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New Cytotoxic Polyacetylenes from the Marine Sponge *Petrosia*

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Abstract: New polyacetylenic alcohols with a C_{45} carbon skeleton (**2**) and with an enone moiety in the alkyl chain (C_{46}, **1**) were isolated from the marine sponge *Petrosia* sp. The gross structures of **1** and **2** were established by spectral methods, and the absolute stereochemistry was determined by the modified Mosher's method. Compounds **1** and **2** displayed considerable cytotoxicity against a small panel of human solid tumor cell lines. Significant inhibitions on DNA replication by **1** and **2** were also observed which could be explanative of their cytotoxicity.

Polyacetylenes with significant biological activity have been isolated from marine sponges of the genus *Petrosia*.[1] Novel C_{30} and C_{46} polyacetylenic alcohols possessing significant cytotoxic activity against a small panel of human tumor cell lines have been reported by the authors.[2,3] In continuation of our search for more bioactive novel polyacetylenes from the same sponge, *Petrosia* sp. (family Petrosiidae), an unprecedented C_{45} analogue has been isolated, which was given the trivial name nor-3*S*,14*S*-petrocortyne A (**2**). A new C_{46} analogue named petrotetrayndiol C (**1**) was also isolated. The structure elucidation, stereochemistry, and biological activities of these compounds are described herein.

A methanol extract of the frozen marine sponge showed cytotoxicity in the in vitro P388 assay. Guided by this assay, the methanol extract was successively fractionated employing reversed-phase flash column chromatography, ODS and CN HPLC to afford **1** and **2** as the causative constituents. Nor-3*S*,14*S*-petrocortyne A (**2**) is chemically unique for its linear C_{45} skeleton. Except for the C_{47} polyacetylenic acids with a terminal carboxyl group such as nepheliosyne A[4] and osirisynes,[5] most of the long-chain ($> C_{40}$) polyacetylenes isolated from marine sponges possess C_{46} linear carbon skeletons. While **2** possessed the same partial structures and absolute configurations as 3*S*,14*S*-petrocortyne A,[3] its C_{45} skeleton is unprecedented.

Petrotetrayndiol C (**1**), which possess a characteristic enone moiety, was isolated as a colorless oil. The molecular formula of **1** was established as $C_{46}H_{68}O_3$ on the basis of HRFABMS and NMR data (Tables 1 and 2). The ^{1}H and ^{13}C NMR data were reminiscent of those of 3*S*,-14*S*-petrocortyne A.[3] However, certain differences in the ^{1}H NMR data were noticed that indicated one of the cis double bonds had changed to a trans double bond (δ 6.12, 1H, dd, J = 15.9, 4.7 Hz; δ 6.93, 1H, dt, J = 15.9, 7.0 Hz). And additional signals for a methylene group were observed at δ 2.59 which might be located adjacent to a carbonyl group. This carbonyl function was also detected in the ^{13}C NMR spectrum as a signal at δ 203.7, accompanied by a substantial downfield shift (δ 149.6) of one of the trans olefinic carbon signals, indicating that this carbonyl group is located at the allylic position to the trans double bond comprising an enone system. By analysis of the COSY spectral data of **1**, two terminal partial structures **a** and **b** have been easily deduced (Figure 1). The structure of long-chain subunit **c** was deduced by careful examination of COSY, TOCSY, and FAB-CID tandem mass spetral data (Figures 1 and 2). The long-range ^{1}H−^{1}H correlations between the allylic (H-23) protons and the α-keto methylene protons (H-26) were clearly observed in TOCSY spectrum of **1**. Another pair of allylic methylene protons (H-20) showed correlation with methylene protons (H-17) next to acetylenic function. Weak correlations between olefinic protons (H-21,22) and H-17, H-26, respectively, were also observed. Thus **1** might be biogenetically related to 3*S*,14*S*-petrocortyne A, of which the double bond was oxidized at C-27 with subsequent 1,2-migration of the double bond. The gross structure deduced by NMR spectral analysis was further confirmed by FAB−CID tandem mass spectrometry. The fragmentation of **1** showed a characteristic pattern of a linear aliphatic chain compound. A prominent fragment produced by loss of H_2O from the [M +

