Additional Cytotoxic Polyacetylenes from the Marine Sponge *Petrosia* Species

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Ten new polyacetylenic alcohols (**1**−**6**, **8**−**11**), along with a known compound, petrocortyne C (**7**), were isolated from the marine sponge *Petrosia* sp. The gross structures were established based on NMR and MS data, and the absolute configuration was determined by the modified Mosher's method. These compounds displayed considerable cytotoxicity against a small panel of human solid tumor cell lines. Compounds **1**−**11** were further evaluated for in vitro inhibitory activity on DNA replication.

Marine sponges of the genus *Petrosia* are known as a source of diverse bioactive polyacetylenic compounds.[1] These polyacetylenic compounds exhibit various chain lengths and functional groups as illustrated by C_{30} petrosynol,[2] C_{46} petroformynes,[3] brominated C_{18} acids,[4] and corticatic acids.[5] Most of them have shown interesting biological activities, including antifungal activity,[4,5] antimicrobial activity,[6] HIV reverse transcriptase inhibition,[7] and cytotoxicity.[8] In earlier papers we reported new C_{30} and C_{46} polyacetylenic alcohols from the *Petrosia* sp., which displayed significant cytotoxicity against five human cell lines.[9−11] In our continuing search for bioactive metabolites from the same sponge, additional new C_{45}, C_{46}, and C_{47} congeners (**1**−**6**, **8**−**11**) have been isolated, along with petrocortyne C (**7**). Herein we report the structure elucidation and biological evaluation of these compounds.

Results and Discussion

The methanolic extract of the frozen sponge showed cytotoxicity in the in vitro P-388 assay. Guided by this assay, the MeOH extract was successively fractionated employing reversed-phase flash column chromatography and HPLC to afford compounds **1**−**11** as the causative constituents. Compounds **1**−**11** were further tested for cytotoxicity against five human solid tumor cell lines to display significant to moderate activities (Table 1). The cytotoxicity of these compounds was comparable to that of cisplatin, with the exception of **5**. The potency of these compounds was also comparable to those of C_{46} polyacetylenic alcohols, (3*S*,14*S*)-petrocortyne A[10] and petrotetrayndiols A−B,[10] and somewhat less than those of C_{30} polyacetylenic alcohols, dideoxypetrosynols A−E,[9,10] which were previously isolated from the same sponge. Compounds **1**−**11** also displayed significant inhibition on the DNA replication in a concentration-dependent mode, which could explain their cytotoxicity (Table 2). Structural analysis of the compounds revealed that they are congeners of (3*S*,-14*S*)-petrocortyne A,[10] possessing diverse partial structures **a**−**m**, as depicted in Figure 1.

Petrotetrayndiol C (**1**), which possesses 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 3 and 5). The $[M + Na]^+$ ion was observed at *m/z* 691.5054 ($C_{46}H_{68}O_3Na$, Δ −1.8 ppm). The ^1H and ^{13}C NMR data were reminiscent of those of (3*S*,14*S*)-petrocortyne A. However, certain differences in the ^1H NMR data were noticed. The signal of one of the cis-disubstituted double bonds was replaced by a trans-disubstituted double bond (δ 6.12, dd, J = 15.9, 4.7 Hz; δ 6.93, dt, J = 15.9, 7.0 Hz). An additional signal for a methylene α to a carbonyl group was observed at δ 2.59. This keto carbonyl was also detected in the ^{13}C NMR

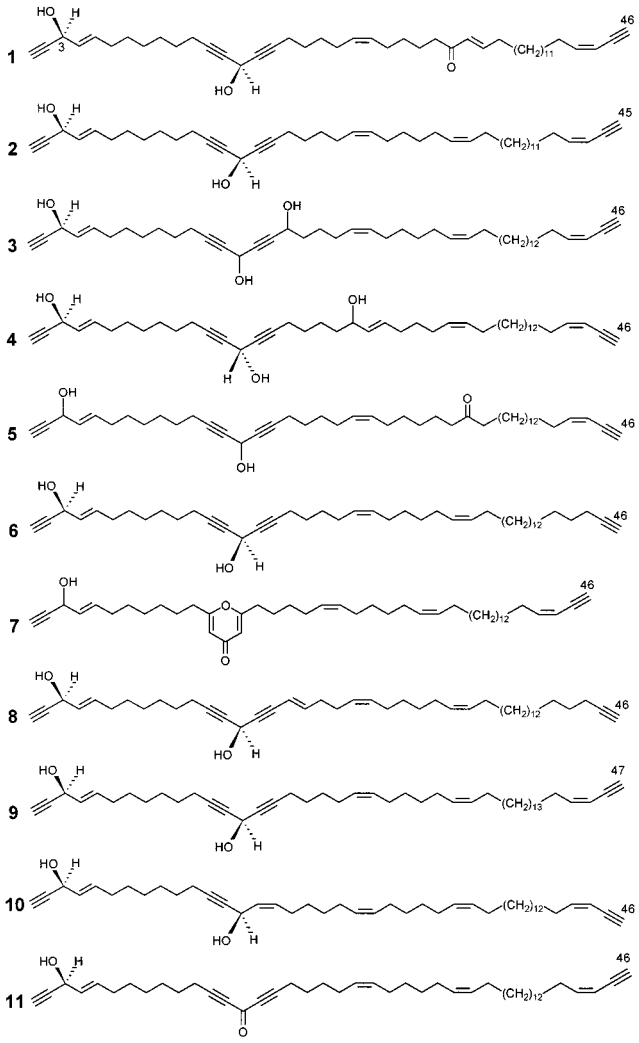

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Table 1. In Vitro Cytotoxicity Data of **1**−**11** Against a Panel of Human Solid Tumor Cell Lines[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	>10	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
3	>30	4.2	3.9	18.5	12.9
4	26.3	1.9	2.3	8.0	5.0
cisplatin	0.9	1.6	1.0	0.9	1.9
5	>10	>10	>10	>10	>10
6	>10	1.5	1.5	5.8	2.5
cisplatin	0.8	1.2	1.5	0.7	1.5
7	>10	0.7	2.4	>10	7.5
8	24.5	1.7	1.1	3.4	1.8
9	11.3	2.2	0.8	2.5	1.7
10	1.8	0.8	0.6	1.3	0.8
11	>30	4.6	5.2	>30	>30
cisplatin	0.6	0.9	0.7	0.6	0.6

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

Table 2. The Percent Inhibitions of **1**−**11** on SV40 DNA Replication[a]

concn (μM)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
125	45	12	63	79	66	40	89	62	59	62	76
250	46	47	77	94	80	70	100	83	86	90	100
500	81	70	100	100	100	100	100	100	100	100	100

[a] The bioactivity of compounds **1** and **2** was assessed as a separate batch from others.

spectrum 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 conjugated with the trans-double bond comprising an enone moiety (**k** in Figure 1). By analysis of the COSY spectral data of **1**, the partial structures **a**, **b**, **c**, and **e** have been easily deduced (Figure 1). The structure of the long-chain subunit was deduced by careful examination of the COSY and TOCSY spectral data (Figure 2). The long-range $^1H-^1H$ correlation between the allylic protons (δ 2.06−2.11, H-20/23) and the α-keto methylene protons (δ 2.59, H-26) was clearly observed in the TOCSY spectrum of **1**. This pair of allylic methylene protons (H-20/23) showed a correlation with another pair of allylic methylene protons (δ 2.22, H-17). The gross structure deduced by NMR spectral analysis was further confirmed by FAB−CID tandem mass spectrometry (Figure 3). The fragmentation of **1** showed the characteristic pattern of a linear aliphatic chain compound. A prominent fragment produced by a loss of H_2O from the $[M + Na]^+$ was observed at *m*/*z* 673. Major fragmentations of the $[M + Na]^+$ ion were observed as odd mass ions due to the remote charge fragmentation, which is characteristic of collisional activation of an alkali-metal-cationized ion.[12] 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.[13] The FAB−CID tandem mass spectrometry was found useful for determining double-bond location of these polyacetylenic alcohols, inasmuch as the migration of the double bonds was significantly suppressed. The absolute configuration of **1** was determined by the modified Mosher's method as 3*S*,14*S*, which was the same as that of (3*S*,14*S*)-petrocortyne A (Table 7).

