Cytotoxic Polyacetylenes from the Marine Sponge *Petrosia* sp.

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Three C_{46} (**1**–**3**) and three C_{30} (**4**–**6**) polyacetylenic alcohols with cytotoxic activity against a small panel of human solid-tumor cell lines have been isolated from the marine sponge *Petrosia* sp. Although compound **1** was identified as the stereoisomer of petrocortyne A, the structures of compounds **2**–**5** have not been previously reported and were established by spectral methods. Compound **6** was identified as the known compound petrosiacetylene D. The stereochemistry of compounds **1**–**5** was determined by the modified Mosher's method.

Marine sponges of the genera *Petrosia* are known to afford biologically active polyacetylenes.[1–7] These bioactive metabolites have been associated with H^+- and K^+-ATPase inhibitory,[8] antifungal,[9,10] antifouling,[11] antimicrobial,[12] HIV protease inhibitory,[13] HIV reverse transcriptase inhibitory,[14] immunosuppressant,[15] and antitumor activities.[16] We have recently reported four novel polyacetylenic alcohols, dideoxypetrosynols A–D from *Petrosia* sp.[17] The significant cytotoxic activity against a panel of human tumor cell lines of these compounds has prompted further isolation of novel biologically active acetylenic analogues from this same marine sponge, collected near Komun Island, Korea. Guided by the P-388 cytotoxicity assay, three C_{46} analogues (**1**–**3**) have been isolated. By employing the brine shrimp lethality assay to monitor fractionation,[18] two minor C_{30} metabolites (**4** and **5**) together with petrosiacetylene D (**6**)[19] have been obtained from the same fraction that gave dideoxypetrosynols A–D.[17] The structure elucidation, stereochemistry, and cytotoxic activities of compounds **1**–**6** (Chart 1) are described herein.

Results and Discussion

A methanol-soluble extract of the frozen marine sponge showed significant activity in the brine shrimp larvae lethality bioassay (LD_{50} 30 μg/mL).[18] Guided by this assay, the methanol extract was further fractionated between water and CH_2Cl_2, followed by partitioning of the CH_2Cl_2 solubles between 90% methanol and *n*-hexane. The 90% MeOH fraction was then partitioned again between water and CH_2Cl_2. The CH_2Cl_2 layer was subjected to reversed-phase flash column chromatography to afford eight fractions. Both the brine shrimp lethality assay and the *in vitro* P-388 assay were performed on these fractions. Fraction 7 showed activity in the P-388 assay (ED_{50} 4.7 μg/mL), and separation on a reversed-phase flash column and reversed-phase HPLC monitored by the P-388 assay resulted in the purification of three polyacetylenic compounds, **1**–**3**. From fraction 4, which showed potent brine shrimp lethality (LD_{50} < 1 μg/mL),[17] three minor polyacetylenic alcohols **4**–**6** were isolated. These compounds were, in general, labile at room temperature but were stable at −20 °C. No ultraviolet absorption was observed above 230 nm. Compounds **1**–**5** all showed ^{1}H and ^{13}C NMR spectral features similar to those polyacetylenic alcohols reported previously (Tables 1–4).[17,19,20] The structures of the novel compounds **1**–**5** were characterized based on analysis of their ^{1}H and ^{13}C NMR and MS data, with the modified Mosher's method employed to determine the absolute stereochemistry. The spectral properties as well as stereochemistry of compound **6** were identical to those previously described for petrosiacetylene D.[19]

Compound **1** was isolated as a yellow oil. The ^{1}H and ^{13}C NMR data were reminiscent of those of petrocortyne A,[19] with the absence of terminal methyls and evidence for long, unbranched alkyl chains (a strong signal at δ 1.30–1.51 was observed) and the presence of isolated double bonds (δ 5.33–5.38). Thus, the partial structures **a**, **b**, and **c** (Figure 1) could be proposed. Detailed ^{1}H NMR analysis (COSY and TOCSY) with the addition of Eu(fod)$_3$, a lanthanide-induced shift reagent (Figure 2), and EIMS analysis of its TMS derivative (Figure 3), confirmed the gross structure of compound **1**. However, the absolute stereochemistry determined by the modified Mosher's method at 600 MHz was 3*S*,14*S* (Table 5), revealing compound **1** to be an enantiomer of petrocortyne A, which was reported to have the 3*R*,14*R* configuration.[19]

Compound **2** was also a yellow oil that showed similar NMR data to compound **1**, differing in the additional unsaturation at C-17, as indicated in its ^{1}H (δ 5.53, 6.14) and ^{13}C NMR data (δ 110.5, 145.9) (partial structure **d**, Figure 1). The molecular ion observed from the EIMS data of its TMS derivative showed that the molecular formula of **2** possessed two fewer hydrogens than **1**. The fragmentation pattern further confirmed the structure **2** by showing several fragments with a difference of 2 mass units when compared with **1** (Figure 3). The absolute stereochemistry determined by the modified Mosher's method was 3*S*,14*S* (Table 5).