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Table 1. ^{1}H NMR Data of **1** and **2** (CD_3OD)[a]

position	1	2
1	2.87 (d, 2.2)	2.86 (d, 2.4)
3	4.74 (br d, 6.2)	4.74 (br d, 6.3)
4	5.56 (dd, 15.2, 6.2)	5.55 (ddt, 15.1, 6.3, 1.5)
5	5.85 (dt, 15.2, 6.8)	5.85 (dtd, 15.1, 6.8, 1.0)
6	2.07 (m)	2.02−2.05 (m)
7	1.41 (m)	1.30−1.51 (m)
8−9	1.30−1.60 (m)	1.30−1.51 (m)
10	1.50 (m)	1.30−1.51 (m)
11	2.21 (td, 6.7, 1.7)	2.21 (td, 4.9, 2.0)
14	5.01 (brs)	5.00 (quint, 2.0)
17	2.22 (td, 6.7, 1.7)	2.21 (td, 4.9, 2.0)
18	1.50 (m)	1.30−1.51 (m)
19	1.30−1.60 (m)	1.30−1.51 (m)
20	2.06 (m)[b]	2.02−2.05 (m)
21−22	5.37 (m), 5.39 (m)	5.33−5.37 (m)
23	2.11 (m)[b]	2.02−2.05 (m)
24	1.30−1.60 (m)	1.30−1.51 (m)
25	1.59 (m)	1.30−1.51 (m)
26	2.59 (q, 7.2)	2.02−2.05 (m)
27	–	5.33−5.37 (m)
28	6.12 (dd, 15.9, 4.7)	5.33−5.37 (m)
29	6.93 (dt, 15.9, 7.0)	2.02−2.05 (m)
30	2.26 (quint, 7.9)	1.30−1.51 (m)
31	1.56 (m)	1.30−1.51 (m)
32−40	1.30−1.60 (m)	1.30−1.51 (m)
41	1.42 (m)	2.31 (q, 6.8)
42	2.32 (q, 7.3)	5.99 (dtd, 10.7, 7.3, 1.0)
43	5.99 (dt, 10.7, 7.6)	5.44 (ddt, 10.7, 2.0, 1.0)
44	5.44 (br d, 10.7)	–
45	–	3.39 (br d, 2.0)
46	3.40 (br d, 1.9)	

[a] Multiplicities and coupling constants in parentheses. Compound **1** was measured at 600 MHz, and compound **2** was measured at 200 MHz. [b] Assignments may be interchanged.

Table 2. ^{13}C NMR Data of **1** and **2** (CD_3OD)[a]

position	1	2
1	74.5	74.5
2	84.8	84.8
3	63.2	63.2
4	130.7[b]	130.5[b]
5	134.1	134.0
6	32.9	32.9
7−10	29.5−30.7	29.2−30.9
11	19.2[d]	19.2[d]
12	84.5[c]	84.5[c]
13	79.9	79.9[f]
14	52.6	52.6
15	79.9	79.8[f]
16	84.4[c]	84.4[c]
17	19.3[d]	19.3[d]
18−19	29.5−30.7	29.2−30.9
20	27.7[e]	27.7[e]
21	131.5[b]	130.75[b]
22	131.3[b]	131.1[b]
23	27.6[e]	28.1[e]
24	29.5−30.7	29.2−30.9
25	23.7	29.2−30.9
26	40.5	28.04[e]
27	203.7	130.9[b]
28	130.3[b]	130.74[b]
29	149.6	28.03[e]
30	33.5	29.2−30.9
31−40	29.5−30.7	29.2−30.9
41	29.5−30.7	31.1
42	31.1	146.4
43	146.4	109.3
44	109.3	81.2
45	81.2	82.7
46	82.7	

[a] Compound **1** was measured at 150 MHz, and compound **2** was measured at 50 MHz. [b−f]Assignments with the same superscript in the same column may be interchanged.

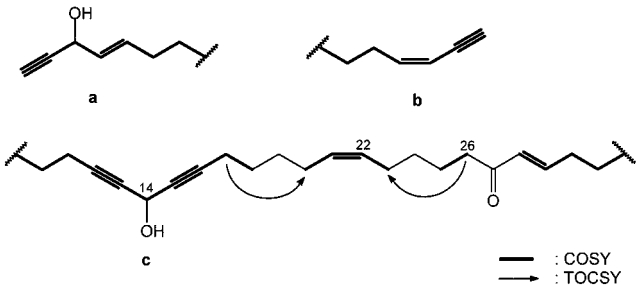

Figure 1. Partial structures of **1** with diagnostic COSY and TOCSY correlations (600 MHz).

Na]$^+$ was observed at *m*/*z* 673. Major fragmentations of the [M + Na]$^+$ of **1** were observed as odd mass ions due to a remote charge fragmentation which is characteristic of the collisional activation of an alkali-metal-cationized ion.[6] The terminal allylic cleavage was observed as a base peak at *m*/*z* 625. From this base fragment, sequential losses of methylene units continued until the fragmentation at the double bond. Allylic cleavages were observed as enhanced peaks at *m*/*z* 471 and 307. Fragment ions of *m*/*z* 443 and 431 were observed as relatively weak peaks, with 12 amu difference, augmenting the evidence for the location of the double bond.[7] The FAB−CID tandem mass spectrometry was found useful for determining double bond location of these polyacetylenic alcohols since the migration of the double bonds was significantly suppressed. The absolute stereochemistry of **1** was determined by the modified Mosher's method as (3*S*,14*S*), which is the same as that of 3*S*,14*S*-petrocortyne A (Table 3).