Nor-(3*S*,14*S*)-petrocortyne A (**2**) and homo-(3*S*,14*S*)-petrocortyne A (**9**) were isolated as yellow oils. The ^1H and ^{13}C NMR data of **2** and **9** were indistinguishable from those of (3*S*,14*S*)-petrocortyne A (Tables 3 and 5, Experimental Section). However, when coeluted in HPLC, they showed retention times distinct from that of (3*S*,14*S*)-petrocortyne A. In the LRFABMS, **2** showed the $[M + Na]^+$ ion at *m*/*z* 663, which was 14 amu less than that of (3*S*,14*S*)-petrocortyne A, and **9** showed the $[M + Na]^+$ ion at *m*/*z* 691, which was 14 amu higher than that of (3*S*,14*S*)-petrocortyne A. Based on the FABMS and NMR data, the molecular formulas of **2** and **9** were established as $C_{45}H_{68}O_2$ (*m*/*z* 663.5109, $C_{45}H_{68}O_2Na$, Δ −1.3 ppm) and $C_{47}H_{72}O_2$ (*m*/*z* 691, $C_{47}H_{72}O_2Na$), respectively. Their gross structures were further confirmed by FAB−CID tandem mass spectrometry (Figure 3). The fragmentation of **2** and **9** showed the same

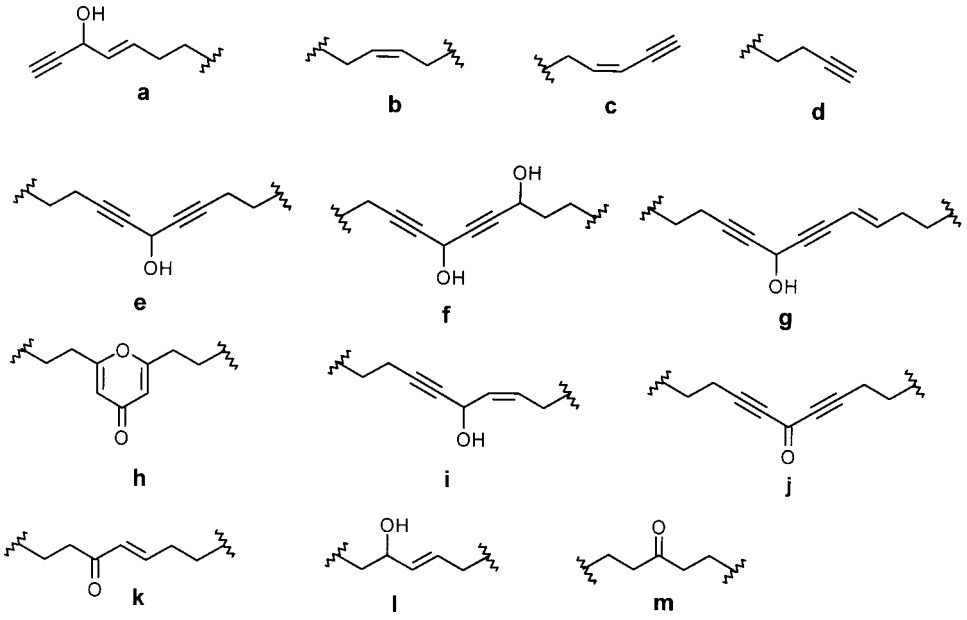

Figure 1. Partial structures of compounds **1**−**11**.

Table 3. ^{1}H NMR Data of (3*S*,14*S*)-Petrocortyne A and Compounds **1**−**4** (CD_3OD, 600 MHz)[a]

position	(3*S*,14*S*)-petrocortyne A	**1**	**2**	**3**	**4**
1	2.83 (d, 2.2)	2.87 (d, 2.2)	2.86 (d, 2.4)	2.86 (d, 2.1)	2.87 (d, 2.2)
3	4.74 (br d, 5.9)	4.74 (br d, 6.2)	4.74 (br d, 6.3)	4.75 (br d, 6.0)	4.75 (br d, 6.0)
4	5.55 (ddt, 15.2, 5.9, 1.3)	5.56 (dd, 15.2, 6.2)	5.55 (ddt, 15.1, 6.3, 1.5)	5.56 (ddt, 15.2, 6.2, 1.0)	5.56 (ddt, 15.2, 6.2, 1.0)
5	5.85 (dtd, 15.2, 6.6, 1.0)	5.85 (dt, 15.2, 6.8)	5.85 (dtd, 15.1, 6.8, 1.0)	5.86 (dtd, 15.2, 7.1, 1.0)	5.85 (dtd, 15.2, 6.7, 1.0)
6	2.02−2.05 (m)	2.07 (m)	2.02−2.05 (m)	2.07 (m)	2.07 (m)
7	1.30−1.51 (m)	1.41 (m)	1.30−1.51 (m)	1.42 (m)	1.42 (m)
8−9	1.30−1.51 (m)	1.30−1.60 (m)	1.30−1.51 (m)	1.30−1.38 (m)	1.30−1.36 (m)
10	1.30−1.51 (m)	1.50 (m)	1.30−1.51 (m)	1.52 (m)	1.52 (m)
11	2.21 (td, 7.0, 2.0)	2.21 (td, 6.7, 1.7)	2.21 (td, 4.9, 2.0)	2.23 (td, 6.7, 2.0)	2.21 (t, 6.7)
14	5.01 (quint, 2.0)	5.01 (br s)	5.00 (quint, 2.0)	5.09 (q, 1.7)	5.02 (m)
17	2.21 (td, 7.0, 2.0)	2.22 (td, 6.7, 1.7)	2.21 (td, 4.9, 2.0)	4.33 (td, 5.2, 1.5)	2.22 (t, 6.7)
18	1.30−1.51 (m)	1.50 (m)	1.30−1.51 (m)	1.65 (m)	1.52 (m)
19	1.30−1.51 (m)	1.30−1.60 (m)	1.30−1.51 (m)	1.49 (m)	1.30−1.36 (m)
20	2.02−2.05 (m)	2.06−2.11 (m)	2.02−2.05 (m)	2.05 (m)	1.48 (m), 1.55 (m)
21	5.33−5.38 (m)	5.37−5.39 (m)	5.33−5.37 (m)	5.34−5.38 (m)	3.97 (quint, 5.9)
22	5.33−5.38 (m)	5.37−5.39 (m)	5.33−5.37 (m)	5.34−5.38 (m)	5.47 (dd, 15.2, 7.1)
23	2.02−2.05 (m)	2.06−2.11 (m)	2.02−2.05 (m)	2.05 (m)	5.61 (dt, 15.2, 7.1)
24	1.30−1.51 (m)	1.30−1.60 (m)	1.30−1.51 (m)	1.30−1.38 (m)	2.05 (m)
25	1.30−1.51 (m)	1.59 (m)	1.30−1.51 (m)	1.30−1.38 (m)	1.36 (m)
26	2.02−2.05 (m)	2.59 (q, 7.2)	2.02−2.05 (m)	2.05 (m)	2.05 (m)
27	5.33−5.38 (m)		5.33−5.37 (m)	5.34−5.38 (m)	5.36−5.37 (m)
28	5.33−5.38 (m)	6.12 (dd, 15.9, 4.7)	5.33−5.37 (m)	5.34−5.38 (m)	5.36−5.37 (m)
29	2.02−2.05 (m)	6.93 (dt, 15.9, 7.0)	2.02−2.05 (m)	2.05 (m)	2.05 (m)
30	1.30−1.51 (m)	2.26 (quint, 7.9)	1.30−1.51 (m)	1.30−1.38 (m)	1.30−1.36 (m)
31	1.30−1.51 (m)	1.56 (m)	1.30−1.51 (m)	1.30−1.38 (m)	1.30−1.36 (m)
32−40	1.30−1.51 (m)	1.30−1.60 (m)	1.30−1.51 (m)	1.30−1.38 (m)	1.30−1.36 (m)
41	1.30−1.51 (m)	1.42 (m)	2.31 (q, 6.8)	1.43 (m)	1.42 (m)
42	2.32 (q, 6.6)	2.32 (q, 7.3)	5.99 (dtd, 10.7, 7.3, 1.0)	2.32 (qd, 7.5, 1.3)	2.32 (q, 7.3)
43	5.98 (dtd, 10.8, 7.4, 1.0)	5.99 (dt, 10.7, 7.6)	5.44 (ddt, 10.7, 2.0, 1.0)	5.99 (dt, 10.8, 7.5)	6.00 (dt, 10.8, 7.3)
44	5.43 (ddt, 10.8, 2.0, 1.3)	5.44 (br d, 10.7)		5.44 (br d, 10.8)	5.45 (br d, 10.8)
45			3.39 (br d, 2.0)		
46	3.36 (d, 2.0)	3.40 (br d, 1.9)		3.39 (br d, 2.0)	3.40 (br d, 1.6)

