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Isolation of Two New Meroterpenoids from *Sargassum siliquastrum*

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In the course of our continuing phytochemical investigation of the algal genus *Sargassum*, we have recently published antioxidant and cytotoxic effects of organic extract of *S. siliquastrum*,[1,2] leading to the isolation of several chromanol derivatives. Herein we report the isolation and structure determination of two new meroterpenoids (**2**, **3**), together with one known compound (**1**) obtained on the reinvestigation of this extract. The isolated compounds have been tested in human cancer cell lines to assess their cytotoxic activity.

The chemical structure of isolated compound (**1**) was identified as sargachromanol J by comparison of its NMR spectral data with those reported in the literature.[3]

Sargachromanol Q (**2**) was isolated as a colorless gum, having the molecular formula $C_{27}H_{40}O_4$ as established on the basis of its molecular ion (HREIMS *m/z*: 428.2915, calcd, 428.2927). The IR absorption at 3350 and 1621 cm^{-1} showed the presence of a hydroxyl group and a α,β-unsaturated carbonyl group, which was supported by a carbon signal at δ 203.3 in the ^{13}C NMR spectrum. Furthermore, the NMR spectra of 2 were similar to those of **1** except for the signals due to an additional double bond. However, careful examination of the ^{1}H and ^{13}C NMR spectral data exhibited that there were significant shifts of carbons and protons at the terminal part (C-7'-C-15') of the prenyl chain. Combined 2-D NMR spectral analysis defined the hydroxy group (C-9') and the carbonyl group (C-10') which has α,β-unsaturation at C-11' and -12', respectively. This assignment was further supported by comparison with known compounds.[4-6] Relative configurations of C-8' and -9' were assigned as 8*R** and 9*R** on the basis of spectral data of structurally similar compounds.[4] However, absolute stereochemistry of C-9' could not be assigned definitely due to the limited amount of isolated material. Thus, the structure of **2** was determined as (8*R**,9*R**)-13-(3,4-dihydro-6-hydroxy-2,8-dimethy-2*H*-1-benzopyran-2-yl)-2,6,10-trimethyltrideca-(2*E*,10*E*)-dien-5-hydroxy-4-one.

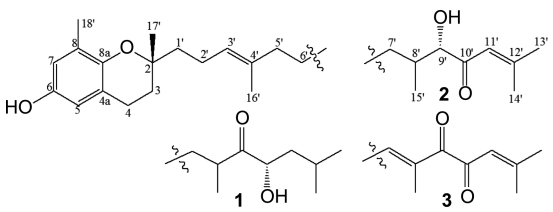

Figure 1. Chemical structure of compounds **1-3** isolated from *S. siliquastrum*.

Sargachromanol R (**3**) was isolated as a colorless gum which was analyzed for $C_{27}H_{38}O_3$ by high resolution mass and ^{13}C NMR spectrometry. The NMR spectra of compound **3** were closely related to those of sargachromanol Q (**2**). Taking into account the molecular formula, **3** has two more degrees of unsaturation than **2**, which can be accommodated by additional existence of both a double bond (δ_c 153.0 and 134.6) and a carbonyl function (δ_c 198.7). Locations of the double bond at C-7',C-8' and carbonyl function at C-9' were determined by combined 2-D NMR analyses and by comparison of NMR spectral data with the related compounds.[7] The E geometry of the double bond at C-7',C-8' was supported by the NOESY correlation between the ofefinic proton H-7' and the methyl protons H-15'. Thus far, the structure of sargachromanol R was defined as 13-(3,4-dihydro-6-hydroxy-2,8-dimethy-2*H*-1-benzopyran-2-yl)-2,6,10-trimethyltrideca-(2,6*E*,10*E*)-triene-4,5-dione.

All isolated meroterpenoids (**1-3**) were tested for their cytotoxicities against human cancer cell lines *in vitro* using MTT assay. The results are shown in Table 1. Among them, Sargachromanol R (**3**) revealed the strongest cytotoxicites against AGS, HT-29, and HT-1080 cell lines with IC_{50} values of 6.5, 3.4, and 13.9 μg/mL, respectively, compared with paclitaxel and doxorubicin.

