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Isolation and Structures of Haterumalides NA, NB, NC, ND, and NE, Novel Macrolides from an Okinawan Sponge *Ircinia* sp.

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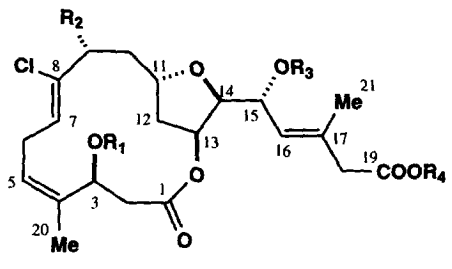
Abstract: Novel macrolides, haterumalides NA (**1**), NB (**2**), NC (**3**), ND (**4**), and NE (**5**), were isolated from an Okinawan sponge *Ircinia* sp. The absolute stereostructure of **1** was determined by spectroscopic analysis and modified Mosher's method. Haterumalide NA (**1**) showed cytotoxicity against P388 cells and acute toxicity against mice.

As described previously, in our screening for inhibitors of the cell division of fertilized sea urchin eggs, haterumalide B was isolated from an Okinawan ascidian *Lissoclinum* sp.[1] We report here the isolation and structural determination of haterumalides NA (**1**), NB (**2**), NC (**3**), ND (**4**), and NE (**5**), novel cytotoxic macrolides isolated from an Okinawan sponge *Ircinia* sp.



1 : R_1 = Ac, R_2 = H, R_3 = H, R_4 = H
2 : R_1 = Ac, R_2 = H, R_3 = H, R_4 = ^{n}Bu
3 : R_1 = Ac, R_2 = OH, R_3 = H, R_4 = ^{n}Bu
4 : R_1 = Ac, R_2 = OH, R_3 = H, R_4 = H
5 : R_1 = H, R_2 = H, R_3 = H, R_4 = H
6 : R_1 = Ac, R_2 = H, R_3 = H, R_4 = Me
7 : R_1 = Ac, R_2 = H, R_3 = (*R*)-MTPA, R_4 = Me
8 : R_1 = Ac, R_2 = H, R_3 = (*S*)-MTPA, R_4 = Me

The acetone extract of an Okinawan sponge *Ircinia* sp., collected on Hateruma Island in Okinawa Prefecture, Japan, was partitioned between EtOAc and H_2O. The EtOAc extract was subjected to fractionation guided by acute toxicity against mice using column chromatography (SiO_2, $CHCl_3$-MeOH) and preparative thin layer chromatography (SiO_2, $CHCl_3$-MeOH) to give haterumalides NA[2] (**1**, 0.065% yield based on wet wt), NB[3] (**2**, 0.024% yield based on wet wt), NC[3] (**3**, 0.027% yield based on wet wt), ND[3] (**4**, 0.023% yield based on wet wt), and NE[3] (**5**, 0.030% yield based on wet wt) as colorless oils. Haterumalide NA (**1**) exhibited cytotoxicity against P388 cells, with an IC_{50} of 0.32 μg/mL, and moderate acute toxicity against mice, with an LD_{99} of 0.24 g/kg.

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Table 1 NMR Data for Haterumalide NA (**1**) in CD_3OD

position	^1H (ppm)[a]	^{13}C (ppm)[a]	position	^1H (ppm)[a]	^{13}C (ppm)[a]
1		169.5 s	12a	1.52 br dd (12.1, 12.2)	38.7 t
2a	2.77 dd (11.5, 11.6)	38.9 t	12b	2.09 br d (12.1)	
2b	2.82 dd (4.6, 11.6)		13	5.29 m	76.7 d
3	5.79 dd (4.6, 11.5)	68.7 d	14	3.89 dd (3.7, 8.7)	84.6 d
4		134.7 s	15	4.52 dd (8.5, 8.7)	66.5 d
5	5.70 dd (6.6, 9.7)	130.9 d	16	5.34 d (8.5)	129.8 d
6a	2.45 m	35.8 t	17		136.8 s
6b	3.50 m		18a	2.97 d (14.9)	47.8 t
7	5.30 m	126.9 d	18b	3.01 d (14.9)	
8		133.2 s	19		179.2 s
9a	2.28 m	35.5 t	20	1.88 br s 3H	18.5 q
9b	2.45 m		21	1.82 br s 3H	17.4 q
10a	1.38 m	29.0 t	22		171.3 s
10b	2.28 m		23	2.02 s 3H	20.9 q
11	3.93 br dd (12.1, 12.2)	78.1 d			

[a] Recorded at 400 MHz. Coupling constants (Hz) are in parentheses.