[a] Multiplicity and coupling constants (Hz) in parentheses. Compound **2** and (3*S*,14*S*)-petrocortyne A were measured at 200 MHz.

Table 4. ^{1}H NMR Data of Compounds **5**, **8**, **10**, and **11** (CD_3OD, 600 MHz)[a]

position	**5**	**8**	**10**	**11**
1	2.87 (d, 2.2)	2.85 (d, 2.1)	2.86 (d, 2.2)	2.86 (d, 2.2)
3	4.75 (br d, 5.6)	4.74 (br d, 6.0)	4.74 (br d, 6.2)	4.75 (br d, 6.2)
4	5.56 (dd, 15.2, 6.2)	5.56 (dd, 15.0, 6.3)	5.54 (ddt, 15.3, 5.9, 1.5)	5.56 (ddt, 15.2, 6.1, 1.3)
5	5.85 (dt, 15.2, 7.0)	5.85 (dt, 15.0, 6.9)	5.85 (dtd, 15.3, 6.8, 1.1)	5.86 (dtd, 15.2, 6.8, 1.3)
6	2.07 (m)	2.05 (m)	2.06 (m)	2.05−2.10 (m)
7	1.42 (m)	1.42 (m)	1.42 (m)	1.42 (m)
8−9	1.30−1.37 (m)	1.29−1.37 (m)	1.30−1.33 (m)	1.29−1.37 (m)
10	1.52 (m)	1.50 (m)	1.49 (quint, 8.1)	1.61 (m)
11	2.22 (td, 6.3, 1.9)	2.22 (td, 6.6, 1.8)	2.20 (td, 6.8, 1.9)	2.44 (t, 7.0)
14	5.02 (quint, 1.9)	5.13 (q, 1.8)	5.06 (dt, 7.4, 1.9)	
15			5.48 (ddt, 10, 7.4, 0.5)[c]	
16			5.46 (dt, 10, 5)[c]	
17	2.23 (td, 6.9, 1.9)	5.53 (br d, 15.9)	2.12 (m)	2.45 (t, 7.0)
18	1.52 (m)	6.13 (dt, 15.9, 6.6)	1.43 (m)	1.61 (m)
19	1.30−1.37 (m)	2.15 (m)	1.36−1.38 (m)	1.48 (m)
20	2.06 (m)	2.15 (m)	2.03−2.50 (m)	2.05−2.10 (m)
21−22	5.37 (m)	5.33−5.38 (m)	5.33−5.37 (m)	5.35−5.39 (m)
23	2.06 (m)	2.05 (m)	2.03−2.05 (m)	2.05−2.10 (m)
24−25	1.30−1.37 (m)	1.29−1.37 (m)	1.36−1.38 (m)	1.38 (m)
26	1.55 (m)	2.05 (m)	2.03−2.05 (m)	2.05−2.10 (m)
27	2.46 (t, 7.5)[b]	5.33−5.38 (m)	5.33−5.37 (m)	5.35−5.39 (m)
28		5.33−5.38 (m)	5.33−5.37 (m)	5.35−5.39 (m)
29	2.45 (t, 7.5)[b]	2.05 (m)	2.03−2.05 (m)	2.05−2.10 (m)
30	1.55 (m)	1.29−1.37 (m)	1.36−1.38 (m)	1.38 (m)
31−40	1.30−1.37 (m)	1.29−1.37 (m)	1.36−1.38 (m)	1.30−1.34 (m)
41	1.43 (m)	1.29−1.37 (m)	1.36−1.38 (m)	1.42 (m)
42	2.32 (q, 7.3)	1.29−1.37 (m)	2.33 (qd, 7.3, 1.3)	2.32 (q, 7.2)
43	5.99 (dt, 10.9, 7.6)	1.48 (m)	5.99 (dt, 10.8, 7.4)	5.99 (dt, 10.8, 7.6)
44	5.44 (br d, 10.9)	2.15 (m)	5.44 (br d, 10.9)	5.44 (br d, 10.8)
46	3.40 (br d, 1.8)	2.15 (m)	3.39 (br d, 2.0)	3.39 (br d, 1.8)

[a] Multiplicity and coupling constants (Hz) in parentheses. Compound **8** was measured at 300 MHz. [b] Assignments with the same superscript in the same column may be interchanged. [c] The values were estimated by simulation.

characteristic pattern of linear aliphatic chain compounds as in the case of **1**. Prominent fragment ions generated by a loss of H_2O from the $[M + Na]^+$ were observed at *m*/*z* 645 for **2** and at *m*/*z* 673 for **9**. The terminal allylic cleavages were observed as base peaks at *m*/*z* 597 and 625 for **2** and **9**, respectively. From these base fragments, sequential losses of methylene units continued until the fragmentations at the double bonds for each compound. Allylic cleavages of each compound were observed as enhanced peaks at *m*/*z* 443, 389, 361, and 307. Each