Experimental

General Experimental Procedures. NMR spectra were recorded in CD_3OD on a Varian Mercury 300 spectrometer.

Table 1. Cytotoxic activity of compounds **1-3** (IC_{50}, μg/mL)

IC_{50} (μg/mL)	AGS	HT-29	HT-1080	MCF-7
1	27.8	29.3	31.3	31.1
2	42.8	> 50	> 50	> 50
3	6.5	3.4	13.9	> 50
Paclitaxel	6.5	0.5	28.3	15.6
Doxorubicin	0.4	3.7	5.62	23.7

Table 2. ^{1}H and ^{13}C NMR data for compounds **2** and **3** isolated from *Sargassum siliquastrum*

No	**2**		**3**	
	δ_H	δ_C	δ_H	δ_C
2		76.2 s		76.1 s
3	1.76 (2H, m)	32.8 t	1.75 (2H, m)	23.3 t*
4	2.67 (2H, t, *J* = 6.9 Hz)	23.5 t	2.68 (2H, t, *J* = 6.8 Hz)	32.8 t
4a		122.2 s		122.3 s
5	6.30 (1H, d, *J* = 2.0 Hz)	113.5 d	6.31 (1H, d, *J* = 2.6 Hz)	113.5 s
6		150.2 s		150.2 s
7	6.39 (1H, d, *J* = 2.0 Hz)	116.5 d	6.39 (1H, q, *J* = 2.6 Hz)	116.5 d
8		127.6 s		127.6 s
8a		146.2 s		146.2 s
1'	1.62 (1H, m), 1.55 (1H, m)	40.6 t	1.56 (2H, m)	40.4 t
2'	2.13 (2H, dt, *J* = 8.0, 7.7 Hz)	23.3 t	2.13 (2H, m)	23.5 t*
3'	5.15 (1H, tt, *J* = 7.7, 1.1 Hz)	125.6 d	5.15 (1H, t, *J* = 7.3 Hz)	127.1 d
4'		135.9 s		134.4 s
5'	1.98 (2H, t, *J* = 7.4 Hz)	40.8 t	2.10 (2H, m)	38.8 t
6'	1.44 (2H, m)	26.6 t	2.43 (2H, q, *J* = 7.2 Hz)	28.6 t
7'	1.45 (2H, m)	34.5 t	6.52 (1H, tq, *J* = 7.2, 1.4 Hz)	153.0 d
8'	1.90 (1H, m)	37.3 d		134.6 s
9'	4.04 (1H, d, *J* = 3.0 Hz)	80.5 d		198.7 s
10'		203.3 s		200.4 s
11'	6.25 (1H, dq, *J* = 1.2, 1.1 Hz)	120.9 d	6.17 (1H, m)	122.0 d
12'		159.3 s		162.8 s
13'	1.94 (3H, d, *J* = 1.2 Hz)	28.0 q	1.96 (3H, d, *J* = 1.5 Hz)	28.3 q
14'	2.16 (3H, d, *J* = 1.1 Hz)	21.3 q	2.15 (3H, d, *J* = 1.0 Hz)	21.7 q
15'	0.70 (3H, d, *J* = 6.8 Hz)	13.7 q	1.78 (3H, d, *J* = 1.0 Hz)	10.5 q
16'	1.58 (3H, s)	15.8 q	1.59 (3H, s)	15.7 q
17'	1.23 (3H, s)	24.5 q	1.24 (3H, s)	24.5 q
18'	2.07 (3H, s)	16.4 s	2.07 (3H, s)	16.4 s

Measured in $CDCl_3$ at 300 and 75 MHz, respectively. Assignments were aided by ^{1}H gDQCOSY, TOCSY, DEPT, gHMQC, and gHMBC experiments.

^{1}H and ^{13}C NMR spectra were measured using standard Varian pulse sequence programs at 300 MHz and 75 MHz, respectively. Optical rotations were taken on a Perkin-Elmer polarimeter 341 using a 5 cm cell. All chemical shifts were recorded with respect to residual CD_3OD peaks. Mass spectra were obtained at the Korean Basic Science Institute, Seoul, Korea. HPLC was performed using a Dionex P580 isocratic pump equipped with a Shodex RI detector. All solvents used were spectral grade or were distilled from glass prior to use.

