Isolation and Structures of Hedaols A, B, and C, New Bisnorditerpenes from a Japanese Brown Alga

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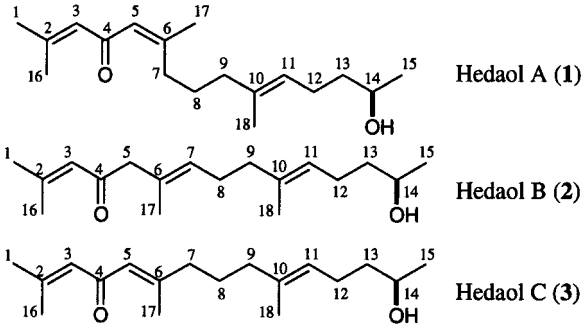
New bisnorditerpenes, hedaols A (**1**), B (**2**), and C (**3**), were isolated from the Japanese brown alga *Sargassum* sp. Their structures were determined by spectroscopic analysis. The absolute stereocenter of **1** was determined by the modified Mosher's method. Hedaols showed low cytotoxicity against P388 cells.

In our continuing search for marine bioactive metabolites, we isolated three novel α,β-unsaturated ketones, hedaols A (**1**), B (**2**), and C (**3**), from the Japanese brown alga *Sargassum hemiphyllum*. We report here the isolation and structure determination of hedaols A (**1**), B (**2**), and C (**3**). Although polyphenols, benzoquinones, and hydroquinones bearing a diterpenoid side chain, cyclopentenones, glycerides bearing a methacrylic acid moiety, and bisnorditerpene derivatives have been isolated from the alga *Sargassum* sp., the hedaols described here are new bisnorditerpenes that are derived biogenetically from the geranyl geraniol precursor.[1]

The EtOAc-soluble fraction of the aqueous 80% EtOH extract of the brown alga *S. hemiphyllum*, collected off the Heda coast of the Izu Peninsula, was subjected to fractionation using SiO_2 column chromatography and HPLC (SiO_2) to give hedaols A (**1**), B (**2**), and C (**3**) as colorless oils. Hedaols A (**1**), B (**2**), and C (**3**) showed low cytotoxicity against P388 cells, with IC_{50} values of 5.1, 2.2, and 50 μg/mL, respectively.



The molecular formula of **1** was determined to be $C_{18}H_{30}O_2$ by HRFABMS. The IR spectrum indicated the presence of hydroxyl (3600, 3500 cm^{-1}) and conjugated ketonic (1670 cm^{-1}) functionalities. The NMR data for **1** are summarized in Table 1. The ^1H NMR and HMQC spectra of **1** showed the presence of five methyl carbons, five methylene carbons, three olefinic methine carbons (δ_C 125.2, 127.1, 127.1 ppm), and one methine carbon (δ_C 68.3 ppm) connected to an oxygen. A detailed analysis of the COSY and HOHAHA spectra of **1** allowed four partial structures, C-1 to C-3 including C-16, C-5 to C-6 including C-17, C-7 to C-9, and C-10 to C-15 including C-18, to be connected. The remaining connectivities of **1** were clarified by the HMBC correlations H-3/C-4, H-5/C-4, H-7/C-6, H-17/C-7, H-9/C-10, H-9/C-11, and H-18/C-9. Finally, the NOESY correlations H-5/H-17 and H-9/H-11 suggested that the geometries of the C-5 and C-10 olefins in **1** were 5*Z* and 10*E*. The assignments of 5*Z* and 10*E* geometries were also supported by the ^{13}C shifts of the vinyl methyl groups [δ_{C-17} 28.2 ppm (i.e., >20); δ_{C-18} 16.0 ppm (i.e., <20)]. Therefore, the connectivities of the entire framework were established to be as shown in **1**.