Table 5. ^{13}C NMR Data of (3*S*,14*S*)-Petrocortyne A and Compounds **1**−**4** (CD_3OD, 50 MHz)[a]

position	(3*S*,14*S*)-petrocortyne A	**1**	**2**	**3**	**4**
1	74.5	74.5	74.5	74.5	74.5
2	84.8	84.8	84.8	84.9	84.8
3	63.1	63.2	63.2	63.2	63.2
4	130.5[e]	130.7[e]	130.5[f]	130.5[e]	130.5[e]
5	134.0	134.1	134.0	134.0	134.0
6	32.9	32.9	32.9	32.9	32.9
7−10	29.2−30.9	29.5−30.7	29.2−30.9	29.2−30.8	29.2−30.8
11	19.2[c]	19.2[c]	19.2[c]	19.2	19.28[c]
12	84.5[b]	84.5[b]	84.5[b]	85.4[b]	84.5[b]
13	79.9[d]	79.9	79.9[e]	79.9[d]	79.9
14	52.6	52.6	52.6	52.5	52.6
15	79.8[d]	79.9	79.8[e]	79.4[d]	79.9
16	84.3[b]	84.4[b]	84.4[b]	83.6[b]	84.4[b]
17	19.3[c]	19.3[c]	19.3[c]	62.7	19.31[c]
18	29.2−30.7	29.5−30.7	29.2−30.9	38.8	29.2−30.8
19	29.2−30.7	29.5−30.7	29.2−30.9	26.1	29.2−30.8
20	27.8[f]	27.7[d]	27.7[d]	27.7[c]	38.0
21	130.7[e]	131.5[e]	130.75[f]	130.7[e]	73.6
22	131.1[e]	131.3[e]	131.1[f]	131.1[e]	134.7
23	28.22[f]	27.6[d]	28.1[d]	28.1[c]	132.3
24	29.2−30.9	29.5−30.7	29.2−30.9	29.2−30.8	33.1
25	29.2−30.9	23.7	29.2−30.9	29.2−30.8	29.2−30.8
26	28.20[f]	40.5	28.04[d]	28.05[c]	28.2[d]
27	131.0[e]	203.7	130.9[f]	131.0[e]	131.0[e]
28	130.7[e]	130.3[e]	130.74[f]	130.8[e]	130.8[e]
29	28.1[f]	149.6	28.03[d]	28.02[c]	27.7[d]
30	29.2−30.9	33.5	29.2−30.9	29.2−30.8	29.2−30.8
31−40	29.2−30.9	29.5−30.7	29.2−30.9	29.2−30.8	29.2−30.8
41	29.2−30.9	29.5−30.7	31.1	29.2−30.8	29.2−30.8
42	31.2	31.1	146.4	31.1	31.1
43	146.3	146.4	109.3	146.4	146.4
44	109.4	109.3	81.2	109.3	109.3
45	81.2	81.2	82.7	81.2	81.3
46	82.8	82.7		82.7	82.7

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

fragment ion at *m*/*z* 415 and 333 showed a 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 configuration of **2** was determined by the modified Mosher's method as 3*S*,14*S*, which was the same as those of **1** and (3*S*,14*S*)-petrocortyne A (Table 7). The stereochemistry of **9** was also determined as 3*S*,14*S* by comparing the ^1H NMR data of the (*R*)-MTPA ester with that of **2**. Nor-(3*S*,14*S*)-petrocortyne A (**2**) and homo-(3*S*,14*S*)-petrocortyne A (**9**) were chemically unique for their linear C_{45} and C_{47} skeletons, respectively. Except for the C_{47} polyacetylenic acids with a terminal carboxyl group, such as nepheliosyne A,[14] osirisynes,[15] and haliclonyne,[16] most of the long-chain (> C_{40}) polyacetylenes isolated from marine sponges possess C_{46} linear carbon skeletons. Although **2** and **9** possess the same partial structures and absolute configurations as (3*S*,14*S*)-petrocortyne A, their C_{45} and C_{47} skeletons are unprecedented.

Petrotetrayntriol A (**3**) was isolated as a yellow oil. The molecular formula of **3** was deduced to be $C_{46}H_{70}O_3$ based on LRFABMS and NMR data. The ^1H and ^{13}C NMR data show that **3** has a structure similar to (3*S*,14*S*)-petrocortyne A (Tables 3 and 5; **a**, **b**, and **c** in Figure 1). However, an additional hydroxyl group at the α-position of diacetylenic carbinol moiety was indicated by the ^1H NMR (δ 4.33, 1H, td, J = 5.2, 1.5 Hz) and ^{13}C NMR (δ 62.7) data. And an additional methylene signal was also observed in the ^1H NMR (δ 1.65, 2H, m) and ^{13}C NMR (δ 38.8) data, which may be ascribed to the α-hydroxymethylene group (**f** in Figure 1). By analysis of the COSY and TOCSY spectral data of **3**, the structure of the long-chain subunit was deduced (Figure 2). The long-range $^1H-^1H$ correlation between the α-hydroxymethylene protons (δ 1.65, H-18) and the allylic protons (δ 2.05, H-20) was observed in the

Table 6. ^{13}C NMR Data of Compounds **5**, **8**, **10**, and **11** (CD_3OD, 75 MHz)[a]

position	**5**	**8**	**10**	**11**
1	74.5	74.5	74.5	74.5
2	84.9	*h*	84.7	84.8
3	63.2	63.2	63.1	63.1
4	130.7[g]	130.8[d]	130.7[c]	130.76[c]
5	134.1	134.1	134.0	134.0
6	32.9	32.9	32.9	32.9
7−10	29.2−30.7	29.5−30.8	29.7−30.8	29.5−30.8
11	19.25[c]	19.3	19.4	19.4[d]
12	84.51[b]	85.0[b]	85.4	95.7
13	79.9[b]	79.5[b]	81.9	83.2
14	52.6	52.9	58.8	162.4
15	79.9[b]	87.1[b]	131.9	83.2
16	84.49[b]	*h*	132.2	95.7
17	19.31[c]	110.5	28.3	19.5[d]
18	29.2−30.7	145.9	29.7−30.8	29.5−30.8
19	29.2−30.7	34.1	29.7−30.8	29.5−30.8
20	27.7[d]	27.5[c]	28.00[b]	28.01[b]
21	129.9[g]	129.4[d]	130.8[c]	131.1[c]
22	130.8[g]	131.8[d]	131.0[c]	131.4[c]
23	28.0[d]	28.1[c]	28.1[b]	28.3[b]
24−25	29.2−30.7	29.5−30.8	29.7−30.8	29.5−30.8
26	24.9[e]	28.04[c]	28.03[b]	28.1[b]
27	43.5[f]	131.0[d]	130.9[c]	130.76[c]
28	214.4	130.8[d]	130.8[c]	130.84[c]
29	43.4[f]	28.0[c]	28.03[b]	28.04[b]
30	24.5[e]	29.5−30.8	29.7−30.8	29.5−30.8
31−41	29.2−30.7	29.5−30.8	29.7−30.8	29.5−30.8
42	31.1	29.5−30.8	31.1	31.1
43	146.4	29.5−30.8	146.4	146.4
44	109.3	19.0	109.3	109.3
45	81.3	85.6	81.1	81.3
46	82.7	69.3	82.7	82.7

[a] Compounds **5** and **10** were measured at 50 MHz. [b−g] Assignments with the same superscript in the same column may be interchanged. [h] Not observed due to low intensity.