Plant Material. The brown alga *Sargassum siliquastrum* was collected by hand in February 2007, along the shore of Cheju Island, Korea and identified by Dr. Jee Hee Kim. A voucher specimen (No J07-11) was deposited at the Division of Marine Environment & Bioscience, Korea Maritime University, Korea.

Extraction and Isolation. Shade-dried samples of *S. siliquastrum* were ground to powder and extracted successively for 24 h with a mixture (1:1) of acetone-CH_2Cl_2 (2 L × 2) and MeOH (2 L × 2). The combined crude extract (59.6 g) was evaporated under reduced pressure and then the residue was partitioned between CH_2Cl_2 and water. The organic layer was further partitioned between 85% aq. MeOH and *n*-hexane, and the aqueous layer was fractionated with *n*-BuOH and H_2O. The resulting four fractions were evaporated to dryness *in vacuo*, to yield *n*-hexane (7.8 g), 85% aq. MeOH (8.7 g), *n*-BuOH (3.3 g), and water (41.9 g) fractions, respectively. The 85% aq. MeOH fraction was separated into six subfractions by C_{18} (YMC-GEL ODS-A, 12 nm, S-75 mm) reversed-phase vacuum flash chromatography eluting with stepwise gradient mixtures of MeOH and water (50%, 60%, 70%, 80%, and 90% aq. MeOH, and 100% MeOH). Fraction 6 was separated by reversed-phase HPLC (ODS-A, 73% aq. MeOH) to give 6 subfractions (6-1~4), in order of elution. Subfraction 6-1 was further separated by reversed-phase HPLC (ODS-A, 75% aq. CH_3CN) to give **3** (5.5 mg). Subfraction 6-2 was also further separated by reversed-phase HPLC with 78% aq. CH_3CN to yield **1** (3.5 mg) and **2** (3.3 mg).

Sargachromanol J (1): A colorless gum; $[\alpha]_D^{25}$: +27.27° (*c* 0.13, MeOH); IR (NaCl): ν_{max} = 3350, 1711 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 7 and 8; HREIMS *m/z* 430.3080 $[M]^+$ (calcd for $C_{27}H_{42}O_4$, 430.3083).

Sargachromanol Q (2): A colorless gum; $[\alpha]_D^{25}$: +42.86° (*c* 0.73, MeOH); IR (NaCl): ν_{max} = 3350, 1621 cm^{-1}; UV (MeOH) λ_{max}(log ε) 224 (3.86) nm; ^{1}H and ^{13}C NMR, see

Tables 7 and 8; HREIMS *m/z* 428.2915 $[M]^+$ (calcd for $C_{27}H_{40}O_4$, 428.2927).

Sargachromanol R (3): A colorless gum; $[\alpha]_D^{20}$: +12.67° (*c* 0.12, MeOH); IR (NaCl): ν_{max} = 3350, 1682, 1620 cm^{-1}; UV (MeOH) λ_{max} (log ε) 237 (3.77) nm; ^1H and ^{13}C NMR, see Table 6; HREIMS *m/z* 424.2609 $[M]^+$ (calcd for $C_{27}H_{38}O_3$, 424.2614).

Cell Cytotoxicity: Cytotoxic levels of the isolated compounds on cultured cells were measured using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [6b], which is based on the conversion of MTT to MTT-formazan by mitochondrial enzyme. The cells were grown in 96-well plates at a density of 5×10^3 cells/well. After 24 h, cells were washed with fresh medium and treated with different concentrations of samples. After incubation for 48 h, the cells were rewashed and incubated with 100 μL of MTT (1 mg/mL) for 4 h. Finally, 150 μL of DMSO was added to solubilize the formed formazan crystals. The amount of formazan was determined by measuring the absorbance at 540 nm using a multidetection microplate fluorescence spectrophotometer synergy HT (Bio-Tek instruments Inc., Winooski, VT). Relative cell viability was determined by the amount of MTT converted into formazan. Viability of cells was quantified as a percentage compared with the control, and dose response curves were developed.

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