Hedaol B (**2**) was found to be an isomer of hedaol A (**1**) by HRFABMS. The NMR spectrum of **2** resembled that of **1**, but the signal of a singlet methylene (δ_H 3.03 ppm) suggested that **2** was a regioisomer of **1**. The NMR data for **2** are summarized in Table 1. As expected, a similar analysis of the COSY, HOHAHA, HMQC, and HMBC spectra of **2** allowed us to construct the entire framework. In NOE experiments (800 MHz) on hedaol B (**2**), irradiation of the signal at H-7 (δ_H 5.23) and H-11 (δ_H 5.15) enhanced the signals for H-5 (δ_H 3.03, 3.5%) and H-9 (δ_H 2.04, 2.6%), respectively. These results suggested that the geometries of the C-6 and C-10 olefins in **2** were 6*E* and 10*E*. These geometries were also supported by the ^{13}C shift of the vinyl methyl groups in **2**.

Hedaol C (**3**) was also found to be an isomer of hedaol A (**1**) by HRFABMS. The NMR spectrum of **3** resembled that of **1**, but the signals of H-7 (δ_H 2.09 ppm) and C-17 methyl (δ_C 20.0 ppm) were different. This implied that **3** was a stereoisomer of the C-5 olefin in **1**. The NMR data for **3** are summarized in Table 1. Analysis of the COSY, HOHAHA, HMQC, and HMBC spectra of **3** allowed us to derive the gross structure. In NOE experiments (800 MHz) on hedaol C (**3**), irradiation of the signal at H-5 (δ_H 6.01) and H-18 (δ_H 1.63) enhanced the signals for H-7 (δ_H 2.09, 1.6%) and H-12 (δ_H 2.10, 0.8%), respectively. These results suggested that the geometries of the C-5 and C-10 olefins in **3** were 5*E* and 10*E*. These geometries were also supported by the ^{13}C shift of the vinyl methyl groups in **3**.

The absolute stereochemistry in **1** was determined using the modified Mosher's method.[2] Treatment of **1** with (*R*)- or (*S*)-MTPACl gave (*S*)- or (*R*)-MTPA esters **4** and **5**, respectively, the ^1H NMR signals of which were assigned based on the 2D NMR spectra. The $\Delta\delta$ values ($\delta_S - \delta_R$, Hz) (Figure 1), calculated carefully, suggested that the absolute stereochemistry of C-14 is 14*R*.

Experimental Section

General Experimental Procedures. ^1H NMR spectra were recorded on 400 MHz spectrometers. 2D NMR spectra were recorded on 800 MHz spectrometers. FABMS spectra

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Table 1. NMR Data for Hedaols A (**1**), B (**2**), and C (**3**)[a]

hedaol A (**1**)			hedaol B (**2**)			hedaol C (**3**)		
atom	^1H[b]	^{13}C[c]	atom	^1H[b]	^{13}C[c]	atom	^1H[b]	^{13}C[c]
1	1.86 d (1.1) 3H	26.0 q	1	1.87 d (1.2) 3H	28.0 q	1	1.87 d (1.1) 3H	27.8 q
2		154.0 s	2		156.0 s	2		155.9 s
3	6.02 br s	127.1 d	3	6.11br s	123.8 d	3	6.02 m	127.5 d
4		191.0 s	4		200.2 s	4		193.0 s
5	6.04 dq (1.1, 1.1)	127.1 d	5	3.03 s 2H	56.0 t	5	6.01 m	127.0 d
6		158.2 s	6		130.1 s	6		158.4 s
7a	2.54 dt (12.9, 8.0)	33.4 t	7	5.23 br t (7.8)	129.8 d	7	2.09 br t (7.7) 2H	41.5 t
7b	2.61 dt (12.9, 8.0)							
8	1.55 m 2H	26.8 t	8	2.14 m 2H	26.6 t	8	1.57 m 2H	26.5 t
9	2.03 br t (8.0) 2H	40.3 t	9	2.04 br t (7.7) 2H	39.5 t	9	1.99 br t (7.9) 2H	39.0 t
10		135.0 s	10		136.0 s	10		137.0 s
11	5.17 br dt (7.6)	125.2 d	11	5.15 br t (7.8)	125.0 d	11	5.15 br t (7.3)	125.2 d
12	2.08 dt (7.6, 7.6) 2H	24.8 t	12	2.10 m 2H	24.4 t	12	2.10 m 2H	25.3 t
13	1.50 dt (7.1, 7.6) 2H	39.7 t	13	1.50 m 2H	39.6 t	13	1.50 m 2H	39.0 t
14	3.81 m	68.3 d	14	3.81 m	68.3 d	14	3.81 m	68.2 d
15	1.18 d (6.5) 3H	23.6 q	15	1.19 d (6.5) 3H	24.6 q	15	1.19 d (6.9) 3H	24.0 q
16	2.14 d (1.0) 3H	20.9 q	16	2.14 d (1.0) 3H	20.8 q	16	2.17 d (1.2) 3H	20.9 q
17	1.87 d (1.1) 3H	28.2 q	17	1.62 br s 3H	17.0 q	17	2.15 d (1.2) 3H	20.0 q
18	1.64 br s 3H	16.0 q	18	1.62 br s 3H	15.8 q	18	1.63 s 3H	16.0 q