Nor-3*S*,14*S*-petrocortyne A (**2**) was isolated as a yellow oil. The ^{1}H and ^{13}C NMR data of **2** were indistinguishable from those of 3*S*,14*S*-petrocortyne A (Tables 1 and 2). However, when coeluted in HPLC it clearly showed distinct retention time from that of 3*S*,14*S*-petrocortyne A. In the LRFABMS data it showed the [M + Na]$^+$ at *m*/*z* 663 which is 14 amu less than that of 3*S*,14*S*-petrocortyne A. The molecular formula of **2** was established as $C_{45}H_{68}O_2$ based on the HRFABMS and NMR data. The gross structure was thus deduced as shown in Figure 2, and it was further confirmed by FAB-CID tandem mass spectrometry. The fragmentation of **2** showed a characteristic pattern of a linear aliphatic chain compound as in the case of **1**. A prominent fragment generated by loss of H_2O from the [M + Na]$^+$ was observed at *m*/*z* 645. The terminal allylic cleavage was observed as a base peak at *m*/*z* 597. From this base fragment, sequential losses of methylene units continued until the fragmentation at the double bond. Allylic cleavages were observed as enhanced peaks at *m*/*z* 443, 389, 361, and 307. Each fragment at *m*/*z* 415 and 333 showed 12 amu difference from the next lower mass ions as relatively weak peaks augmenting the evidence for the location of the double bonds. The absolute stereochemistry of **2** was determined by the modified Mosher's method as (3*S*,14*S*), which is the same as those of **1** and 3*S*,14*S*-petrocortyne A (Table 3).

The cytotoxicities of **1** and **2** were comparable to that of cisplatin (Table 4). Compounds **1** and **2** further displayed significant inhibitions on the DNA replication in SV40, which could be explanative of their cytotoxicity. The % inhibitions of **1** on the DNA replication at various concentrations were 45 (125 μM), 46 (250 μM), and 81 (500 μM), those for **2** were 12 (125 μM), 47 (250 μM), and 70 (500 μM).

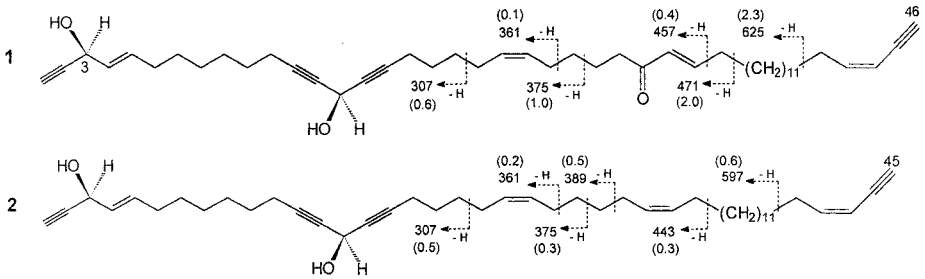

Figure 2. Key FAB-CID tandem mass fragmentations of $[M + Na]^+$ of **1** and **2** (% relative abundance).

Table 3. $\Delta\delta$ ($\delta_S - \delta_R$) Values (ppm) Obtained for the MTPA Esters of **1** and **2**[a]

position	**1**	**2**
1	+0.04	+0.04
4	−0.10	−0.10
5	−0.07	−0.06
11	−0.03	−0.03
17	+0.01	+0.01

[a] Spectra recorded at 200 MHz.

Table 4. *In Vitro* Cytotoxicity Data of Compounds **1** and **2** Against a Panel of Human Solid Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	>10.0	4.2	4.1	12.7	5.7
2	7.3	4.4	3.8	6.1	3.5
Cisplatin	0.4	0.6	0.9	0.2	1.8

[a] Data as expressed in ED_{50} values (μg/mL). A549, human lung carcinoma; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200 and DMX600 instruments. Chemical shifts are reported with reference to the respective residual solvent peaks (δ 3.30 and 49.0 for CD_3OD). FABMS data were obtained using a JEOL JMS-HX110/110A. HPLC was performed with a YMC ODS-H80 and YMC CN column using a Shodex RI-71 detector.

Animal Material. The sponge *Petrosia* sp. was collected in July 1995 (15−25 m depth), off Komun Island, Korea.[2] A voucher specimen (J95K-11) was deposited in the Natural History Museum, Hannam University, Taejeon, Korea.