Compound **3**, isolated as a colorless oil, also exhibited structural similarities to **1**. In particular, **3** lacked two olefinic protons assignable to C-4 and C-5 (Table 1, partial structure **e**, Figure 1) and showed two additional methylene carbon peaks corresponding to C-4 and C-5 (δ 39.0, 26.3) in the ^{13}C NMR spectrum compared to compound **1**.

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Chart 1

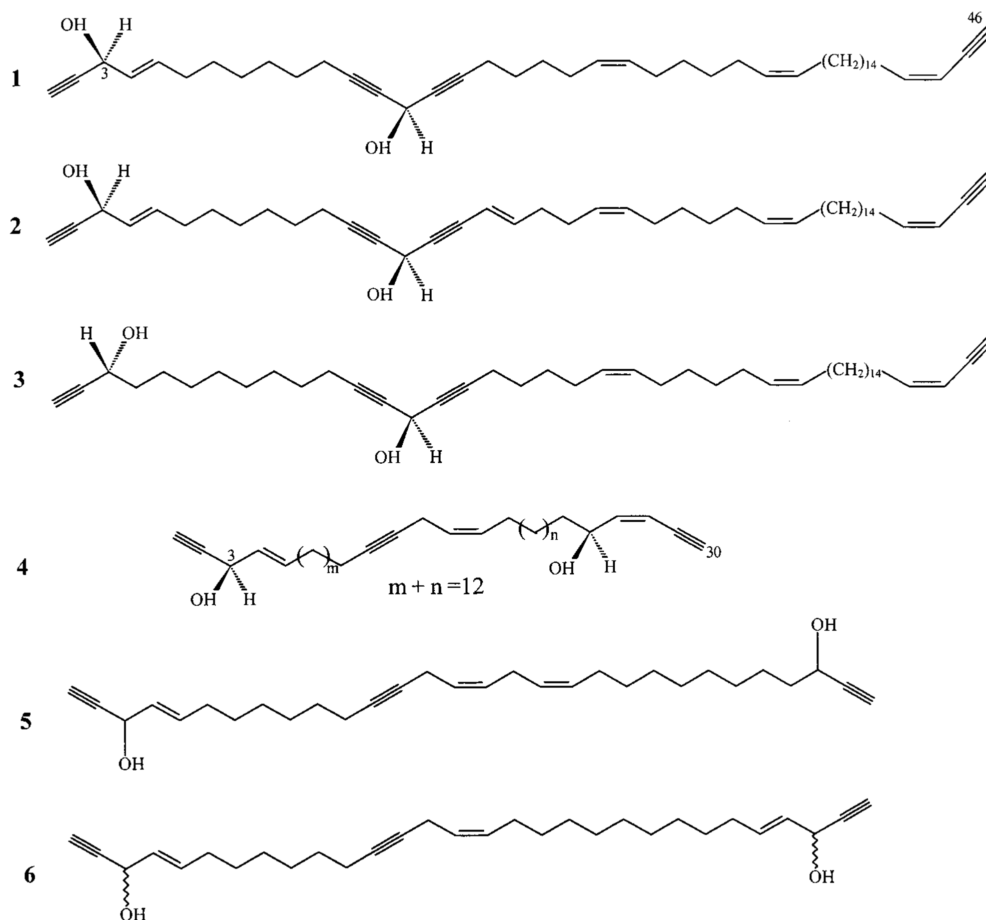

Table 1. ^{1}H NMR Data of Compounds **1**–**3** (200 MHz, CD_3OD)[a]

position	1	2	3
1	2.83 (d, 2.2)	2.83 (d, 2.2)	2.75 (d, 2.0)
3	4.74 (br d, 5.9)	4.74 (br d, 5.9)	4.26 (td, 6.1, 1.9)
4	5.55 (ddt, 15.2, 5.9, 1.3)	5.55 (ddt, 15.0, 5.9, 1.3)	1.63 (m)
5	5.85 (dtd, 15.2, 6.6, 1.0)	5.85 (dtd, 15.0, 7.3, 1.0)	1.30–1.50 (m)
6	2.02–2.05 (m)	2.02–2.05 (m)	1.30–1.50 (m)
7–10	1.30–1.51 (m)	1.29–1.50 (m)	1.30–1.50 (m)
11	2.21 (td, 7.0, 2.0)	2.22 (td, 7.0, 1.8)	2.22 (m)
14	5.01(quint, 2.0)	5.13 (quart, 1.8)	5.01(quint, 1.9)
17	2.21 (td, 7.0, 2.0)	5.53 (br d, 16.0)	2.22 (m)
18	1.30–1.51 (m)	6.14 (dt, 16.0, 7.0)	1.30–1.50 (m)
19	1.30–1.51 (m)	2.15 (m)	1.30–1.50 (m)
20	2.02–2.05 (m)	2.15 (m)	2.02–2.05 (m)
21–22	5.33–5.38 (m)	5.30–5.40 (m)	5.35 (m)
23	2.02–2.05 (m)	2.02–2.05 (m)	2.02–2.05 (m)
24–25	1.30–1.51 (m)	1.29–1.50 (m)	1.30–1.50 (m)
26	2.02–2.05 (m)	2.02–2.05 (m)	2.02–2.05 (m)
27–28	5.33–5.38 (m)	5.30–5.40 (m)	5.35 (m)
29	2.02–2.05 (m)	2.02–2.05 (m)	2.02–2.05 (m)
30–41	1.30–1.51 (m)	1.29–1.51 (m)	1.30–1.50 (m)
42	2.32 (quart, 6.6)	2.31 (quart, 6.8)	2.31 (quart, 6.6)
43	5.98 (dtd, 10.8, 7.4, 1.0)	5.99 (dtd, 10.8, 7.4, 1.0)	5.99 (dtd, 10.8, 7.4, 1.0)
44	5.43 (ddt, 10.8, 2.0, 1.3)	5.43 (ddt, 10.8, 2.1, 1.3)	5.43 (ddt, 10.8, 2.1, 1.3)
46	3.36 (d, 2.0)	3.38 (d, 2.1)	3.39 (d, 2.1)