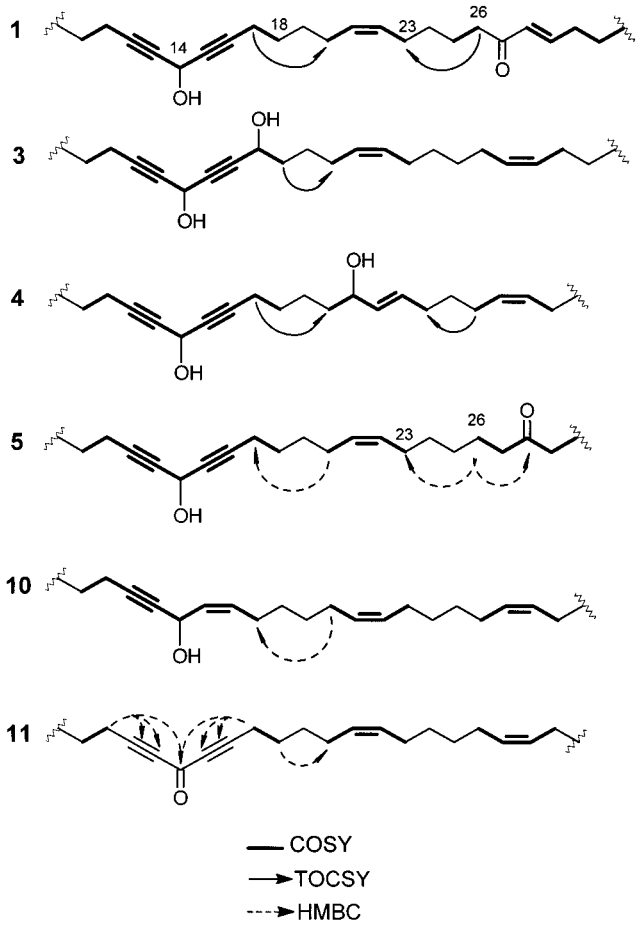

Figure 2. Diagnostic COSY, TOCSY, and HMBC correlations of **1**, **3**−**5**, **10**, and **11** (600 MHz, CD_3OD).

Table 7. Selected $\Delta\delta$ ($\delta_S - \delta_R$) Values (ppm) of the MTPA Esters of **1**−**4**, **6**, and **10** ($CDCl_3$)[a]

position	1	2	3	4	6	10
1	+0.04	+0.04	+0.10	+0.04	+0.04	+0.04
4	−0.10	−0.10	−0.07	−0.11	−0.10	−0.10
5	−0.07	−0.06	−0.06	−0.07	−0.06	−0.08
11	−0.03	−0.03		0.00	−0.02	
15						+0.04
17	+0.01	+0.01		−0.04	+0.03	

[a] Spectra were recorded at 200 MHz for **1**−**4**, **10**, and at 300 MHz for **6**.

TOCSY spectrum. The gross structure deduced by NMR spectral analysis was further confirmed by FAB−CID tandem mass spectrometry (Figure 3). The absolute configuration of C-3 was determined by the modified Mosher's method as *S*, however, those of C-14 and C-17 could not be determined due to their decomposition during analysis (Table 7).

(3*S*,14*R*)-Petrocortyne E (**4**) was isolated as a yellow oil. The molecular formula of **4** was deduced to be $C_{46}H_{70}O_3$ based on LRFABMS and NMR data. The ^1H and ^{13}C NMR data showed partial structures **a**−**c**, **e**, and **l** (Figure 1, Tables 3 and 5). Careful analysis of the COSY, TOCSY, HMQC, and FAB−CID tandem mass spectral data confirmed that **4** has a gross structure identical to petrocortyne E.[17] The absolute stereochemistry of **4** was determined by the modified Mosher's method as 3*S*,14*R*, although the absolute configuration of C-21 could not be determined due to its decomposition during analysis (Table 7). The absolute stereochemistry of petrocortyne E was not previously reported because of its decomposition during esterification reaction.

Petrotetrayndiol D (**5**), with a molecular formula of $C_{46}H_{70}O_3$ (LRFABMS and NMR), was isolated as a yellow oil. The presence of the partial structures **a**−**c** and **e** was immediately recognized. In addition, a keto group was indicated by ^{13}C (δ 214.4, C-28) and ^1H (δ 2.46, t, J = 7.5, H-27/29; δ 2.45, t, J = 7.5, H-27/29) NMR data. The location of this ketone moiety was deduced by an HMBC experiment (Figure 2). The allylic carbon at δ 27.7/ 28.0 (C-20, 23) showed correlations with the β-carbonyl protons at δ 1.55 (H-26) and the allylic protons at δ 2.23 (H-17). The FAB−CID tandem mass data further confirmed this interpretation (Figure 3). The absolute stereochemistry of **5** could not be determined by the modified Mosher's method due to decomposition during esterification.

The ^1H and ^{13}C NMR data of (3*S*,14*S*)-petrocortyne B (**6**) were similar to those of (3*S*,14*S*)-petrocortyne A[10] (Experimental Section). However, the proton and carbon signals of C-43 (δ_H 5.99, δ_C 146.4) and C-44 (δ_H 5.44, δ_C 109.3) have shifted upfield to δ_H 1.49, δ_C 29.2−30.8 (C-43) and δ_H 2.15, δ_C 19.1 (C-44), indicating that **6** has a saturated structure at C-43 (**d** in Figure 1). This interpretation was supported by the $[M + Na]^+$ ion of **6** at *m*/*z* 679, which was 2 amu higher than that of (3*S*,14*S*)-petrocortyne A. The gross structure of **6** was the same as that of the known compound petrocortyne B.[18] However, the absolute configuration of **6** was determined to be 3*S*,14*S*, whereas that of petrocortyne B was reported to be 3*R*,14*R* (Table 7).

Compound **7** was deduced to have the same gross structure as that of petrocortyne C,[18] by comparison of the ^1H and ^{13}C NMR and LRFABMS data with the reported data. The stereochemistry of C-3 could not be determined due to its decomposition during esterification.