***In Vitro* SV40 DNA Replication.** The reaction mixtures (40 μL) included 40 mM creatine phosphate-di-Tris salt (pH 7.7), 1 μg of creatine kinase, 7 mM $MgCl_2$, 0.5 mM DTT, 4 mM ATP, 200 μM UTP, GTP, and CTP, 100 μM dATP, dGTP, and dCTP, 25 μM [^{3}H]dTTP (300 cpm/pmol), 0.6 μg of SV40 T-Ag, 0.3 μg of SV40 origin-containing DNA (pUC), and the indicated amounts of replication proteins. The reactions ran for 90 min at 37 °C, after which the acid-insoluble radioactivity was measured. Replication products were analyzed using [α-^{32}P]dATP (30,000 cpm/pmol) instead of [^{3}H]dTTP in the reactions just described. After incubation, the reactions were stopped by the addition of 40 μL of a solution containing 20 mM EDTA, 1% sodium dodecyl sulfate, and *Escherichia coli* tRNA (0.5 mg/mL). One-tenth of the reaction mixture was used to measure the acid-insoluble radioactivity. DNA was isolated and electrophoretically separated in a 1.0% agarose gel for 12−14 h at 2 V/cm. The gel was subsequently dried and exposed to X-ray film.

Extraction and Isolation. The frozen sponge (14.5 kg) was extracted with MeOH at room temp. The MeOH solubles were fractionated between water and CH_2Cl_2. The CH_2Cl_2 solubles were further partitioned between 90% methanol and *n*-hexane to yield 58.15 and 61.5 g of residues, respectively. The 90% methanol fraction was then partitioned again between water and CH_2Cl_2 to afford 34 g of the CH_2Cl_2 residue which was subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh), eluting with a solvent system of 25 → 0% H_2O/MeOH followed by acetone, to obtain eight fractions. These fractions were evaluated for activity in the in vitro P388 assay (MTT method). Fraction 7 (7 g) was active in the P388 assay (ED_{50} 4.7 μg/mL, doxorubicin 4.0 μg/mL). Guided by the P388 assay, fraction 7 was further separated by a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh), eluting with 10 → 0% H_2O/MeOH, ethyl acetate, and chloroform to produce 13 fractions. Fraction 7−7 (1.283 g, ED_{50} 0.5 μg/mL, doxorubicin 2.0 μg/mL) was further separated on the same flash column eluting with 16.7 → 0% H_2O/MeOH to yield fraction 7−7−5 (1.073 g, ED_{50} 4.5 μg/mL, doxorubicin 6.0 μg/mL), which upon another reversed-phase flash column chromatography eluting with 4.8% H_2O/MeOH afforded fraction 7−7−5−4 (484 mg, ED_{50} 0.5 μg/mL, doxorubicin 6.0 μg/mL). Fraction 7−7−5−4 was separated on a reversed-phase HPLC (YMC ODS−H80, 250 × 20 mm i.d., 4 μm, 80 Å) eluting with 100% MeOH to afford crude compounds **1** and **2**. Pure compounds **1** (0.96 mg) and **2** (10.53 mg) were obtained upon purification on a CN column (YMC CN, 250 × 10 mm i.d., 5 μm, 120 Å) eluting with 37% and 33.3% aqueous CH_3CN, respectively.

Petrotetrayndiol C (1): colorless oil; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRFABMS *m/z* 691 $[M + Na]^+$, HRFABMS *m/z* 691.5054 (calcd. for $C_{46}H_{68}O_3Na$, 691.5066).

Nor-3*S*,14*S*-petrocortyne A (2): yellow oil; $[\alpha]^{23}_D$ +10° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (3.9) nm; IR (film) ν_{max} 3297, 2925, 2853, 1452, 995 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRFABMS *m/z* 663 $[M + Na]^+$, HRFABMS *m/z* 663.5109 (calcd. for $C_{45}H_{68}O_2Na$, 663.5117).

Preparation of MTPA Ester. The (*R*)-MTPA and (*S*)-MTPA esters of **1** and **2** were prepared as described previously.[3] To solutions of **1** and **2** in dry pyridine (20μL) were added four times the molar excess of (*R*)(−)- or (*S*)(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (paying attention to the fact that (*R*)-MTPA-Cl gives (*S*)-MTPA ester and *vice versa*). Each mixture was allowed to stand at room temperature for 16 h and an equimolar amount of 3-(dimethylamino)propylamine was added. After standing for 10 min, the solvent was evaporated. The residue was purified on Si gel in a Pasteur pipet eluting with CH_2Cl_2 and characterized by ^{1}H NMR.

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