Cytotoxic Oxylipins from a Marine Sponge *Topsentia* sp.

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Received September 21, 2005

By a bioactivity-guided fractionation, seven new oxylipins, topsentolides A_1−C_2 (**1**−**7**), were isolated from the MeOH extract of a marine sponge *Topsentia* sp. Detailed NMR and MS analyses established the planar structures of these structurally related oxylipins, which are proposed to be biosynthesized by lipoxygenation followed by cyclization of unsaturated fatty acids. Acetonide derivatives and MTPA esters were prepared to elucidate the stereochemistry of topsentolides B_1 (**3**), B_2 (**4**), and C_2 (**7**). All compounds were tested against a panel of five human solid tumor cell lines and displayed moderate cytotoxicity.

Secondary metabolites from sponges of the genus *Topsentia* cover bisindole alkaloids,[1] steroids,[2−11] and a nitrogen-containing terpenoid.[12] In the course of our study on cytotoxic compounds from the marine sponge *Topsentia* sp. (family Halichondriidae, order Halichondrida), seven new oxylipins (**1**−**7**) were isolated. Although fatty acid derivatives are reported from various marine sponges[13−17] and algae,[18−20] there was no previous report on this class of compounds from *Topsentia* sp. Halicholactone (**12**) and neohalicholactone (**13**) from the marine sponge *Halichondria okadai*[21,22] are the only precedents which share the same structural frame with these new oxylipins (**1**−**7**). A C_{15}-epimer of neohalicholactone has also been isolated from the brown alga *Laminaria sinclairii*.[23] Work on the total synthesis of halicholactone and neohalicholactone has been widely conducted[24−31] owing to their unique structural features and potential bioactivity such as inhibition of lipoxygenase.[21] We report, herein, the structure elucidation, plausible biosynthetic pathway, and cytotoxicity evaluation of these oxylipins.

Results and Discussion

The sponge *Topsentia* sp., collected off the coast of Jeju Island, Korea, was extracted with MeOH, and the extract was partitioned between H_2O and CH_2Cl_2. The latter portion was further partitioned between aqueous MeOH and *n*-hexane. The aqueous MeOH extract, active to brine shrimp larvae (LD_{50} 30 μg/mL),[32] was subjected to reversed-phase flash column chromatography followed by repeated reversed-phase HPLC separation of the subfractions to yield compounds **1**−**7**. By detailed NMR and MS analyses, the planar structures of the seven oxylipins were elucidated. The absolute stereochemistry at C-12 in topsentolide C_2 (**7**) was determined as *S* by Mosher's method.[33]

Topsentolide A_1 (**1**) was isolated as a colorless oil. Its HR-FABMS showed a pseudomolecular ion peak at *m/z* 339.1927 ([M + Na]$^+$, Δ −0.9 mmu), suggesting a molecular formula of $C_{20}H_{28}O_3$ with 7 degrees of unsaturation. The IR absorption at 1735 cm^{-1} and the carbon signal at δ 175.6 in the ^{13}C NMR spectrum revealed the presence of a carbonyl carbon. The ^{13}C NMR data supported the presence of eight vinylic carbons. Three oxymethines were indicated by the signals at δ 73.8, 57.3, and 59.3. In the HSQC spectrum, the relatively upfield-shifted oxymethine carbon signals (δ 57.3, 59.3), which were correlated to the proton signals at δ 3.48 and 3.12, respectively, indicated an epoxy group.[34] The above interpretation accounted for six unsaturations, leaving one for a ring system. In the HMBC spectrum, the signal of H-8 (δ 5.28) showed a long-range correlation with C-1, and correlations from H-8 to C-6 and C-7, from H-4 to C-2, C-3, C-5, and C-6, and from H-3 to C-1, C-2, C-4, and C-5 were observed. Thus, by combination of the HMBC and COSY experiments, the nine-membered lactone ring was confirmed. In the other direction, the proton signal of H-8 showed correlations to two vinylic carbon signals of C-9 and C-10, and the H-8 signal was also coupled to a vinylic proton signal of H-9 in the COSY spectrum. The vinylic proton signal at H-10 was coupled to an oxymethine proton signal of H-11, which was in turn coupled to another oxymethine proton signal of H-12. Hence, the structure of **1** was established as a nine-membered lactone with an epoxide side chain.