Petrotetrayndiol E (**8**), with a molecular formula of $C_{46}H_{70}O_2$ by LRFABMS and NMR, was isolated as a yellow oil. Comparison of the spectral data with those of petrotetrayndiol A[10] revealed that **8** differed from petrotetrayndiol A by an additional saturation at C-43. This was further supported by FAB−CID tandem mass spectrometry (Figure 3). The stereochemistry of **8** was determined to be 3*S*,14*S* by comparing the ^1H NMR data of the (*R*)-MTPA ester with that of petrotetrayndiol A.[10]

Petrotriyndiol A (**10**) was isolated as a yellow oil. The molecular formula of **10** was deduced as $C_{46}H_{72}O_2$, based on LRFABMS and NMR data. The ^1H and ^{13}C NMR data showed that **10** has a structure similar to that of (3*S*,14*S*)-petrocortyne A (Tables 4 and 6, **a**−**c** in Figure 1). However, the signals of the acetylenic carbinol moiety showed differences in chemical shifts and splitting patterns in ^1H and ^{13}C NMR. The acetylenic carbinol proton (H-14) and one of the allylic methylene protons (H-17) have shifted downfield to δ 5.06 (dt, J = 7.4, 1.9) and δ 2.12 (m), respectively. Additional olefinic protons were detected at δ 5.48 (H-15) and δ 5.46 (H-16), which showed a second-order splitting. In the ^{13}C NMR data, diacetylenic carbinol carbon (C-14) has shifted downfield to δ 58.8. Two of the acetylenic carbons have disappeared, and a pair of additional olefinic carbons was detected at δ 131.9 (C-15) and δ 132.2 (C-16). These data indicated that one of the triple bonds of the diacetylenic carbinol moiety was saturated to a double bond to compose an acetylenic enol group (**i** in Figure 1). The geometry of this acetylenic enol group was determined to be *Z* based on the $^1H-^1H$ coupling constant (J = 10 Hz), which was analyzed by spin simulation. The *Z*-geometry of C-15 was further confirmed by the chemical shift of the allylic carbon (C-17) at δ 28.3.[19] The entity of the long-chain subunit was deduced by careful analysis of HMBC and

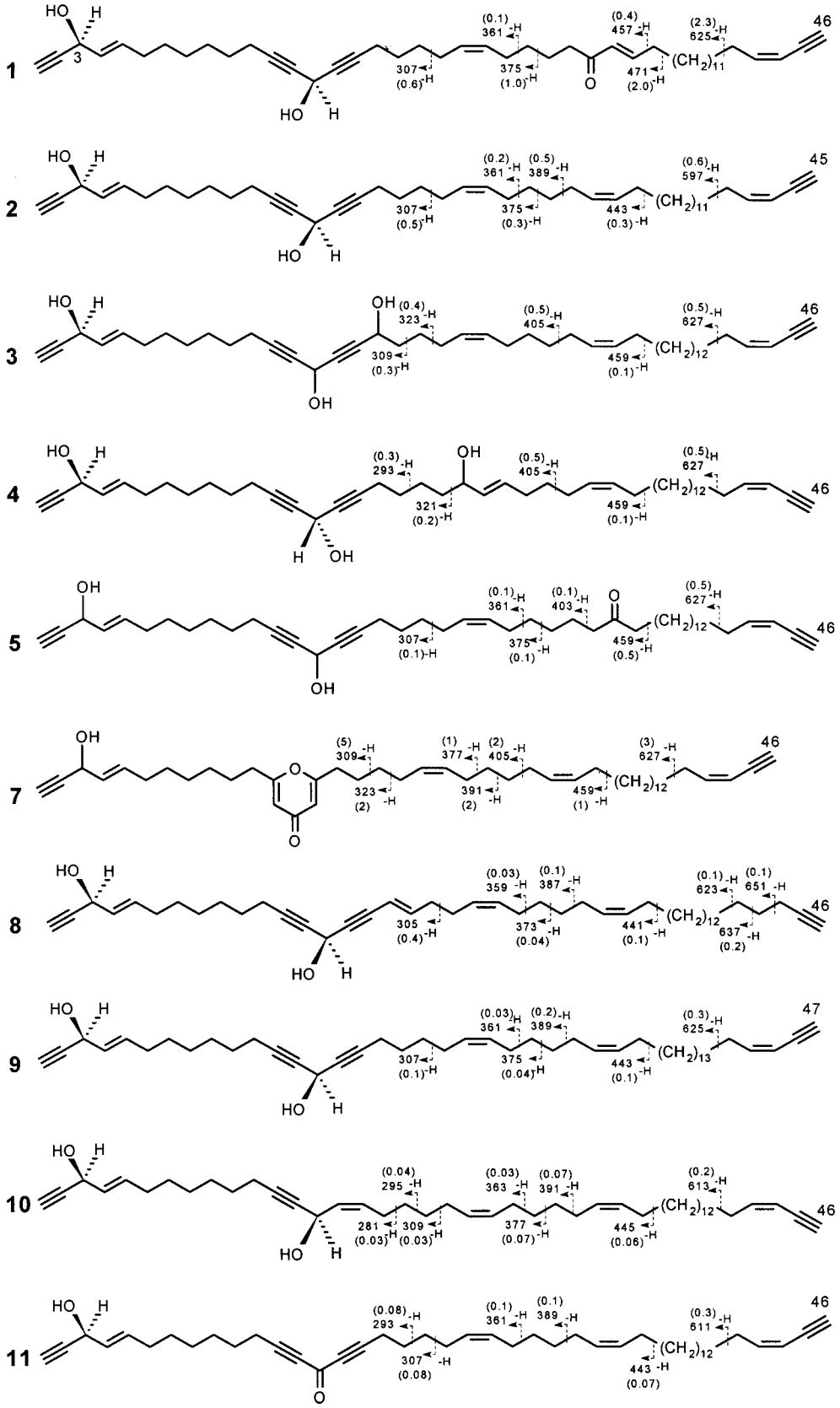

Figure 3. Key FAB−CID tandem mass spectral fragmentations of the $[M + Na]^+$ of **1**−**5** and **7**−**11**. Fragmentation of **11** is that of di-deuterated $[M + Na]^+$ at *m*/*z* 677. The percent relative abundance is given in parentheses.

FAB−CID tandem mass spectral data. The allylic protons of the acetylenic enol group at δ 2.12 (H-17) showed correlation with the allylic carbon of C-20 (δ 28.00/ 28.03/ 28.1) in the HMBC spectrum (Figure 2). The allylic cleavage of the acetylenic enol group was observed at *m*/*z* 281 as an enhanced peak with almost the same relative intensity as other allylic cleavages at *m*/*z* 309 and 363 (Figure 3). The absolute configuration was determined to be 3*S*,14*R* by the modified Mosher's method (Table 7).

Petrotetraynol A (**11**), isolated as a yellow oil, showed an ^{1}H NMR spectrum similar to that of (3*S*,14*S*)-petrocortyne A. In fact, compound **11** differed from (3*S*,14*S*)-

petrocortyne A only by the absence of a carbinol proton (H-14) and by the downfield shift of H-11, -17 (δ 2.44, 2.45), and H-10, -18 (δ 1.61) (Table 4). These data indicated the presence of a ketone at C-14 instead of a hydroxyl group. Likewise, the ^{13}C NMR data of **11** showed a carbonyl signal at δ 162.4 and a substantial downfield shift of C-12 and C-16 (δ 95.7) (Table 6). The structure of the long-chain subunit was deduced by the analysis of COSY and HMBC spectral data (Figure 2). The allylic protons at δ 2.44 (H-11) and δ 2.45 (H-17) showed correlations with the carbonyl carbon (C-14, δ_C 162.4), α-acetylenic carbons (C-13/15, δ_C 83.2), and β-acetylenic carbons (C-12/16, δ_C 95.7). The correlation between the homoallylic methylene protons (H-18, δ_H 1.61) and the allylic carbon (C-20, δ_C 28.01) was also observed. In the FABMS, the ion cluster of $[M + Na]^+$ was observed between *m/z* 675 and 679, with the *m/z* 677 peak being most intense, which was 2 mass unit higher than the expected $[M + Na]^+$ (*m/z* 675). It was considered that the acidic protons at C-11 and C-17 have been partially exchanged with deuterium during NMR measurements. The labile hydroxyl deuterium (−OD) might be easily reexchanged by triturating with CH_3OH prior to mass measurement, while the deuteriums at C-11 and C-17 were not readily reexchanged by CH_3OH trituration. Careful examination of the ^{1}H NMR spectrum revealed that the allylic protons (H-11, -17) showed diminished integral (ca. 60%). FAB−CID tandem mass spectral fragmentation of the *m/z* 677 ion, in accordance with the proposed gross structure (Figure 3). The absolute stereochemistry was determined to be 3*S* by comparison of the chemical shifts of the (*R*)-MTPA ester of **11** with those of the (*R*)-MTPA ester of **2**.