[a] Recorded in $CDCl_3$. [b] Recorded at 400 MHz. Coupling constants (Hz) are in parentheses. [c] Chemical shifts and multiplicity were based on HMQC and HMBC spectra.

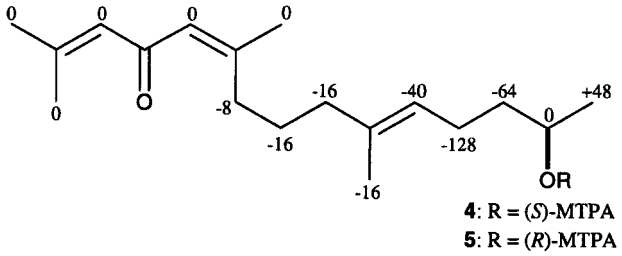

Figure 1. $\Delta\delta$ values ($\delta_S - \delta_R$) for the MTPA esters **4** and **5** in Hz (800 MHz).

were recorded using *p*-nitrobenzyl alcohol as a matrix in positive mode. The starting materials were azeotropically dried with benzene before use. All reactions were conduced under a nitrogen atmosphere.

Cytotoxic Activity. Growing cells of murine P388 lymphocytic leukemia were suspended in RPMI-1640 medium containing 10% fetal bovine serum, 5 μM 2-hydroxyethyl disulfide, and kanamycin (100 μg/mL) at 2×10^4 cells/mL, and samples dissolved in acetone were added. The mixture was incubated at 37 °C for 4 days in a CO_2 incubator with a humidified atmosphere containing 5% CO_2. The cells were counted by the MTT method.[3] The IC_{50} value (concentration required for 50% inhibition of cell growth) was determined using the growth curve.

Plant Material. The brown alga *Sargassum hemiphyllum* (Turner) C. Agardh was collected off the Heda coast of the Izu Peninsula.

Isolation. This alga (160 g, wet wt) was crushed and extracted with aqueous 80% EtOH. The aqueous EtOH extract was filtered, concentrated, and then extracted with EtOAc to give 1.50 g of EtOAc extract. The EtOAc extract was diluted with aqueous 90% MeOH (100 mL) and washed with hexane (100 mL × 3) to give 0.59 g of aqueous 90% MeOH extract. The aqueous 90% MeOH extract was subjected to fractionation using SiO_2 column chromatography [$CHCl_3$ → $CHCl_3$/MeOH (100/1→9/1) → MeOH] to give three fractions.