Topsentolide A_1 (**1**) possessed four double bonds, which were located at C-5, C-9, C-14, and C-17. The geometry of Δ^9 was defined as *trans* from the large coupling constant ($J_{9,10}$ = 15.5 Hz), while the geometry of Δ^5 was determined as *cis* by comparison of the ^{13}C NMR data of allylic carbons C-4 and C-7 with those of halicholactone (**12**).[21] The geometry of Δ^{14} and Δ^{17} was assigned as *cis* on the basis of the chemical shift of the bisallylic carbon C-16 (δ 27.1), since those between *cis*−*cis*, *cis*−*trans*, and *trans*−*trans* olefins are reported to be approximately δ 25, 30, and 35, respectively.[35] The *cis* configuration of Δ^{17} was also suggested by the relatively upfield-shifted penultimate allylic carbon signal of C-19 (δ 21.5); otherwise, it would be around δ 26 in the *trans* configuration. The relative stereochemistry of the epoxy group in **1** was presumed to be *cis* from the large coupling constant ($J_{11,12}$ = 4.0 Hz) between the vicinal protons H-11 and H-12 (J_{trans} = 1−3 Hz, J_{cis} = 2−5 Hz).[36] The stereochemistry at C-8 might be expected to be the same as halicholactone (**12**) and neohalicholactone (**13**). For similar lactones such as mueggelones,[37,38] constanolactones,[19] and solandelactones,[39] the optical activity seems to be mostly modulated by the stereochemistry of the lactone ring. Therefore, all topsentolides (**1**−**7**), with the same sign of optical rotation, should share the same configuration of the lactone ring.

Topsentolide A_2 (**2**) was isolated as a colorless oil. Its HR-FABMS showed the [M + Na]$^+$ ion peak at *m/z* 341.2078 (Δ −1.5 mmu), which matched well with the expected molecular formula of $C_{20}H_{30}O_3Na$. Compared to **1**, the ^{1}H NMR data of **2** revealed an absence of one double bond in the side chain. The locations of double bonds at C-9 and C-14 were determined on the basis of COSY and HMBC correlations. The geometry of double bonds was defined as 5*Z*,9*E*, the same as that of **1**. The 14*Z* configuration was deduced from the allylic carbon (C-16) signal, which resonated at δ 27.0.[35] On the basis of the vicinal coupling constant between

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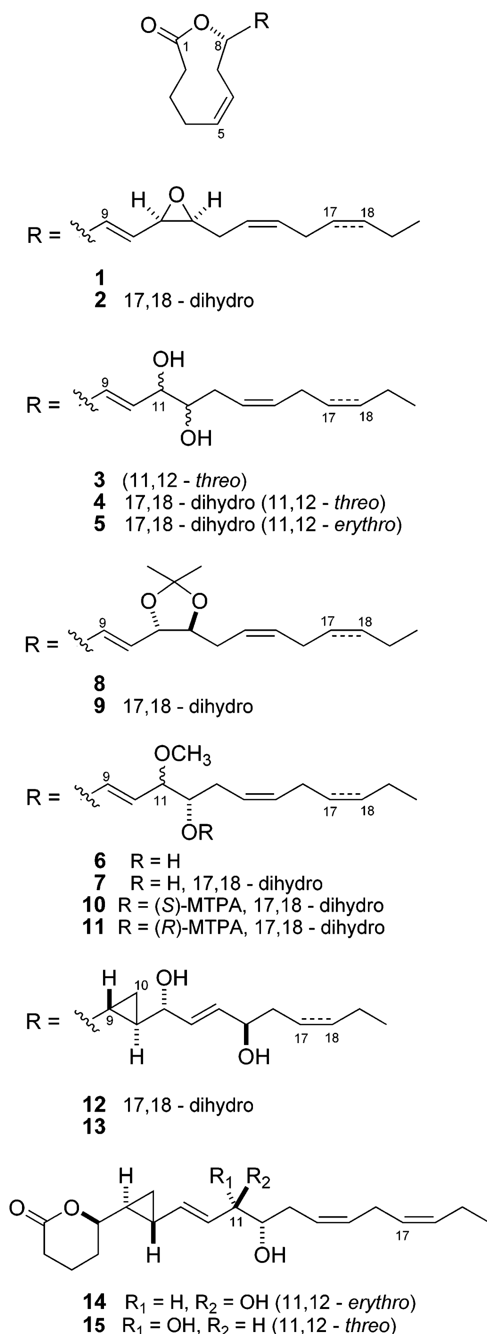
10.1021/np0503552 CCC: $33.50 © 2006 American Chemical Society and American Society of Pharmacognosy
Published on Web 03/16/2006



H-11 and H-12 ($J_{11,12}$ = 4.0 Hz), the relative configuration of the epoxy group was defined as *cis*.