[a] Multiplicities and coupling constants in parentheses.

Furthermore, the fragmentation pattern of its TMS derivative in the EIMS (Figure 3) supported the gross structure of compound **3**. The stereochemistry of **3** was determined as 3*R*,14*S* (Table 5).

The C_{30} compounds **4** and **5** were isolated as minor metabolites together with the previously reported dideoxypetrosynols[17] and petrosiacetylene D (**6**).[19] A common partial structure **a** (Figure 1) was present in compounds **4**–**6**. The ^{1}H NMR spectrum of compound **4**, however, showed an additional signal at δ 3.50 in place of one of the terminal acetylenic proton signals at δ 2.86 in the spectrum of dideoxypetrosynol A[17] and petrosiacetylene D.[19] Moreover, two olefinic carbon signals observed at δ 109.3 (C-28) and 148.6 (C-27) indicated the presence of partial structure **f**, which was not found in the structures of dideoxypetrosynols A–D. Partial structure **f** was confirmed by a NMR decoupling experiment and by comparison with the NMR data of isopetroformyne 1.[21] Based on the

Table 2. ^{1}H NMR Data of Compounds **4** and **5** (200 MHz, CD_3OD)[a]

position	4	5
1	2.86 (d, 2.2)	2.86 (d, 2.2)
3	4.76 (br d, 6.0)	4.76 (br d, 6.0)
4	5.54 (ddt, 15.2, 6.0, 1.0)	5.54 (ddt, 15.3, 6.0, 1.3)
5	5.85 (dtd, 15.2, 6.0, 1.0)	5.85 (dtd, 15.3, 6.7, 1.3)
6	2.06 (m)	2.08 (m)
7	1.30–1.50 (m)	1.28–1.50 (m)
8	1.30–1.50 (m)	1.28–1.50 (m)
9	1.30–1.50 (m)	1.28–1.50 (m)
10	1.30–1.50 (m)	1.28–1.50 (m)
11	2.11 (m)	2.12 (m)
14	2.89 (m)	2.90 (m)
15–16	5.37–5.40 (m)	5.33–5.41 (m)
17	2.06 (m)	2.80 (t, 5.5)
18–19	1.30–1.50 (m)	5.33–5.41 (m)
20	1.30–1.50 (m)	2.08 (m)
21	1.30–1.50 (m)	1.28–1.50 (m)
22	1.30–1.50 (m)	1.28–1.50 (m)
23	1.30–1.50 (m)	1.28–1.50 (m)
24	1.30–1.50 (m)	1.28–1.50 (m)
25	1.60 (m)	1.28–1.50 (m)
26	4.59 (dt, 8.0, 6.0)	1.28–1.50 (m)
27	5.92 (ddd, 11.0, 8.0, 0.6)	1.63 (m)
28	5.51 (ddd, 11.0, 2.0, 0.8)	4.26 (td, 6.7, 2.2)
30	3.50 (d, 2.0)	2.75 (d, 2.2)

[a] Multiplicities and coupling constants in parentheses.

Table 3. ^{13}C NMR Data of Compounds **1**–**3** (50 MHz, CD_3OD)