[b] Recorded at 100 MHz. Multiplicity was based on the HSQC spectrum.

The molecular formula of **1** was determined to be $C_{23}H_{31}ClO_8$ by HRFABMS (*m/z* 493.1616, calcd for $C_{23}H_{31}ClNaO_8$ $[M+Na]^+$ 493.1605). The IR spectrum [$CHCl_3$ in the presence of Et_3N] showed bands at 3650-2900 (br), 1735, and 1600 cm^{-1} that were assigned to ester and carboxylic acid functionalities. The NMR data for **1** are summarized in Table 1. The ^1H NMR, ^{13}C NMR, and HSQC spectra of **1** showed the presence of three methyl groups combined to quaternary carbons, six methylene carbons, five oxymethine carbons (δ 66.5, 68.7, 76.7, 78.1, and 84.6), three carbonyl carbons (δ 169.5, 171.3, and 179.2) of ester or carboxylic acid, and six olefinic carbons (δ 126.9, 129.8, 130.9, 133.2, 134.7, and 136.8). A detailed analysis of the phase-sensitive DQF-COSY and HOHAHA spectra of **1** allowed three partial structures, C2-C3, C5-C7, C9-C16, to be constructed (Figure 1). The connectivities of the foregoing partial structures were clarified by the HMBC correlations H2/C1, H20/C3, H20/C4, H20/C5, H6/C8, H7/C8, and H9/C8. Furthermore, the HMBC

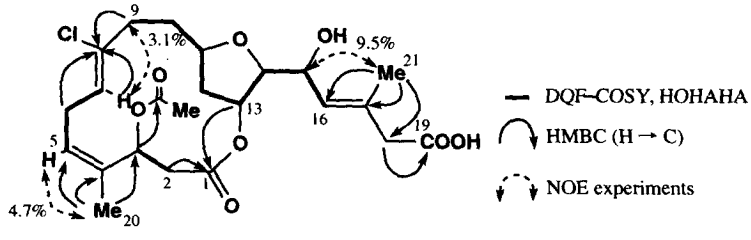

Figure 1 Partial structures of haterumalide NA (**1**), based on 2D NMR correlations.

correlations H21/C16, H21/C17, H21/C18, and H18/C19 suggested the connectivities of C16, 17, 18, 19, and 21. Therefore, the connectivities of the entire carbon framework were established as shown in Figure 1. The HMBC correlations H3/C22 and H13/C1 also suggested that both C1 and C22 were ester carbonyl carbons.

Therefore, the remaining C19 carbonyl carbon must be a carboxyl carbon. The presence of a tetrahydrofuran ring was suggested by the characteristic chemical shifts of C11 (δ 78.1) and C14 (δ 84.6)[4]. Based on the molecular formula and degree of unsaturation of **1**, the chlorine atom must be connected to C8. Finally, the stereochemistries of the three olefins in **1** were clarified to be 4*Z*, 7*Z*, and 16*E* by NOE experiments. Thus, the gross structure of haterumalide NA (**1**) was determined as shown in Figure 1.

The relative stereochemistries of C3, C11, C13, and C14 in **1** were determined as follows. In the tetrahydrofuran ring, NOESY correlations H11/H12b, H12a/H13, and H13/H14 suggested that the relative stereochemistries at C11, C13, and C14 were 11*R**, 13*R**, and 14*R**. The magnitude of $J_{2a,3}$ = 11.5 Hz and $J_{2b,3}$ = 4.6 Hz and the NOESY correlations H2a/H20, H2b/H3, H3/H6b, H5/H6a, H5/H7, H6a/H7, H7/H12b, H11/H12b, H12a/H13, and H13/H14 suggested the plausible conformation of the macro ring in **1** shown in Figure 2. Therefore, both H3 and H6b were directed inside the macro ring, and the stereochemistry of C3 was determined to be 3*R**.

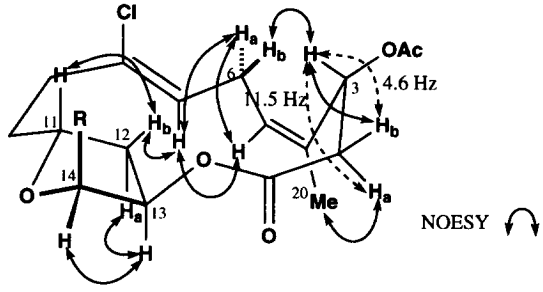

Figure 2 A plausible conformation of macro ring, based on coupling constants and NOESY correlations.