Topsentolide B1 (**3**) was also isolated as a colorless oil. Its molecular formula was defined as $C_{20}H_{30}O_4$ with 6 degrees of unsaturation on the basis of FABMS and NMR data. Its HRFABMS showed the pseudomolecular ion peak at *m/z* 357.2076 ([M + Na]$^+$, Δ +3.4 mmu). Comparison of its ^{1}H NMR data with those of **1** indicated that it possessed the same nine-membered lactone ring and the identical number of double bonds. The major differences were observed in the oxymethine proton signals, which were shifted downfield to δ 3.97 (H-11) and 3.48 (H-12). The HSQC spectrum showed that the corresponding carbon signals of C-11 and C-12 were also shifted downfield to δ 74.2 and 74.3, respectively. These observations indicated that two hydroxyl groups were attached to C-11 and C-12, and this was further proved by the IR absorption band at 3331 cm^{-1}. In compound **1**, the vinylic protons H-9 and H-10 appeared as clear doublets of doublets with the coupling constant of 15.5 Hz, whereas in compound **3**, they are magnetically almost equivalent and appeared as a collapsed triplet with two small side peaks (higher order splitting). Therefore, the geometry of Δ^9 was indirectly deduced by the presence of an IR absorption for the *trans* (974 cm^{-1}) configuration. The geometry of Δ^5, Δ^{14}, and Δ^{17} was defined to be *cis*, the same as that of **1**. The relative stereochemistry of C-11 and C-12 in **3** was deduced to be *threo* (11*R**,12*R**) by comparison of the NMR data of its acetonide derivative (**8**) with those of analogous compounds, topsentolide B2 (**4**), constanolactone E (**14**), and constanolactone F (**15**)[19] (*vide infra*). The absolute stereochemistry could not be defined due to paucity of material.

Topsentolide B2 (**4**) was isolated as a colorless oil. Its molecular formula was established as $C_{20}H_{32}O_4$ on the basis of HRFABMS and NMR analyses. The HRFABMS spectrum showed the [M + Na]$^+$ ion peak at *m/z* 359.2185 (Δ −1.3 mmu). The almost identical NMR features except the absence of the signals of one double bond in the side chain suggested that compound **4** was a 17,18-dihydro analogue of **3**. This was further corroborated by the [M + Na]$^+$ ion, which was 2 amu higher than that of **3**, as well as the COSY and HMBC analyses. The geometry of double bonds and the relative stereochemistry of C-11 and C-12 were defined on the basis of its NMR and optical rotation data and were the same as those of **3**. The acetonide derivatives of compounds **3** and **4** were prepared for further investigation of the stereochemistry at the diol position. The large coupling constant ($J_{11,12}$ = 8.0 Hz) and the almost identical magnetic environment of the acetonide methyls (Δδ ≈ 0) revealed the diol configuration in derivatives **8** and **9** as *threo*.[19]

Topsentolide B3 (**5**) was also isolated as a colorless oil. Its FABMS and NMR data suggested the molecular formula $C_{20}H_{32}O_4$. The LRFABMS showed a fragment ion peak at *m/z* 319 [MH − H_2O]$^+$. The geometry of double bonds in **5** was defined to be the same as that of **2**. The identical molecular formula and ^{1}H NMR spectral features similar to those of topsentolide B2 (**4**) implied that they possess the same planar structure other than the stereochemistry at C-11 and C-12. As expected, there was difference in the chemical shifts of H-11 and H-12. Compared to those of compound **4**, the signals of H-11 (δ 4.41) and H-12 (δ 3.68) were shifted downfield. This observation was in line with the relationship between compounds **14** and **15**. Those of constanolactone E (**14**, *erythro*) were reported downfield-shifted compared to those of constanolactone F (**15**, *threo*). The difference in the coupling constants between compounds **4**/**3** ($J_{11,12}$ = 5.0 Hz) and **5** ($J_{11,12}$ = 4.0 Hz) also supported the *threo* and *erythro* relationship between them.[19] An attempt to prepare the acetonide derivative of compound **5** failed, even after increased catalyst and reaction time. The unsuccessful formation may be due to the *erythro* diol configuration and the unfavorable formation of the *cis* product due to steric hindrance.[40] Therefore, the diol stereochemistry in compound **5** was presumed to be *erythro* (11*R**,12*S**). The absolute stereochemistry at the diol position in compounds **3**−**5** could not be defined due to paucity of material. The diols from the same organism (**3**−**5**) with mixed stereochemistry are suspected to be artifacts produced by nonenzymatic hydrolysis of the corresponding epoxides (**1** and **2**).

Topsentolide C1 (**6**) was also isolated as a colorless oil. Its molecular formula was determined as $C_{21}H_{32}O_4$ on the basis of the HRFABMS and NMR data. The HRFABMS data of the [M + Na]$^+$ ion (*m/z* 371.2182, Δ −1.6 mmu) matched well with the expected molecular formula of $C_{21}H_{32}O_4Na$. Compound **6** showed almost the same NMR data as those of **5** except for a noticeable difference in those of the side chain. A new methoxyl signal (δ 3.30) in the ^{1}H NMR spectrum and the corresponding carbon signal (δ 56.0) in the HSQC spectrum were observed. The location of the methoxyl group at C-11 was confirmed by the HMBC correlation between the methoxy proton signal at δ 3.30 and the oxymethine carbon signal at δ 84.2 (C-11). The geometry of the double bonds was deduced to be the same as that in compound **1**. The stereochemistry of **6** could not be studied due to paucity of material, while it may be presumed to be the same as that of compound **7** (*vide infra*).