The first fraction was subjected to fractionation using HPLC [Develosil 30-3, hexane/EtOAc (4/1), flow rate 2 mL/min, detection at 254 nm] to give hedaol A (**1**) as a colorless oil (2.8 mg; 0.0018% yield based on wet wt). The second fraction was subjected to fractionation using HPLC [Develosil 30-3, hexane/EtOAc (6/1), flow rate 1 mL/min, detection at 254 nm] to give hedaol B (**2**) as a colorless oil (1.9 mg; 0.0013% yield based on wet wt). Finally, the third fraction was subjected to fractionation using HPLC [(1) Develosil 30-3, hexane/EtOAc (6/1), flow rate 2 mL/min, detection at 254 nm; (2) Develosil 30-3, hexane/$CHCl_3$ (1/1), flow rate 1 mL/min, detection at 254 nm] to give hedaol C (**3**) as a colorless oil (2.0 mg; 0.0013% yield based on wet wt).

Hedaol A (1): $[\alpha]^{29}_D$ −1.9° (*c* 0.057, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 3500, 1670 cm^{-1}; FABMS *m/z* 301 $[M + Na]^+$; HRFABMS *m/z* 301.2124 (calcd for $C_{18}H_{30}O_2Na$, 301.2144).

Hedaol B (2): $[\alpha]^{29}_D$ −79° (*c* 0.047, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 3500, 1680 cm^{-1}; FABMS *m/z* 301 $[M + Na]^+$; HRFABMS *m/z* 301.2166 (calcd for $C_{18}H_{30}O_2Na$, 301.2144).

Hedaol C (3): $[\alpha]^{28}_D$ −3.0° (*c* 0.047, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3520, 1670 cm^{-1}; FABMS *m/z* 301 $[M + Na]^+$; HRFABMS *m/z* 301.2150 (calcd for $C_{18}H_{30}O_2Na$, 301.2144).

(*S*)-MTPA Ester 4. To a solution of hedaol A (0.3 mg) in pyridine was added (*R*)-MTPACl (10.5 mg) at room temperature. The reaction mixture was stirred at room temperature for 14 h and concentrated. The oily residue was purified by SiO_2 column chromatography using hexane/$CHCl_3$ to give (*S*)-MTPA ester **4** (0.1 mg) as a colorless oil: ^1H NMR ($CDCl_3$, 800 MHz) δ 7.55 (m, 2H), 7.38 (m, 3H), 6.04 (s, 1H, H-5), 6.02 (s, 1H, H-3), 5.11 (m, 1H, H-14), 5.05 (br t, J = 6.5 Hz, 1H, H-11), 3.52 (s, 3H, OMe), 2.55 (m, 2H, H-7), 2.14 (s, 3H, H-16), 2.00 (br t, J = 7.5 Hz, 2H, H-9), 1.88 (s, 3H, H-1), 1.88 (m, 2H, H-12), 1.86 (s, 3H, H-17), 1.65 (m, 2H, H-13), 1.54 (m, 2H, H-8), 1.52 (s, 3H, H-18), 1.32 (d, J = 8.2 Hz, 3H, H-15); FABMS *m/z* 517 $[M + Na]^+$.

(*R*)-MTPA Ester 5. Hedaol A (0.3 mg) was reacted with (*S*)-MTPACl (8.4 mg) as described above and chromatographed to give (*R*)-MTPA ester **5** (0.1 mg) as a colorless oil: ^1H NMR ($CDCl_3$, 800 MHz) δ 7.55 (m, 2H), 7.38 (m, 3H), 6.04 (s, 1H, H-5), 6.02 (s, 1H, H-3), 5.11 (m, 1H, H-14), 5.10 (br t, J = 7.1 Hz, 1H, H-11), 3.52 (s, 3H, OMe), 2.56 (m, 2H, H-7), 2.14 (s, 3H, H-16), 2.04 (m, 2H, H-12), 2.02 (br t, J = 6.8 Hz, 2H, H-9), 1.88 (s, 3H, H-1), 1.86 (s, 3H, H-17), 1.73 (m, 2H, H-13), 1.56 (m, 2H, H-8), 1.54 (s, 3H, H-18), 1.26 (d, J = 8.0 Hz, 3H, H-15); FABMS *m/z* 517 $[M + Na]^+$.

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