The absolute stereochemistry of C15 in **1** was determined using modified Mosher's method[5]. Methylation of **1** with CH_2N_2 gave methyl ester **6**, which was transformed into the (*R*)- and (*S*)-MTPA esters, **7** and **8**. The ^{1}H NMR signals of the two esters, **7** and **8**, were assigned based on the 2D NMR spectra, and the

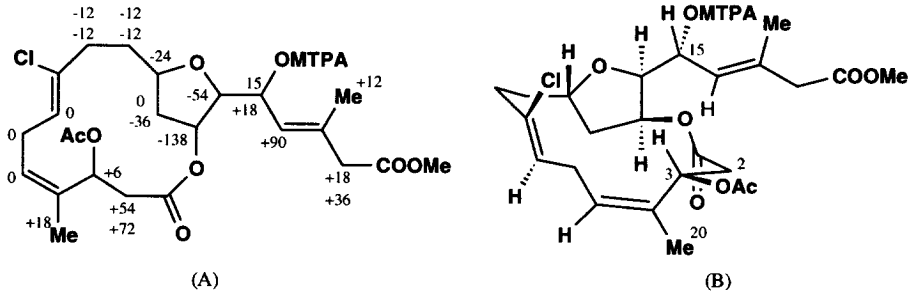

Figure 3 (A) Δδ values (δ_S-δ_R) for the MTPA esters **7** and **8** in Hz (600 MHz), and (B) conformation of the MTPA esters **7** and **8**.

Δδ values (δ_S-δ_R, Hz) were then calculated. The results, shown in Figure 3A, established that the absolute stereochemistry of C15 in **1** was *R*. The magnitude of $J_{14,15}$ = 8.7 Hz suggested that H14 and H15 were located in an *anti* arrangement. The Δδ values of C2, C3, and C20 indicated that H2, H3, and H20 were

on the right of the MTPA plane. This information suggested that **1** adopted the conformation shown in Figure 3B, and that the relative stereochemistry between C14 and C15 was *threo*. Thus, the absolute stereochemistries of the five stereocenters in **1** were determined to be 3*S*, 11*S*, 13*S*, 14*S*, and 15*R*.

The gross structures of haterumalides NB, NC, ND, and NE were determined in the same manner as descrived above for **1**. The relative stereostructures of haterumalides NB, NC, ND, and NE, except for C15, were also elucidated by NOESY data as depicted in formulas **2**, **3**, **4**, and **5**, respectively. The absolute stereochemistries of haterumalides NB, NC, ND, and NE including C15 were deduced as shown in formulas **2**, **3**, **4**, and **5**, respectively based on the similarity of their NMR data to **1** and in view of their biosynthesis.

In conclusion, haterumalides NA, NB, NC, ND, and NE, novel cytotoxic macrolides, were isolated from an Okinawan sponge *Ircinia* sp. The structures of these haterumalides were determined by using 2D NMR spectra and modified Mosher's method. The framework of these haterumalides resembled those of halichlorine[6], pinnaic acid[7], and attenols A, B, and C[8], which were recently isolated in our laboratory. This implies that these polyketides may be formed by similar biosynthetic processes.

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References and Notes

1) Ueda, K.; Hu, Y. See preceding paper.

2) $[\alpha]^{26}_{D}$ = -3.0 (*c* 0.053, MeOH). IR ($CHCl_3$ in the presence of Et_3N) 3650 (br), 1735, 1600 cm^{-1}.