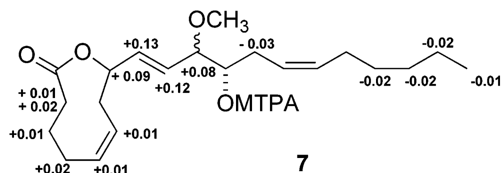


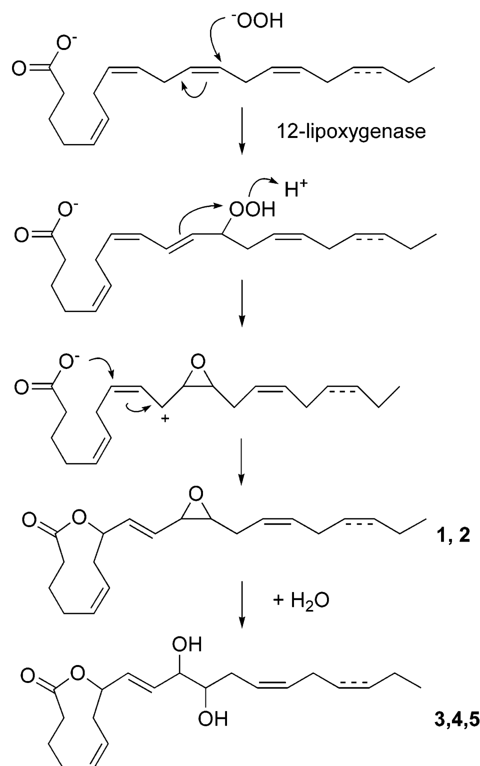
Figure 1. $\Delta\delta$ ($\delta_S - \delta_R$) values for the MTPA esters of compound **7**.

Topsentolide C_2 (**7**) was also isolated as a colorless oil. Its molecular formula was established as $C_{21}H_{34}O_4$ on the basis of HRFABMS and NMR analyses. The exact mass of the $[M + Na]^+$ ion (*m/z* 373.2341, Δ −1.4 mmu) matched well with the expected molecular formula of $C_{21}H_{34}O_4Na$. The NMR data of **7** were almost identical to those of **6** except for the absence of one double bond in the side chain, which implied that compound **7** was a 17,18-dihydro analogue of **6**. The $[M + Na]^+$ ion, which was 2 amu higher than that of **6**, as well as the COSY and HMBC correlations further confirmed the above interpretation. The absolute stereochemistry at C-12 was determined as *S* by the Mosher's method.[33] The (*S*)- and (*R*)-MTPA esters (**10** and **11**) were prepared, and $\Delta\delta$ ($\delta_S - \delta_R$) values for all assignable protons were observed (Figure 1). Compounds **6** and **7**, the methyl ether analogues, are suspected to be artifacts formed during the process of extraction with MeOH.

The plausible biosynthetic pathway of **1** and **2** is thought to be analogous to those proposed for halicholactone (**12**) and neohalicholactone (**13**).[23] However, it would be initiated by 12-lipoxygenase rather than 15-lipoxygenase (Scheme 1).

Topsentolides (**1**−**7**) were evaluated for cytotoxicity against a panel of five human solid tumor cell lines and showed moderate cytotoxicity (Table 3). Compound **1** exhibited the most potent cytotoxic profile to all tumor cell lines tested. Compounds **4**, **5**, and **7** showed selective cytotoxicity to SK-OV-3 and SK-MEL-2 cell lines. Therefore, the cytotoxicity of topsentolides seems to be of interest for further investigation.

Scheme 1. Plausible Biosynthetic Pathway of Topsentolides A_1−B_3 (**1**−**5**)



Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. The IR spectra were obtained using a JASCO FT/IR-410 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity Plus 300, Varian Unity Inova 400, and Varian Unity Inova 500 spectrometers. Chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS data were obtained on a JEOL JMS SX-102A spectrometer. HRFABMS data were obtained on a JEOL JMS SX-101A spectrometer. HPLC was performed with a C18-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å) and a YMC packed ODS column (250 × 10 mm, 5 μm, 120 Å) using a Shodex RI-71 detector.

