dextrorotation represented by manzamines A (**2**) and B (**3**), while the other enantiomer may be correlated to those with raevorotation like ircinols A (**6**) and B (**7**). The ratio of the total amount of manzamine-related alkaloids with dextrorotation vs. those with raevorotation in this sponge was found to be about 15:1, which approximately corresponded to the ratio of (+)- and (-)-keramaphidin B (**1**).

A plausible biogenetic path of manzamines A (**2**) and B (**3**) is shown in Scheme 1, which was elucidated on the basis of the structures and ratios of **1** ~ **10** isolated from this sponge. The Baldwin and Whitehead *bis*-3-alkyldihydropyridine (**a**) might be a biogenetic precursor of both enatiomers of keramaphidin B (**1**), and 2,3-iminium form of the major enantiomer (**1a**) may be hydrolyzed to generate (+)-ircinals A (**4**) or B (**5**), which are probably converted through Pictet-Spengler cyclization with tryptamine into manzamines D (**10**), H (**9**), and L (**8**), respectively, and then dehydrogenated into manzamines A (**2**) and B (**3**), respectively, while the minor enantiomer (**1b**) may be associated with some antipodes such as ircinols A (**6**) and B (**7**). The ratio (15:1) of amount of manzamine-related alkaloids with dextrorotation vs. those with raevorotation may indicate an incomplete stereoselectivity in *endo*-type Diels-Alder-type [4+2] intramolecular cycloaddition of the *bis*-3-alkyldihydropyridine (**a**) to afford a major and a minor enantiomers of keramaphidin B (**1a** and **1b**, respectively).

Recently keramaphidin B (**1**) was isolated from the sponge *Xestospongia ingens* together with ingenamine.[3] Since the optical rotation ($[\alpha]_D$ +22.2°) of **1** from *Amphimedon* sp. was close to that ($[\alpha]_D$ +29.8°) of **1** from *X. ingens*, both samples might possess the same absolute configurations. The absolute configuration of **1** from each sponge, however, remains to be determined.

Manzamine L (**8**) exhibited cytotoxicity against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells (IC_{50} 3.7 and 11.8 μg/mL, respectively) and antibacterial acitivity against bacteria, *Sarcina lutea*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium* 607 (MIC 10, 10, 10, and 5 μg/mL, respectively).

Experimental

Isolation. The sponge *Amphimedon* sp. (1.5 kg) collected off Kerama Islands, Okinawa, was extracted with MeOH (1 L x 2), and the extract was partitioned between EtOAc (300 mL x 3) and water (500 mL), and the aqueous layer was subsequently extracted with *n*-BuOH (300 mL x 3). A part (960 mg) of the *n*-BuOH soluble material (5.60 g) was chromatographed on silica gel columns ($CHCl_3$/MeOH, 85:15 and then cyclohexane/acetone/Et_2NH, 95:5:0.5) to give a residue (37 mg), a part of which (6.5 mg) was purified by silica gel column chromatography (*n*-hexane/isopropanol/Et_3N, 95:5:0.5) to yield manzamine L (**8**, 4.2 mg, 0.0056 %, t_R 12.8 min) together with manzamine H (**9**, 2.0 mg, 0.0028 % t_R 14.9 min). Keramaphidins B (**1**, 0.0054 %, wet weight) and C^{12} (0.0022 %), manzamines A (**2**, 0.081 %), B (**3**, 0.0072 %), C (0.002%), and D (**10**, 0.012 %), ircinals A (**4**, 0.0009 %) and B (**5**, 0.0006 %), keramamine C (0.0026 %), ircinols A (**6**, 0.006 %) and B (**7**, 0.0003 %), 6-hydroxymanzamine A (0.005 %), 3,4-dihydromanzamine A (0.002 %), and 8-hydroxymanzamine A (0.002%) were isolated from other EtOAc and *n*-BuOH soluble fractions.

Determination of the Ratio of (+)- and (-)-Keramphidin B ((+)- and (-)-1). The HPLC system consisted of a Chiralpak OP (+) column (4.6 i.d. x 250 mm, Daicel Chemical Ind., Ltd.), Shodex RI SE-61 and OP-1 detectors (Showa Denko Ltd.). A racemic form ((±)-**1**) of keramaphidin B crystalized from CH_3CN was subjected to chiral HPLC analysis (eluent MeOH/H_2O, 80:20; flow rate, 0.3 mL/min; sample injection, 50 μg/100 μL in MeOH) and peaks due to (+)- and (-)-forms were detected at t_R 11.5 and 13.9 min in the ratio of 1:1, respectively. The mother liquid of keramaphidin B (**1**, $[\alpha]_D$ +22.2°)

were also analyzed under the same condition as described above and the ratio of (+)- and (-)-forms of keramaphidin B (**1**) was estimated to be ca. 20:1.

Manzamine L (8). A colorless amorphous solid; mp 143 °C; $[\alpha]_D^{24}$ -15° (*c* 0.42, $CHCl_3$); IR (NaCl) ν_{max} 3400 and 2990 cm^{-1}; UV (MeOH) λ_{max} 223 (ε 34000) and 283 (6400) nm; CD (MeOH) λ_{ext} 202 (Δε +13.6), 222 (-10.8), and 270 (+4.2) nm; ^1H NMR ($CDCl_3$) δ 7.82 (1H, brs), 7.48 (1H, d, *J* = 7.7 Hz), 7.31 (1H, d, *J* = 8.1 Hz), 7.14 (1H, t, *J* = 8.0 Hz), 7.09 (1H, t, *J* = 8.0 Hz), 5.64 (1H, s), 5.58 (1H, m), 5.43 (1H, m), 5.32 (1H, m), 5.19 (1H, m), 4.65 (1H, m), 3.74 (1H, brs), and 3.6 ~ 1.0 (36H, m); ^{13}C NMR (see Table 1); EIMS *m/z* 554 (M^+); HREIMS *m/z* 554.3975 (M^+, calcd for $C_{36}H_{50}N_4O$, 554.3984).

DDQ Oxidation of Manzamine L (8). To a stirred solution of manzamine L (**8**, 5.0 mg) in EtOH (0.25 mL) and $CHCl_3$ (0.75 mL) at room temperature was added DDQ (3.3 mg). The mixture was stirred for 60 min. After evaporation of the solvent, the residue was purified by preparative silica gel TLC with $CHCl_3$/MeOH (97:3) to afford Manzamine J (**11**, 2.0 mg): colorless amorphous solid; mp 138 ~ 139 °C; $[\alpha]_D^{24}$ +45° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.03 (1H, brs), 8.44 (1H, d, *J* = 5.4 Hz), 8.11 (1H, d, *J* = 7.8 Hz), 7.83 (1H, d, *J* = 5.4 Hz), 7.55 (2H, m), 7.26 (1H, m), 6.24 (1H, s), 5.64 (1H, m), 5.45 (1H, m), 5.35 (1H, m), 5.25 (1H, m), 3.90 (1H, s), and 3.32 (1H, m); EIMS *m/z* 550 (M^+); HREIMS *m/z* 550.3696 (M^+, calcd for $C_{36}H_{46}N_4O$, 550.3672).

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19. 1*S*-Isomer of manzamine D has not been isolated from this sponge and the sponge of the genus *Ircinia*, from which ircinals A and B and manzamines H and J were isolated.

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