position	1	2	3
1	74.5	74.5	73.4
2	84.8	84.8[b]	86.3
3	63.1	63.2	62.6
4	130.5[a]	130.8[a]	39.0
5	134.0	134.1	26.3
6	32.9	32.9	29.2–30.8
7–10	29.2–30.9	29.5–30.8	29.2–30.8
11	19.2[c]	19.3	19.2[c]
12	84.5[b]	85.0[b]	84.8[b]
13	79.9[e]	79.4[b]	80.0[e]
14	52.6	52.9	52.6
15	79.8[e]	87.1[b]	79.9[e]
16	84.3[b]	82.6[b]	84.4[b]
17	19.3[c]	110.5	19.3[c]
18	29.2–30.9	145.9	29.2–30.8
19	29.2–30.9	34.1	29.2–30.8
20	27.8[d]	27.5[c]	27.7[d]
21	130.7[a]	129.4[a]	130.6[a]
22	131.1[a]	131.8[a]	131.1[a]
23	28.22[d]	28.1[c]	28.1[d]
24–25	29.2–30.9	29.5–30.8	29.2–30.8
26	28.20[d]	28.14[c]	28.03[d]
27	131.0[a]	131.0[a]	131.0[a]
28	130.7[a]	130.8[a]	130.8[a]
29	28.1[d]	28.05[c]	28.02[d]
30–41	29.2–30.9	29.5–30.8	29.2–30.8
42	31.2	31.1	31.1
43	146.3	146.4	146.4
44	109.4	109.3	109.3
45	81.2	81.3	81.3
46	82.8	82.7	82.7

[a–e] Assignments with the same superscript in the same column may be interchanged.

additional comparison of ^{13}C NMR data with those of the previously reported dideoxypetrosynols, the olefinic group of partial structure **g**, which is also observed in compound **6**, could be located at the center of the C_{30} chain in **4**. Moreover, the methylene groups connecting partial structures **a** and **g** (δ 29.7–30.1) appeared more upfield than those connecting partial structures **g** and **f** (δ 30.3–30.7). This was also observed in similar polyacetylenes isolated by Ochi et al.[22] However, the NMR spectral data and EIMS fragmentation (Figure 3) alone were not sufficient to definitely differentiate the two possible structures in which the two partial structures **a** and **f** are interchanged. Further verification of the gross structure was hampered by lack of material, although the stereochemistry was determined by the modified Mosher's method as 3*R*,26*S* (Table 5).

Table 4. ^{13}C NMR Data of Compounds **4** and **5** (50 MHz, CD_3OD)

position	4	5
1	74.5	74.5
2	83.8	84.8
3	63.2	63.2
4	130.7	130.7
5	134.1	134.1
6	32.9	33.0
7	30.1[a]	30.12[a]
8	30.0[a]	30.10[a]
9	29.70[a]	30.0[a]
10	29.66[a]	29.9[a]
11	19.4	19.4
12	79.2	80.8
13	78.6	79.1
14	17.7	17.8
15	126.4	126.4[c]
16	132.0	130.3[c]
17	28.0	26.4[d]
18	30.66[b]	128.4[c]
19	30.63[b]	131.4[c]
20	30.59[b]	28.1
21	30.6[b]	30.7[b]
22	30.3[b]	30.6[b]
23	30.69[b]	30.34[b]
24	26.2	30.31[b]
25	37.8	30.2[b]
26	70.5	26.3[d]
27	148.6	39.0
28	109.3	62.6
29	80.5	86.3
30	77.2	73.4

[a–d] Assignments with the same superscript in the same column may be interchanged.

Compound **5** was obtained, in the process of purifying compound **6**, as an amorphous solid. This compound was more labile than the other polyacetylenic alcohols isolated from the same sponge material. Even at −20 °C, rapid decomposition was observed when checked by HPLC. This was consistent with the previously reported dideoxypetrosynol C[17] which, like compound **5**, had two double bonds connected by a methylene group. Therefore, although initially 2.4 mg of pure product was obtained, rapid degradation resulted in less than 0.5 mg of intact material for analysis. Based on the HREIMS of the TMS derivative, the molecular formula was determined as $C_{30}H_{44}O_2$. The NMR spectra resembled those of dideoxypetrosynol B and dideoxypetrosynol C.[17] Compound **5** seemed to be either dideoxypetrosynol B saturated at the C-18 position or dideoxypetrosynol C saturated at the C-26 position. The EIMS fragmentation (*m*/*z* 305 and 341) of its TMS derivative suggested gross structure **5** for this compound (Figure 3). The absolute stereochemistry, however, at C-3 and C-28 could not be determined due to degradation during the esterification reaction.

Compounds **1**–**5** were given the trivial names of (*3S*,*14S*)-petrocortyne A, petrotetrayndiol A, petrotetrayndiol B, dideoxypetrosynol E, and dideoxypetrosynol F, respectively.

Compounds **1**–**6** were tested for cytotoxicity against human solid-tumor cells and showed significant activities (Table 6), with the exception of compound **5**. Compound **4** was the most potent in the five cell lines tested. Similar to