Animal Material. The sponge was collected by hand using scuba (20 m depth) in October 2002 off the coast of Jeju Island, Korea. The collected sample was frozen immediately. This specimen was identified as *Topsentia* sp. It was irregular and massive with a lobe or

Table 1. ^{1}H NMR Data of Compounds **1** and **3**−**6** (CD_3OD, 500 MHz)[a]

position	1	3	4	5	6
2	2.20 (ddd, 11.5, 6.0, 2.0)	2.20 (ddd, 11.5, 6.0, 2.0)	2.20 (ddd, 11.5, 6.0, 2.0)	2.20 (ddd, 11.5, 6.0, 2.0)	2.20 (ddd, 11.5, 6.0, 2.0)
	2.35 (m)	2.34 (m)	2.35 (m)	2.35 (dd, 11.5, 7.5)	2.36 (dd, 11.5, 7.5)
3	1.76 (m)	1.76 (m)	1.76 (m)	1.76 (m)	1.76 (m)
	2.07 (m)	2.08 (m)	2.08 (m)	2.08 (m)	2.08 (m)
4	2.05 (m)	2.05 (m)	2.05 (m)	2.05 (m)	2.05 (m)
	2.52 (m)	2.52 (m)	2.52 (m)	2.52 (m)	2.52 (m)
5	5.47 (m)	5.47 (m)	5.46 (m)	5.47 (m)	5.45 (m)
6	5.45 (m)	5.48 (m)	5.48 (m)	5.47 (m)	5.45 (m)
7	2.15 (ddd, 13.5, 7.5, 1.5)	2.12 (m)	2.15 (m)	2.15 (ddd, 13.5, 7.0, 1.5)	2.16 (m)
	2.42 (m)	2.44 (m)	2.42 (m)	2.42 (m)	2.45 (m)
8	5.28 (m)	5.25 (m)	5.25 (m)	5.25 (m)	5.26 (m)
9	6.05 (dd, 15.5, 6.0)	5.85 (m)	5.85 (m)	5.82 (dd, 15.5, 5.0)	5.86 (dd, 15.5, 5.5)
10	5.75 (ddd, 15.5, 7.0, 1.5)	5.86 (m)	5.86 (m)	6.01 (ddd, 15.5, 8.5, 1.5)	5.69 (dd, 15.5, 6.5)
11	3.48 (dd, 7.0, 4.0)	3.97 (dd, 5.0, 4.5)	3.97 (dd, 5.0, 4.0)	4.41 (dd, 8.5, 4.0)	3.51 (m)
12	3.12 (td, 6.5, 4.0)	3.48 (dt, 8.5, 5.0)	3.45 (dt, 7.5, 5.0)	3.68 (ddd, 7.5, 5.5, 4.0)	3.50 (m)
13	2.26 (dd, 10.5, 6.5)	2.15 (m)	2.15 (m)	2.18 (m)	2.14 (m)
	2.36 (m)	2.35 (m)	2.43 (m)	2.40 (m)	2.33 (m)
14	5.43 (m)	5.45 (m)	5.44 (m)	5.43 (m)	5.47 (m)
15	5.45 (m)	5.42 (m)	5.49 (m)	5.50 (m)	5.43 (m)
16	2.80 (m)	2.80 (m)	2.05 (m)	2.05 (m)	2.78 (m)
17	5.27 (m)	5.32 (m)	1.36 (m)	1.38 (m)	5.28 (m)
18	5.38 (m)	5.35 (m)	1.33 (m)	1.38 (m)	5.35 (m)
19	2.07 (m)	2.09 (m)	1.30 (m)	1.30 (m)	2.05 (m)
20	0.96 (t, 7.5)	0.96 (t, 7.5)	0.89 (t, 6.5)	0.90 (t, 7.0)	0.96 (t, 7.0)
OCH_3					3.30 (s)

[a] Multiplicities and coupling constants are in parentheses.

Table 2. ^{13}C NMR Data of Compounds **1** and **3**–**6** (CD_3OD)

position	**1**[a]	**3**[b]	**4**[b]	**5**[b]	**6**[b]
1	175.6	174.5	175.6	174.0	175.0
2	34.4	33.2	33.2	33.5	33.2
3	27.5	26.5	26.5	26.2	26.3
4	26.3	25.3	25.8	25.0	25.0
5	136.2	134.9	134.8	135.0	134.9
6	125.5	124.9	125.5	124.0	124.7
7	35.3	34.4	34.0	34.0	34.2
8	73.8	72.8	72.6	72.1	72.7
9	135.3	130.8	131.0	132.0	132.8
10	127.2	130.8	131.0	129.0	129.0
11	57.3	74.2	74.7	65.8	84.2
12	59.3	74.3	74.5	74.0	73.0
13	26.6	30.4	31.2	32.0	26.6
14	124.9	126.0	126.0	125.0	124.9
15	132.0	129.7	131.5	132.0	132.0
16	27.1	25.6	28.0	27.0	27.0
17	127.9	126.8	29.8	29.0	126.3
18	133.0	131.4	31.0	31.0	131.5
19	21.5	20.5	22.8	22.4	21.0
20	14.6	13.4	13.4	13.0	13.8
OCH_3					56.0

[a] Spectrum was measured at 75 MHz. [b] Signals were assigned by HMBC and HSQC experiments (500 MHz).