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-2401 PC Shimadzu spectrophotometer. IR spectra were recorded on a JASCO FT/IR-410 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC200, DMX600, and Varian Unity Plus 300 instruments. Chemical shifts are reported with reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 7.26 and δ_C 77.0 for $CDCl_3$). HRFABMS data were carried out on a JEOL JMS SX-101A. FAB−CID tandem MS data were obtained using a JEOL JMS-HX110/110A. HPLC was performed with a YMC ODS-H80 (semipreparative, 250 × 10 mm i.d., 4 μm, 80 Å; preparative, 250 × 20 mm i.d., 4 μm, 80 Å) and YMC-Pack CN (250 × 10 mm i.d., 5 μm, 120 Å) 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.[9] A voucher specimen (J95K-11) was deposited in the Natural History Museum, Hannam University, Taejeon, Korea.

Cytotoxicity. In vitro cytotoxicities were determined at the Korea Institute of Chemical Technology following the protocols established by the National Cancer Institute. Five cancer cell lines, A549 (lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-2 (skin cancer), XF498 (CNS cancer), and HCT15 (colon cancer), were employed for the measurement of cytotoxicities.

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 (30000 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 *E. 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 temperature. 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 residues, respectively. The 90% MeOH fraction was then partitioned again between H_2O 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 Me_2CO, to obtain eight fractions. These fractions were evaluated for activity in the in vitro P-388 assay. Fraction 7 (7 g) 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 a 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 afford 13 fractions. Fraction 7-5 (322.9 mg, ED_{50} 0.75 μg/mL), fraction 7-7 (1.283 g, ED_{50} 0.5 μg/mL), fraction 7-8 (417.6 mg, ED_{50} 0.75 μg/mL), and fraction 7-9 (762.1 mg, ED_{50} 3.7 μg/mL), which were eluted with 5%, 3%, and 1% H_2O−MeOH and 100% MeOH, respectively, were more active than other fractions in the in vitro P-388 assay. Fraction 7-7 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 subsequent 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). Fraction 7-7-5-4 was separated on a preparative ODS HPLC 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 eluting with 37% and 33.3% H_2O−CH_3CN, respectively. Fraction 7-5 was further separated on a preparative ODS HPLC eluting with 100% MeOH to afford fractions 7-5-17 and 7-5-8. Pure compound **3** (3.5 mg) from fraction 7-5-17 was obtained upon purification on a CN HPLC eluting with 35.7% H_2O−CH_3CN. Fraction 7-5-8 was purified by the same CN HPLC system to afford compounds **4** (4.2 mg) and **5** (2.8 mg). Fraction 7−8 was separated on a semipreparative ODS HPLC eluting with 3.2% H_2O−MeOH to afford fraction 7-8-6, which was subjected to purification by the same column with 100% MeOH to yield compound **7** (3.9 mg), fraction 7-8-8, fraction 7-8-9, and compound **6** (31.2 mg). Fraction 7-8-8 was subjected to repeated purification on a semipreparative ODS HPLC eluting with 3.2% H_2O−MeOH and 60% MeCN−MeOH to afford compound **8** (2.0 mg). Fraction 7-8-9 was also subjected to repeated purification on a semipreparative ODS HPLC eluting with 100% MeOH and 60% MeCN−MeOH to obtain compounds **9** (1.6 mg) and **10** (3.5 mg). Fraction 7-9 was separated by a semipreparative ODS HPLC eluting with 100% MeOH to obtain 17 fractions. Fraction 7-9-6 was purified by repetitive HPLC using 50% MeCN−MeOH and 60% MeCN−MeOH to yield compound **11** (7.1 mg).

Petrotetrayndiol C (1): colorless oil; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 5; 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 3; ^{13}C NMR data, see Table 5; LRFABMS *m/z* 663 $[M + Na]^+$; HRFABMS *m/z* 663.5109 (calcd for $C_{45}H_{68}O_2Na$, 663.5117).

Petrotetrayntriol A (3): yellow oil; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 5; LRFABMS *m/z* 693 $[M + Na]^+$ ($C_{46}H_{70}O_3Na$).

(3*S*,14*R*)-Petrocortyne E (4): yellow oil; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 5; LRFABMS *m/z* 693 $[M + Na]^+$ ($C_{46}H_{70}O_3Na$).

Petrotetrayndiol D (5): yellow oil; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 6; LRFABMS *m*/*z* 693 $[M + Na]^+$ ($C_{46}H_{70}O_3Na$).

(3*S*,14*S*)-Petrocortyne B (6): yellow oil; $[\alpha]^{23}_D$ +2° (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (3.6) nm; IR (film) ν_{max} 3310, 2925, 2853, 1627, 1462, 1245, 1009, 971 cm^{-1}; ^{1}H NMR data, (CD_3OD, 300 MHz) δ 5.84 (1H, dt, J = 15.1, 6.7, H-5), 5.55 (1H, dd, J = 15.1, 6.1, H-4), 5.35−5.37 (4H, m, H-21, -22, -27, -28), 5.00 (1H, br s, H-14), 4.73 (1H, br d, J = 5.9, H-3), 2.87 (1H, d, J = 2.1, H-1), 2.19−2.22 (4H, m, H-11, -17), 2.15 (3H, m, H-44, -46), 2.05 (10H, m, H-6, -20, -23, -26, -29), 1.49 (6H, m, H-10, -18, -43), 1.29−1.44 (38H, m, H-7−H-9, -19, -24, -25, -30−42); ^{13}C NMR data, (CD_3OD, 75 MHz) δ 134.0 (C-5), 131.1 (C-4/21/22/27/28), 131.0 (C-4/21/22/27/28), 130.8 (C-4/21/22/27/28), 130.6 (C-4/21/22/27/28), 84.5 (C-12/13/15/16), 84.3 (C-12/13/15/16), 80.0 (C-12/13/15/16), 79.9 (C-12/13/15/16), 74.5(C-1), 69.3 (C-46), 63.1 (C-3), 52.6 (C-14), 32.9 (C-6), 29.2−30.8 (C-7−10, 18, 19, 24, 25, 30−43), 28.2 (C-20/23/26/29), 28.1 (C-20/23/26/29), 27.7 (C-20/23/26/29), 19.3 (C-11/17), 19.2 (C-11/17), 19.1 (C-44), (signals of C-2 and C-45 were not detected); LRFABMS *m*/*z* 679 $[M + Na]^+$ ($C_{46}H_{72}O_2Na$).