3) Haterumalide NB (**2**): ^1H NMR (400 MHz, CD_3OD) δ 5.79 (dd, *J* = 4.8, 11.4 Hz, 1H), 5.70 (dd, *J* = 7.0, 10.6 Hz, 1H), 5.37 (d, *J* = 8.8 Hz, 1H), 5.30 (m, 2H), 4.53 (t, *J* = 8.8 Hz, 1H), 4.09 (t, *J* = 6.6 Hz, 2H), 3.93 (m, 1H), 3.89 (dd, *J* = 3.7, 8.8 Hz, 1 H), 3.49 (m, 1H), 3.07 (s, 2H), 2.83 (dd, *J* = 4.8, 11.4 Hz, 1H), 2.77 (t, *J* = 11.4 Hz, 1H), 2.45 (m, 2H), 2.28 (m, 2H), 2.10 (dd, *J* = 3.7, 12.8 Hz, 1 H), 2.02 (s, 3H), 1.88 (s, 3H), 1.81 (s, 3H), 1.62 (m, 2H), 1.52 (ddd, *J* = 3.7, 12.1, 12.8 Hz, 1 H), 1.40 (m, 3H), 0.95 (t, *J* = 7.3 Hz, 3H). Haterumalide NC (**3**): ^1H NMR (400 MHz, CD_3OD) δ 5.78 (dd, *J* = 4.4, 11.4 Hz, 1H), 5.71 (dd, *J* = 7.0, 11.0 Hz, 1H), 5.54 (dd, *J* = 2.6, 8.4 Hz, 1H), 5.37 (d, *J* = 8.4 Hz, 1H), 5.30 (dd, *J* = 3.3, 3.7 Hz, 2H), 4.52 (t, *J* = 8.8 Hz, 1H), 4.14 (dd, *J* = 5.5, 11.0 Hz, 1H), 4.09 (t, *J* = 6.6 Hz, 2H), 3.88 (dd, *J* = 3.7, 8.8 Hz, 1 H), 3.71 (m, 1H), 3.56 (m, 1H), 3.07 (s, 2H), 2.83 (dd, *J* = 4.8, 11.4 Hz, 1H), 2.76 (t, *J* = 11.4 Hz, 1H), 2.53 (dd, *J* = 7.0, 16.1 Hz, 1H), 2.22 (ddd, *J* = 4.0, 11.4, 11.7 Hz, 1H), 2.02 (s, 3H), 1.97 (dd, *J* = 3.3, 12.8 Hz, 1H), 1.88 (s, 3H), 1.81 (s, 3H), 1.70 (dd, *J* = 5.9, 11.7 Hz, 1H), 1.62 (m, 2H), 1.55 (dd, *J* = 3.3, 12.8 Hz, 1H), 1.40 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H).
Since **2** and **3** were isolated without using BuOH, they are not artifacts of **1**. They are perhaps produced by a Baeyer-Villiger-type oxidation.
Haterumalide ND (**4**): ^1H NMR (400 MHz, CD_3OD) δ 5.78 (dd, *J* = 4.8, 11.4 Hz, 1H), 5.70 (dd, *J* = 7.0, 9.9 Hz, 1H), 5.53 (dd, *J* = 2.6, 8.1 Hz, 1H), 5.32 (d, *J* = 9.5 Hz, 1H), 5.29 (dd, *J* = 3.3, 3.7 Hz, 2H), 4.50 (t, *J* = 8.8 Hz, 1H), 4.13 (dd, *J* = 5.5, 11.0 Hz, 1H), 3.88 (dd, *J* = 3.7, 8.8 Hz, 1 H), 3.71 (m, 1H), 3.58 (m, 1H), 2.98 (d, *J* = 14.7 Hz, 1H), 2.92 (d, *J* = 14.7 Hz, 1H), 2.82 (dd, *J* = 4.8, 11.7 Hz, 1H), 2.76 (t, *J* = 11.4 Hz, 1H), 2.52 (dd, *J* = 7.0, 16.1 Hz, 1H), 2.23 (ddd, *J* = 4.0, 11.0, 11.7 Hz, 1H), 2.02 (s, 3H), 1.97 (dd, *J* = 3.3, 13.2 Hz, 1H), 1.88 (s, 3H), 1.82 (s, 3H), 1.68 (ddd, *J* = 5.5, 11.7, 11.7 Hz, 1H), 1.62 (m, 2H), 1.56 (ddd, *J* = 3.3, 12.8, 12.8 Hz, 1H). Haterumalide NE (**5**): ^1H NMR (400 MHz, CD_3OD) δ 5.61 (t, *J* = 7.3 Hz, 1H), 5.37 (d, *J* = 7.3 Hz, 1H), 5.29 (m, 2H), 4.63 (dd, *J* = 4.4, 11.4 Hz, 1H), 4.53 (t, *J* = 8.8 Hz, 1H), 3.91 (m, 1H), 3.88 (dd, *J* = 3.7, 8.8 Hz, 1 H), 3.30 (m, 1H), 3.05 (s, 2H), 2.75 (dd, *J* = 4.0, 11.4 Hz, 1H), 2.64 (t, *J* = 11.4 Hz, 1H), 2.48 (m, 2H), 2.29 (m, 2H), 2.06 (dd, *J* = 3.3, 12.8 Hz, 1H), 1.85 (s, 3H), 1.83 (s, 3H), 1.51 (ddd, *J* = 3.3, 12.1, 12.8 Hz, 1H), 1.38 (m, 1H).

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