Table 3. Cytotoxicity Data of Compounds **1**−**7**[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	4.8	4.6	2.0	3.6	2.4
2	8.9	11.4	6.5	12.1	11.4
3	17.5	12.3	12.4	14.4	14.7
4	4.7	4.2	4.4	4.5	11.6
5	5.6	6.5	4.9	7.8	7.6
6	5.2	13.0	8.6	6.1	4.6
7	4.8	5.1	4.3	7.6	4.4
doxorubicin	0.04	0.13	0.04	0.06	0.06

[a] Data expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT 15, human colon cancer.

semispherical shape of size up to 11 × 16 cm wide and 3 cm thick. The surface of the body was hispid with large oxea, and the texture was tough. It was yellow in life. The skeleton has megascleres, large oxea (2200−3000 μm × 50 μm), medium oxea (1000−1500 μm × 50 μm), and small oxea (70−85 μm × 5 μm). A voucher specimen (registry no. Spo. 46) is deposited in the Natural History Museum, Hannam University, Daejon, Korea.

Extraction and Isolation Procedure. The frozen sponge (8.2 kg) was exhaustively extracted with MeOH at room temperature to afford the MeOH extract, which was partitioned between H_2O and CH_2Cl_2. The latter was further partitioned between aqueous MeOH and *n*-hexane to yield the bioactive aqueous MeOH extract, which showed cytotoxicity to brine shrimp larvae (LD_{50} 30 μg/mL).[32] The aqueous MeOH extract was subjected to stepped gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 400/500 mesh) eluting with 50 to 100% MeOH/H_2O to afford 23 fractions. Fraction 11, one of the bioactive fractions (LD_{50} 6.6 μg/mL), was subjected to reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 80% MeOH to afford six fractions. Compounds **1** (5.6 mg) and **7** (0.5 mg) were obtained by purifying subfraction 1 on reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 92% MeOH. Compound **5** (2.3 mg) was obtained by purifying subfraction 3 on reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å) eluting with 80% MeOH. Fraction 8 was subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å) eluting with 71% MeOH to yield compounds **3** (1.9 mg) and **4** (1.6 mg). Fraction 12 was subjected to reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 80% MeOH, 5% MeCN, and 15% H_2O to afford seven fractions. Subfraction 4 was subjected to reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), with 90% MeOH as mobile phase, to yield compound **2** (2.2 mg). Fraction 9 was subjected to a reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 78% MeOH to afford nine subfractions, and subfractions 5 and 8 were subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å) eluting with 78% MeOH and 77% MeOH, respectively, to afford compounds **4** (0.6 mg) and **6** (0.8 mg), respectively. Fraction 10, one of the bioactive fractions (LD_{50} 4.9 μg/mL), was subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å) eluting with 80% MeOH to afford eight subfractions. Compound **7** (0.8 mg) was obtained by purifying subfraction 8 on reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å) eluting with 95% MeOH.

Acetonides of Topsentolides B_1 (3) and B_2 (4). To a 2,2-dimethoxypropane solution (0.5 mL) of compound **3** (1.0 mg, 2.8 μmol) was added catalyst *p*-toluenesulfonic acid (0.8 mg, 4.6 μmol). After being kept at room temperature for 24 h, the reaction was quenched with triethylamine (50 μL) and evaporated under vacuum. The products were dissolved in MeOH and purified by reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 90% MeOH to yield acetonide product (**8**). Compound **4** (1.0 mg, 2.8 μmol) was treated similarly and yielded acetonide product (**9**).

MTPA Esters of Topsentolide C_2 (7). Compound **7** (1.0 mg, 2.7 μmol) was treated with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (4 μL) in dry pyridine (50 μL) to yield (*S*)-MTPA ester (**10**) and (*R*)-MTPA ester (**11**), respectively. After being kept at room temperature for 24 h, the reaction mixtures were evaporated to dryness under vacuum. The products were dissolved in MeOH and purified by reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 90% MeOH.

Topsentolide A_1 (1): colorless oil; $[\alpha]^{24}_D$ +59.4 (*c* 0.11, MeOH); IR (KBr) ν_{max} 1735, 970, 668 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 339 $[M + Na]^+$, HRFABMS *m/z* 339.1927 (calcd for $C_{20}H_{28}O_3Na$, 339.1936).