Petrocortyne C (7): yellow oil; $[\alpha]^{23}_D$ +3° (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (4.1), 255 (3.8) nm; IR (film) ν_{max} 3310, 2925, 2853, 1719, 1660, 1597, 1151, 1089, 971 cm^{-1}; ^{1}H NMR data, (CD_3OD, 300 MHz) δ 6.15 (2H, s, H-13, -15), 5.98 (1H, dt, J = 11.4, 7.5, H-43), 5.84 (1H, dt, J = 15.3, 8.1, H-5), 5.55 (1H, dd, J = 15.3, 6.3, H-4), 5.42 (1H, br d, J = 11.4, H-44), 5.33−5.37 (4H, m, H-21, -22, -27, -28), 4.73 (1H, br d, J = 5.7, H-3), 3.39 (1H, br d, J = 2.1, H-46), 2.86 (1H, d, J = 1.8, H-1), 2.60 (2H, t, J = 7.5, H-11/17), 2.59 (2H, t, J = 7.5, H-11/17), 2.31 (2H, q, J = 6.6, H-42), 2.03−2.10 (10H, m, H-6, -20, -23, -26, -29), 1.69 (4H, quint, J = 7.5, H-10, -18), 1.28−1.42 (36H, m, H-7−H9, -19, -24, -25, -30−41); ^{13}C NMR data, (CD_3OD, 75 MHz) δ 189.1 (C-14), 173.0 (C-12, -16), 146.4 (C-43), 134.0 (C-5), 131.4 (C-4/21/22/27/28), 131.0 (C-4/21/22/27/28), 130.8 (C-4/21/22/27/28), 130.7 (C-4/21/22/27/28), 130.1 (C-4/21/22/27/28), 113.4 (C-13, 15), 109.3 (C-44), 84.8 (C-2), 82.7 (C-46), 81.3 (C-45), 74.5(C-1), 63.1 (C-3), 34.4 (C-11/17), 34.3 (C-11/17), 32.9 (C-6), 31.1 (C-42), 29.8−30.7 (C-7−C10, -18, -19, -24, -25, -30−41), 28.13 (C-20/23/26/29), 28.05 (C-20/23/26/29), 27.9 (C-20/23/26/29), 27.8 (C-20/23/26/29); LRFABMS *m*/*z* 693 $[M + Na]^+$ ($C_{46}H_{70}O_3Na$).

Petrotetrayndiol E (8): colorless oil; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 6; LRFABMS *m*/*z* 677 $[M + Na]^+$ ($C_{46}H_{70}O_2Na$).

Homo-(3*S*,14*S*)-petrocortyne A (9): yellow oil; ^{1}H NMR data, (CD_3OD, 200 MHz) δ 5.99 (1H, dtd, J = 10.7, 7.3, 1.0, H-44), 5.85 (1H, dtd, J = 15.6, 6.4, 1.0, H-5), 5.55 (1H, ddt, J = 15.6, 5.9, 1.5, H-4), 5.44 (1H, ddt, J = 10.7, 1.9, 1.0, H-45), 5.34−5.36 (4H, m, H-21, -22, -27, -28), 5.00 (1H, quint, J = 2.0, H-14), 4.73 (1H, br d, J = 5.9, H-3), 3.40 (1H, br d, J = 1.9, H-47), 2.86 (1H, d, J = 1.9, H-1), 2.31 (2H, q, J = 6.3, H-43), 2.21 (4H, td, J = 4.4, 2.0, H-11, -17), 2.05 (10H, m, H-6, -20, -23, -26, -29), 1.20−1.51 (42H, m, H-7−H10, -18, -19, -24, -25, -30−42); LRFABMS *m*/*z* 691 $[M + Na]^+$ ($C_{47}H_{72}O_2Na$).

Petrotriyndiol A (10): yellow oil; $[\alpha]^{23}_D$ +7° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (3.7) nm; IR (film) ν_{max} 3311, 2925, 2853, 1458, 1653, 1014, 970 cm^{-1}; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 6; LRFABMS *m*/*z* 679 $[M + Na]^+$ ($C_{46}H_{72}O_2Na$).

Petrotetraynol A (11): yellow oil; ^{1}H NMR data, see Table 4; ^{13}C NMR data, see Table 6; LRFABMS *m*/*z* 675 $[M + Na]^+$ ($C_{46}H_{68}O_2Na$), 679 ($C_{46}H_{64}D_4O_2Na$), 678 ($C_{46}H_{65}D_3O_2Na$), 677 ($C_{46}H_{66}D_2O_2Na$), 676 ($C_{46}H_{67}DO_2Na$)

Preparation of MTPA Ester. The (*R*)-MTPA and (*S*)-MTPA esters of **1**−**11** were prepared as described previously.[3] To solutions of **1**−**11** in dry pyridine (20 μL) 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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References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1998**, *15*, 113−158, and earlier reviews cited therein.
(2) Fusetani, N.; Kato, Y.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1983**, *24*, 2771−2774.
(3) Guo, Y.; Gavagnin, M.; Salierno, C.; Cimino, G. *J. Nat. Prod.* **1998**, *61*, 333−337.
(4) Fusetani, N.; Li, H.-Y.; Tamura, K.; Matsunaga, S. *Tetrahedron* **1993**, *49*, 1203−1210.
(5) Li, H.-Y.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1994**, *57*, 1464−1467.
(6) Fusetani, N.; Shiragaki, T.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1987**, *28*, 4313−4314.
(7) Isaacs, S.; Kashman, Y.; Loya, S.; Hizi, A.; Loya, Y. *Tetrahedron* **1993**, *49*, 10435−10438.
(8) Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *Tetrahedron Lett.* **1989**, *30*, 3563−3566.
(9) Kim, J. S.; Im, K. S.; Jung, J. H.; Kim, Y.-L.; Kim, J.; Sim, C. J.; Lee, C.-O. *Tetrahedron* **1998**, *54*, 3151−3158.
(10) Kim, J. S.; Lim, Y. J.; Im, K. S.; Jung, J. H.; Sim, C. J.; Lee, C.-O.; Hong, J.; Lee, H. *J. Nat. Prod.* **1999**, *62*, 554−559.
(11) Lim, Y. J.; Kim, J. S.; Im, K. S.; Jung, J. H.; Lee, C.-O.; Hong, J.; Kim, D.-K. *J. Nat. Prod.* **1999**, *62*, 1215−1217.
(12) Adams, J.; Gross, M. L. *Anal. Chem.* **1987**, *59*, 1576−1582.
(13) Walkup, R. D.; Jamieson, G. C.; Ratcliff, M. R.; Djerassi, C. *Lipids* **1981**, *16*, 631−646.
(14) Kobayashi, J.; Naitoh, K.; Ishida, K.; Shigemori, H.; Ishibashi, M. *J. Nat. Prod.* **1994**, *57*, 1300−1303.
(15) Shin, J.; Seo, Y.; Cho, K. W.; Rho, J.-R. *Tetrahedron* **1998**, *54*, 8711−8720.
(16) Chill, L.; Miroz, A.; Kashman, Y. *J. Nat. Prod.* **2000**, *63*, 523−526.
(17) Shin, J.; Seo, Y.; Cho, K. W. *J. Nat. Prod.* **1998**, *61*, 1268−1273.
(18) Seo, Y.; Cho, K. W.; Rho, J.-R.; Shin, J.; Sim, C. J. *Tetrahedron* **1998**, *54*, 447−462.
(19) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, 1972.

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