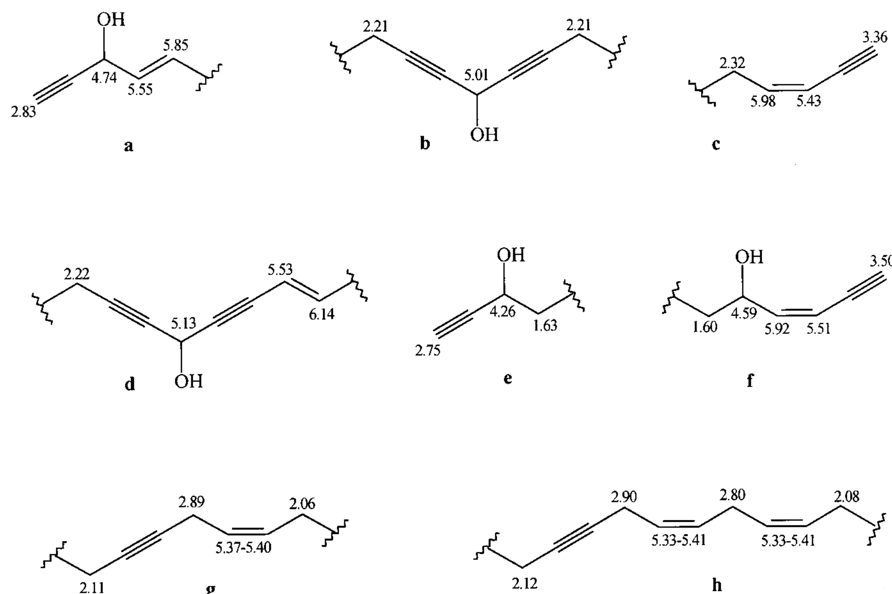

Figure 1. Partial structures of **1**–**5** with diagnostic ^{1}H NMR chemical shifts.

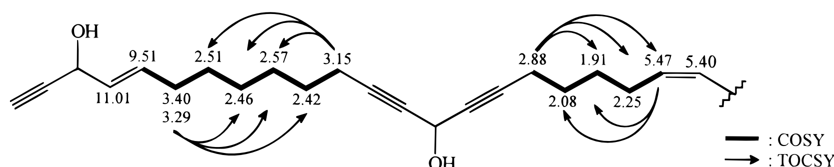

Figure 2. Key COSY and TOCSY NMR correlations of **1** in Eu(fod)$_3$.

dideoxypetrosynols A–D,[17] compounds **1**, **2**, **4**, and **6** showed somewhat selective cytotoxicity against the SK-OV-3 and SK-MEL-2 cell lines and were comparable in potency to doxorubicin. Compound **3** displayed less potent cytotoxicity despite structural similarities to compounds **1** and **2**. The degree of saturation may be related to cytotoxicity in certain cell lines. The least active in the five cell lines used was compound **5**. Consistent with previously reported compounds, an analogue with two double bonds connected by a methylene group (partial structure **h**, Figure 1) such as dideoxypetrosynol C,[17] decomposed more rapidly than other analogues. It is likely that the weak activity of compound **5** is partly due to this chemical instability.

It is interesting to note that this *Petrosia* sp. sponge specimen collected from Korean waters contained polyacetylenes with diverse structures and stereochemistry. The stereochemistry of the C_{30} analogues varies from 3*R*,28*R*; 3*S*,28*S*; and 3*S*,28*R* to 3*R*,28*S*.[17,19] In the case of the C_{46} analogues, although there seems to be a prevalence of the *R* configuration as seen in the 3*R*,14*R* configuration of the petrocortynes,[19] the 3*S*,14*S* and the 3*R*,14*S* configurations were also observed, as in the case of petrotetrayndiols A and B, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH on a JASCO DIP-370 digital polarimeter. UV spectra were obtained in MeOH using a UV-2401PC Shimadzu spectrophotometer. IR spectra were recorded on a BOMEM Michaelson series FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC200 NMR spectrometer. COSY and TOCSY spectra were recorded for compound **1** with the addition of three molar equivalents of Eu(fod)$_3$ on a Bruker DMX 600 spectrometer. Chemical shifts are reported with reference to the respective residual solvent peaks (δ 7.24 and 77.0 for $CDCl_3$, and δ 3.3 and 49.0 for CD_3OD). LREIMS and HREIMS were performed on a JEOL JMS–SX-102A mass spectrometer, while FABMS data were obtained using a JEOL JMS–HX110/110A instrument. TriSil-Z was purchased from Pierce Chemical Company, Rockford, IL. HPLC was performed on a Gilson 370 pump with a YMC ODS–H80 (250 × 4.6 mm i.d., S-4 μm) column and a Perkin–Elmer RP-18 Newguard cartridge (15 × 3.22 mm, 7 μm) or a YMC-Pack CN (250 × 10 mm i.d., S-5 μm, 120 Å) column using a Shodex RI-71 detector at a flow rate of 2 mL/min.