Topsentolide A_2 (2): colorless oil; $[\alpha]^{24}_D$ +84.6 (*c* 0.27, MeOH); ^1H NMR (CD_3OD, 500 MHz) δ 6.03 (1H, dd, J = 15.5, 5.5 Hz, H-9), 5.75 (1H, ddt, J = 15.5, 6.5, 1.5 Hz, H-10), 5.52 (1H, m, H-15), 5.47 (2H, m, H-5, 6), 5.39 (1H, m, H-14), 5.28 (1H, m, H-8), 3.47 (1H, dd, J = 6.5, 4.0 Hz, H-11), 3.11 (1H, td, J = 6.5, 4.0 Hz, H-12), 2.52 (1H, m, H-4), 2.42 (1H, m, H-7), 2.35 (1H, m, H-2), 2.34 (1H, m, H-13), 2.24 (1H, m, H-13), 2.20 (1H, m, H-2), 2.15 (1H, ddd, J = 12.5, 7.5, 1.5 Hz, H-7), 2.07 (1H, m, H-3), 2.05 (3H, m, H-4, 16), 1.76 (1H, m, H-3), 1.35 (2H, m, H-17), 1.31 (2H, m, H-19), 1.28 (2H, m, H-18), 0.90 (3H, t, J = 7.5 Hz, H-20); ^{13}C NMR (CD_3OD, assigned by HMBC and HSQC, 500 MHz) δ 174.5 (C, C-1), 134.8 (CH, C-5), 133.8 (CH, C-9), 132.5 (CH, C-15), 126.0 (CH, C-10), 124.4 (CH, C-6), 123.8 (CH, C-14), 72.5 (CH, C-8), 58.4 (CH, C-12), 56.0 (CH, C-11), 34.2 (CH_2, C-7), 33.2 (CH_2, C-2), 30.2 (CH_2, C-18), 29.0 (CH_2, C-17), 27.0 (CH_2, C-16), 26.3 (CH_2, C-3), 25.6 (CH_2, C-13), 25.0 (CH_2, C-4), 22.3 (CH_2, C-19), 12.8 (CH_3, C-20); FABMS *m/z* 319 $[M + H]^+$, 341 $[M + Na]^+$; HRFABMS *m/z* 341.2078 (calcd for $C_{20}H_{30}O_3Na$, 341.2093).

Topsentolide B_1 (3): colorless oil; $[\alpha]^{23}_D$ +38.2 (*c* 0.27, MeOH); IR (KBr) ν_{max} 3331, 1735, 974, 664 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 357 $[M + Na]^+$; HRFABMS *m/z* 357.2076 (calcd for $C_{20}H_{30}O_4Na$, 357.2042).

Topsentolide B_2 (4): colorless oil; $[\alpha]^{23}_D$ +9.8 (*c* 0.27, MeOH); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 359 $[M + Na]^+$; HRFABMS *m/z* 359.2185 (calcd for $C_{20}H_{32}O_4Na$, 359.2198).

Topsentolide B_3 (5): colorless oil; $[\alpha]^{24}_D$ +44.2 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3279, 1735, 969, 666 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 319 $[MH - H_2O]^+$.

Topsentolide C_1 (6): colorless oil; $[\alpha]^{23}_D$ +40.8 (*c* 0.27, MeOH); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 371 $[M + Na]^+$; HRFABMS *m/z* 371.2182 (calcd for $C_{21}H_{32}O_4Na$, 371.2198).

Topsentolide C_2 (7): colorless oil; $[\alpha]^{23}_D$ +14.5 (*c* 0.27, MeOH); ^1H NMR (CD_3OD, 500 MHz) δ 5.86 (1H, dd, J = 15.5, 5.5 Hz, H-9), 5.67 (1H, ddt, J = 15.5, 7.5, 1.5 Hz, H-10), 5.47 (2H, m, H-5, 6), 5.45 (2H, m, H-14, 15), 5.26 (1H, m, H-8), 3.51 (1H, m, H-11), 3.50 (1H, m, H-12), 3.30 (3H, s, OCH_3), 2.52 (1H, m, H-4), 2.45 (1H, m, H-7), 2.35 (1H, dd, J = 11.5, 7.5 Hz, H-2), 2.34 (1H, m, H-13), 2.20 (1H, ddd, J = 11.5, 6.0, 2.0 Hz, H-2), 2.15 (1H, m, H-7), 2.14 (1H, m, H-13), 2.07 (1H, m, H-3), 2.05 (3H, m, H-4, 16), 1.76 (1H, m, H-3), 1.35 (2H, m, H-17), 1.30 (2H, m, H-19), 1.28 (2H, m, H-18), 0.89 (3H, t, J = 7.0 Hz, H-20); ^{13}C NMR (CD_3OD, assigned by HMBC and HSQC, 500 MHz) δ 174.5 (C, C-1), 134.9 (CH, C-5), 132.8 (CH, C-9), 131.5 (CH, C-15), 129.0 (CH, C-10), 125.4 (CH, C-14), 124.7 (CH, C-6), 84.5 (CH, C-11), 73.6 (CH, C-12), 72.7 (CH, C-8), 56.0