Animal Material. The sponge *Petrosia* species was collected by hand using scuba (15–25 m depth) in July 1995, off Komun Island, Korea. The collected sample was frozen immediately and kept at −20 °C until processed. The sponge was similar to *Petrosia corticata* in spicules, but differed in having only oxeas. A voucher specimen has been deposited in the Natural History Museum, Han Nam University, Taejeon, Korea.[23]

Extraction and Isolation. The frozen sponge (14.5 kg) was extracted with MeOH at room temperature. Guided by the brine shrimp lethality assay, the MeOH solubles were fractionated between H_2O and CH_2Cl_2. The CH_2Cl_2 solubles were further partitioned between 90% MeOH and *n*-hexane to yield 58.15 and 61.5 g of dried extract, respectively. The 90% MeOH fraction was then partitioned between H_2O and CH_2Cl_2 to afford 34 g of the CH_2Cl_2 extract, which was subjected to 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 Me_2CO, to obtain eight fractions. These fractions were evaluated for activity in the P-388 and brine shrimp lethality assays. Fraction 7 (7 g), which did not show significant activity in the brine shrimp lethality assay, was active in the P-388 assay (ED_{50} 4.7 μg/mL, doxorubicin 4.0 μg/mL). Guided by the P-388 assay, fraction 7 was further separated by reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh), eluting with 10 → 0% H_2O–MeOH, EtOAc, and $CHCl_3$ to produce 13 fractions. Fraction 7-7 (1.283 g, ED_{50} 0.5 μg/mL, doxorubicin 2.0 μg/mL)

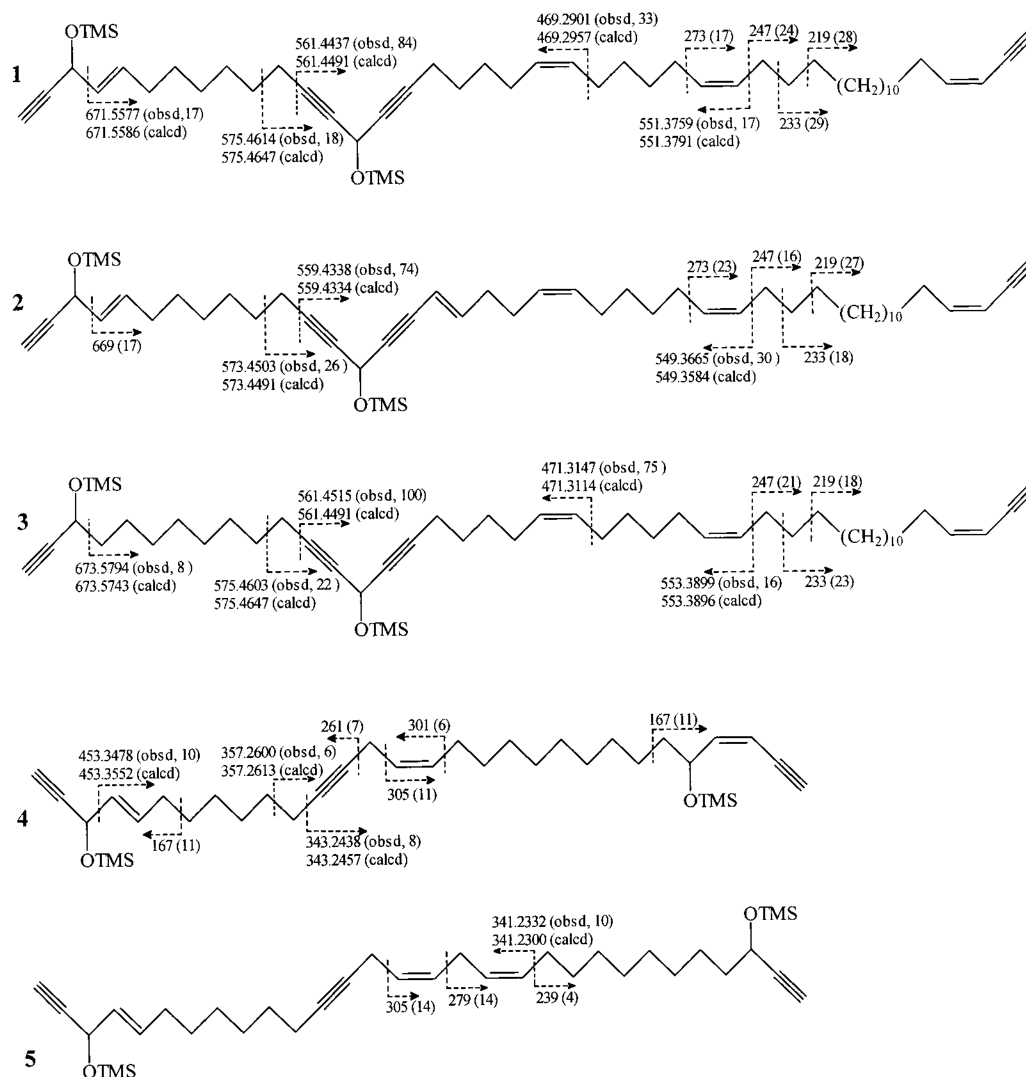

Figure 3. Key EIMS fragmentations of the TMS derivatives of **1**–**5** (*m/z* values; % relative abundance).

Table 5. $\Delta\delta$ ($\delta_S - \delta_R$) Values (ppm) Obtained for the MTPA Esters of **1**–**4**

position	**1**[a]	**2**	**3**[a]	**4**
1	+0.04	+0.04	−0.05	−0.04
4	−0.10	−0.11	+0.06	+0.10
5	−0.06	−0.06		+0.10
6	−0.04			+0.02
11	−0.03	−0.03	−0.03	
17	+0.03	+0.02	+0.02	
18		+0.02		
25				−0.02
26				
27				+0.11
28				+0.04
30				+0.01

[a] Spectra recorded at 600 MHz, otherwise recorded at 200 MHz.

Table 6. *In Vitro* Cytotoxicity Data of Compounds **1**–**6** Against a Panel of Human Solid-Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	1.1	0.6	1.1	1.7	1.0
2	1.6	0.5	0.9	1.7	1.0
3	1.7	2.2	1.9	>3.0	3.7
4	1.3	0.1	0.1	0.6	0.8
5	>3.0	>3.0	>3.0	>3.0	>3.0
6	1.4	0.1	0.2	1.2	1.2
doxorubicin	0.1	0.2	0.2	0.2	0.9

[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.

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), which upon another reversed-phase flash column chromatographic separation eluting with 2% H_2O–MeOH afforded fraction 7-7-5-5 (316 mg, ED_{50} 0.4 μg/mL). Compounds **1** (142.3 mg), **2** (15.5 mg), and **3** (8.0 mg) were obtained by separation of fraction 7-7-5-5 on a reversed-phase HPLC (YMC ODS–H80 column, 4 μm, 80 Å) eluting with 100% MeOH, followed by purification on the same column eluting with 2.4–3.2% aqueous MeOH. Compound **3** was obtained by further purification on a HPLC system with a CN column (YMC CN, 5 μm, 120 Å) eluting with 16.7% aqueous CH_3OH and 33% aqueous CH_3CN and by reversed-phase HPLC (YMC ODS–H80 column) eluting with 100% MeOH.

Compounds **4**–**6** were isolated as minor components from the same fraction that gave dideoxypetrosynols A–D.[17] Fraction 4 (4.65 g), which showed significant brine shrimp lethality (LD_{50} < 1 μg/mL), was further subjected to normal-phase flash column chromatography (Kieselgel 60, 230–400 mesh), eluting with 0 → 100% EtOAc–$CHCl_3$ and 0 → 100% MeOH–EtOAc. Fraction 4-4, which eluted with 10% EtOAc–$CHCl_3$, afforded 355.9 mg of a mixture that showed significant brine shrimp lethality (LD_{50} < 1 μg/mL). Fraction 4-4 was then separated on a reversed-phase column (YMC ODS-H80, 4 μm, 80 Å) eluting with 15% H_2O–MeOH to obtain fractions 4-4-8 (9.1 mg) and 4-4-9 (27 mg). Fraction 4-4-8 was separated again on

a reversed-phase HPLC (YMC ODS-H80, 4 μm, 80 Å) eluting with 10% H_2O-CH_3CN to afford **4** (1.7 mg), upon final purification with a reversed-phase HPLC system (ODS column) eluting with 5% H_2O-CH_3CN. Fraction 4-4-9 was further separated by a reversed-phase HPLC (YMC ODS-H80, 4 μm, 80 Å) column, eluting with 10% H_2O-CH_3CN, to yield **5** (2.5 mg) and **6** (2.6 mg). Compound **6** exhibited physical and spectral data identical to that of petrosiacetylene D.[19]

Preparation of TMS Derivatives. TMS derivatives of compounds **1**−**5** were prepared by treating each dry sample (ca. 0.1 mg) with TriSil-Z (TMSi in dry pyridine, 1.5 mEq/mL, 100 μL) in dry EtOAc (50 μL). After 2 h of standing at room temperature under N_2, the resulting products were subjected to LREIMS and HREIMS.

Preparation of MTPA Esters. The (*R*)-MTPA and (*S*)-MTPA esters of compounds **1**−**4** and **6** were prepared as described previously.[17,24,25] To solutions of **1**−**4** and **6** (0.5−1 mg) in dry pyridine (20 μL) were added four times the molar excess of (*R*)(−)- or (*S*)(+)-α-methoxy-α-trifluoromethylphenylacetic acid chloride (Aldrich) [paying attention to the fact that the (*R*)-MTPA-Cl gives the (*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. Only chemical shifts of protons whose $\Delta\delta$ values are not zero are reported (see Table 5).

(*3S,14S*)-Petrocortyne A (1): yellow oil; $[\alpha]^{23}_D$ +10.8°(*c* 1.9, 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 3; FABMS *m*/*z* 677; HRFABMS *m*/*z* 677.5276 for $C_{46}H_{70}O_2Na$ [M + Na]$^+$ (calcd 677.5273); HREIMS *m*/*z* 798.6205 for $C_{52}H_{86}O_2Si_2^+$ (calcd 798.6166).