(OCH_3), 34.2 (CH_2, C-7), 33.2 (CH_2, C-2), 31.5 (CH_2, C-18), 30.4 (CH_2, C-13), 28.7 (CH_2, C-17), 27.0 (CH_2, C-16), 26.3 (CH_2, C-3), 25.0 (CH_2, C-4), 22.4 (CH_2, C-19), 12.8 (CH_3, C-20); FABMS *m/z* 373 $[M + Na]^+$; HRFABMS *m/z* 373.2341 (calcd for $C_{21}H_{34}O_4Na$, 373.2355).

Acetonide of Topsentolide B_1 (8): colorless oil; ^1H NMR (CD_3-OD, 400 MHz) δ 5.93 (1H, dd, J = 16.0, 6.0 Hz, H-9), 5.76 (1H, dd, J = 16.0, 7.2 Hz, H-10), 5.47−5.31 (6H, m, H-5, 6, 14, 15, 17, 18), 5.24 (1H, m, H-8), 4.07 (1H, t, J = 8.0 Hz, H-11), 3.72 (1H, dt, J = 8.0, 6.4 Hz, H-12), 2.80 (2H, m, H-16), 2.51 (1H, m, H-4), 2.44 (1H, m, H-7), 2.36 (2H, m, H-2, 13), 2.21 (1H, m, H-2), 2.15 (2H, m, H-7, 13), 2.09 (1H, m, H-19), 2.07 (1H, m, H-3), 2.05 (1H, m, H-4), 1.76 (1H, m, H-3), 1.37 (6H, s, $2CH_3$), 0.96 (3H, t, J = 7.6 Hz, H-20).

Acetonide of Topsentolide B_2 (9): colorless oil; ^1H NMR (CD_3-OD, 400 MHz) δ 5.91 (1H, dd, J = 15.6, 6.0 Hz, H-9), 5.75 (1H, ddt, J = 15.6, 7.6, 1.2 Hz, H-10), 5.52−5.39 (4H, m, H-5, 6, 14, 15), 5.23 (1H, m, H-8), 4.07 (1H, t, J = 8.0 Hz, H-11), 3.71 (1H, dt, J = 8.0, 5.6 Hz, H-12), 2.52 (1H, m, H-4), 2.43 (2H, m, H-7, 13), 2.35 (1H, m, H-2), 2.20 (1H, m, H-2), 2.12 (2H, m, H-7, 13), 2.08−2.05 (4H, m, H-3, 4, 16), 1.76 (1H, m, H-3), 1.37 (6H, s, $2CH_3$), 1.35−1.28 (6H, m, H-17, 18, 19), 0.89 (3H, t, J = 6.8 Hz, H-20).

(*S*)-MTPA ester (10): colorless oil; ^1H NMR (CD_3OD, 400 MHz) δ 5.95 (1H, dd, J = 15.6, 5.2 Hz, H-9), 5.64 (1H, ddt, J = 15.6, 7.2, 1.6 Hz, H-10), 5.48 (2H, m, H-5, 6), 5.27 (1H, m, H-8), 3.77 (1H, t, J = 7.2 Hz, H-11), 3.56 (1H, m, H-12), 2.51 (1H, m, H-4), 2.34 (1H, m, H-2), 2.31 (1H, m, H-13), 2.22 (1H, m, H-2), 1.78 (1H, m, H-3), 1.32 (2H, m, H-17), 1.28 (2H, m, H-19), 1.26 (2H, m, H-18), 0.88 (3H, t, J = 7.2 Hz, H-20).

(*R*)-MTPA ester (11): colorless oil; ^1H NMR (CD_3OD, 400 MHz) δ 5.82 (1H, dd, J = 16.0, 6.4 Hz, H-9), 5.52 (1H, dd, J = 16.0, 6.8 Hz, H-10), 5.47 (2H, m, H-5, 6), 5.18 (1H, m, H-8), 3.69 (1H, t, J = 6.8 Hz, H-11), 3.53 (1H, m, H-12), 2.49 (1H, m, H-4), 2.34 (1H, m, H-13), 2.32 (1H, m, H-2), 2.21 (1H, m, H-2), 1.77 (1H, m, H-3), 1.34 (2H, m, H-17), 1.30 (2H, m, H-19), 1.28 (2H, m, H-18), 0.89 (3H, t, J = 7.2 Hz, H-20).

Acknowledgment. This work was supported by a grant from the Basic Research Program of the Korea Science and Engineering Foundation (R01-2004-000-10467-0).

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NP0503552