Petrotetrayndiol A (2): yellow oil; $[\alpha]^{23}_D$ +7.3°(*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (3.3) nm; IR (film) ν_{max} 3300, 2920, 2853, 1452, 996 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.23−1.49 (36H, m, H-7−10, 24, 25, 30−41), 2.00−2.12 (12H, m, H-6, 19, 20, 23, 26, 29), 2.20 (2H, td, *J* = 7.0, 1.9 Hz, H-11), 2.29 (2H, quartet, *J* = 7.1 Hz, H-42), 2.54 (1H, d, *J* = 2.1 Hz, H-1), 3.04 (1H, d, *J* = 2.1 Hz, H-46), 4.81 (1H, br d, *J* = 5.8 Hz, H-3), 5.18 (1H, quartet, *J* = 1.9 Hz, H-14), 5.30−5.35 (4H, m, H-21, 22, 27, 28), 5.41 (1H, ddt, *J* = 10.9, 2.1, 1.3 Hz, H-44), 5.49 (1H, br d, *J* = 16.0 Hz, H-17), 5.58 (1H, dd, *J* = 15.3, 5.8 Hz, H-4), 5.88 (1H, dt, *J* = 15.3, 6.4 Hz, H-5), 5.98 (1H, dtd, *J* = 10.9, 7.6, 0.8 Hz, H-43), 6.18 (1H, dt, *J* = 16.0, 6.8 Hz, H-18); ^{1}H NMR data (CD_3OD), see Table 1; ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.7 (C-11), 26.3−27.2 (C-20, 23, 26, 29), 28.2−29.8 (C-7−10, 24, 25, 30−41), 30.3 (C-42), 31.7 (C-6), 33.2 (C-19), 52.8 (C-14), 62.7 (C-3), 74.0 (C-1), 80.6 (C-45), 81.1 (C-46), 82.7−85.5 (C-12, 13, 15, 16, one of these acetylenic carbon signal overlapped with the solvent peak), 83.3 (C-2), 107.9 (C-44), 108.9 (C-17), 128.1−130.9 (C-21, 22, 27, 28), 128.6 (C-4), 134.2 (C-5), 145.6 (C-18), 146.3 (C-43); ^{13}C NMR data (CD_3OD), see Table 3; FABMS *m*/*z* 675 for $C_{46}H_{68}O_2Na$ [M + Na]$^+$; HREIMS *m*/*z* 796.6008 for $C_{52}H_{84}O_2Si_2$ (calcd 796.6009).

Petrotetrayndiol B (3): colorless oil, $[\alpha]^{23}_D$ +3.8°(*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (3.2) nm; IR (film) ν_{max} 3304, 3019, 2932, 1223, 762 cm^{-1}; ^{1}H NMR data (CD_3OD), see Table 1; ^{13}C NMR data (CD_3OD), see Table 3; HREIMS *m*/*z* 800.6390 for $C_{52}H_{88}O_2Si_2$ (calcd 800.6322).

Dideoxypetrosynol E (4): amorphous solid; $[\alpha]^{23}_D$ +2.6° (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 (3.7) nm; IR (film) ν_{max} 3310, 3000, 2850, 1452, 995 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26−1.61 (24H, m, H-7−10, H-18−25), 2.00 (2H, m, H-6), 2.12 (2H, m, H-17), 2.13 (2H, m, H-11), 2.55 (1H, d, *J* = 2.2 Hz, H-1), 2.88 (2H, t, *J* = 2.5 Hz, H-14), 3.12 (1H, d, *J* = 2.0 Hz, H-30), 4.66 (1H, dt, *J* = 8.0, 6.0 Hz, H-26), 4.82 (1H, d, *J* = 6.0 Hz, H-3), 5.37−5.43 (2H, m, H-15, 16), 5.52 (1H, ddd, *J* = 11.0, 2.0, 0.8 Hz, H-28), 5.59 (1H, ddt, *J* = 15.2, 6.0, 1.0 Hz, H-4), 5.90 (1H, dtd, *J* = 15.2, 6.0, 1.0 Hz, H-5), 5.97 (1H, ddd, *J* = 11.0, 8.0, 0.6 Hz, H-27); ^{1}H NMR data (CD_3OD), see Table 2; ^{13}C NMR (50 MHz, $CDCl_3$) δ 17.2 (C-14), 18.8 (C-11), 27.1 (C-17), 25.1 (C-24), 28.6−28.9 (C-7−10), 29.2−29.5 (C-18−23), 31.9 (C-6), 36.6 (C-25), 62.8 (C-3), 70.1 (C-26), 74.0 (C-1), 77.2 (C-30), 78.5 (C-12), 79.5 (C-13), 82.8 (C-29), 83.8 (C-2), 108.9 (C-28), 125.0 (C-15), 128.5 (C-4), 131.4 (C-16), 134.4 (C-5), 147.5 (C-27); ^{13}C NMR data (CD_3OD), see Table 4; HREIMS *m*/*z* 580.4125 for $C_{36}H_{60}O_2Si_2$ (calcd 580.4132).

Dideoxypetrosynol F (5): amorphous solid; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 4; HREIMS *m*/*z*: 580.4146 for $C_{36}H_{60}O_2Si_2$ (calcd 580.4132).

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