New Acetylenic Enol Ethers of Glycerol from the Sponge *Petrosia* sp.

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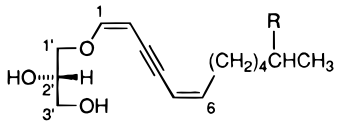
Ten acetylenic enol ethers of glycerols, including six new compounds (**1**−**6**) and a linear acetylenic alcohol (**7**), have been isolated from a sponge of the genus *Petrosia*. The structures of the novel compounds were elucidated by spectroscopic methods. The absolute stereochemistry of **1**−**7** was determined by chemical transformations and the Mosher method. Some of these compounds exhibited weak cytotoxicity against a human leukemia cell-line (K-562).

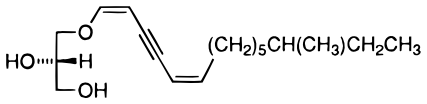
Acetylenic and polyacetylenic compounds are a rapidly growing class of sponge metabolites and exhibit great structural variations in both chain length and functional groups.[1−12] Several sponge-derived acetylenes and polyacetylenes exhibit diverse and potent bioactivity.[2−12]

In the course of a search for bioactive substances from marine organisms of Korean origin, we have reported the structures and bioactivities of several petrocortynes and petrosiacetylenes, novel polyacetylenes from an undescribed sponge of the genus *Petrosia* (Nepheliospongiidae) collected from Keomun Island.[12] In addition to petrocortynes and petrosiacetylenes, ^{1}H NMR analysis of chromatographic fractions of the crude extract of the above sponge revealed the presence of several metabolites of another structural class in more polar fractions. In this paper, we wish to report the structure elucidation and bioactivity of 10 acetylenic enol ethers of glycerol, including six new compounds (**1**−**6**) and a new acetylenic alcohol (**7**). All of the acetylenic enol ethers of glycerol are structurally similar to the raspailynes previously isolated from the sponges *Raspailia pumila* and *Raspailia ramosa*.[13−15] The acetylenic alcohol (**7**) contained a linear C_{12} carbon framework, which is reminiscent of those of the petrocortynes.[12]



1 R = H

2 R = CH_3



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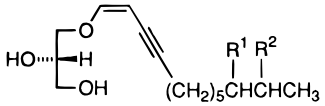
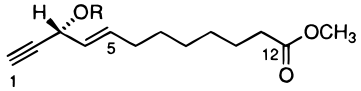


4 $R^1 = R^2 = H$

5 $R^1 = CH_3$, $R^2 = H$

6 $R^1 = H$, $R^2 = CH_3$



7 R = H

7S R = (*S*)-MTPA

7R R = (*R*)-MTPA

Results and Discussion

The sponge was collected and processed as described in the Experimental Section to yield 11 metabolites. The structures of four known metabolites, raspailyne B1, raspailyne B2, isoraspailyne B, and isoraspailyne B1, were determined by a combination of spectroscopic analysis and comparison with reported data for these compounds.[14]

Compound **1** was isolated as a colorless gum. The molecular formula for this compound was deduced as $C_{15}H_{24}O_3$ by a combination of HRMS and ^{13}C NMR analysis. The spectral data of **1** were very similar to those obtained for raspailyne B2, with the absence of NMR signals of the upfield methylene in both of the ^{13}C and ^{1}H NMR spectra as the only noticeable differences (Table 1). A combination of ^{1}H COSY, HMQC, and HMBC NMR experiments allowed the determination of the structure of **1** as a linear glyceryl enol ether containing an yne−diene group.

Compound **1** possessed an asymmetric carbon at C-2′ of the glycerol moiety. Stereochemical assignment at this carbon, as well as confirmation of the whole molecular structure, was achieved by chemical transformations (Scheme 1). Catalytic hydrogenation of **1** quantitatively yielded the perhydrogenated derivative **8**. This compound was also obtained by treatment of (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol with bromododecane in DMF followed by acidic hydrolysis, as indicated by the NMR data obtained. The optical rotation of compound **8** was almost negligible, and, therefore, the absolute configuration at C-2′ was not satisfactorily assigned. However, both samples of compound **8** were acetylated with acetic anhydride in pyridine, and the optical rotations of the 2′,3′-diacetylated products obtained proved that these were indeed the same compound **9**, and the 2′*S* configuration was assigned for **1**. Thus, the structure of **1** was unambiguously determined as an acetylenic enol ether of a glycerol.

A related metabolite (**2**) was isolated as a colorless gum, which analyzed for $C_{16}H_{26}O_3$ by HRMS and ^{13}C NMR

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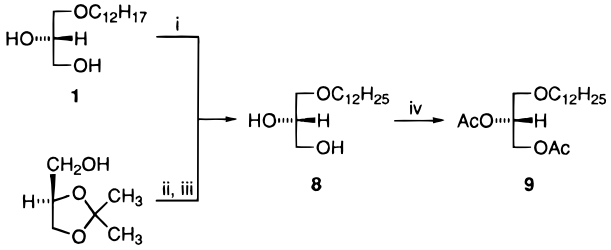
Table 1. ^{13}C NMR Assignments for Compounds 1−6[a]

carbon	1	2	3	4	5	6
1	156.5 d	156.5 d	156.5 d	155.8 d	155.8 d	155.8 d
2	86.3 d	86.3 d	86.3 d	86.6 d	86.6 d	86.7 d
3	89.5 s	89.5 s	89.5 s	76.0 s	76.0 s	76.0 s
4	90.3 s	90.4 s	90.4 s	93.6 s	93.6 s	93.6 s
5	110.7 d	110.7 d	110.7 d	20.3 t	20.3 t	20.3 t
6	143.0 d	142.9 d	143.0 d	30.1 t	30.2 t	30.0 t
7	31.1 t	31.1 t	31.1 t	30.0 t	30.3 t	30.6 t[b]
8	30.0 t[c]	30.3 t	30.0 t	30.4 t[c]	27.8 t	30.1 t[b]
9	29.8 t[c]	28.0 t	30.57 t	30.3 t[c]	37.7 t	28.5 t
10	32.9 t	40.0 t	28.0 t	33.0 t	35.7 d	40.2 t
11	23.7 t	29.2 d	37.8 t	23.7 t	30.6 t	29.2 d
12	14.5 q	23.1 q	35.7 d	14.5 q	11.8 q	23.0 q
13		23.1 q	30.62 t		19.6 q	23.0 q
14			11.8 q			
15			19.7 q			
1′	75.4 t	75.4 t	75.4 t	75.2 t	75.2 t	75.2 t
2′	72.1 d	72.1 d	72.1 d	72.1 d	72.1 d	72.1 d
3′	63.9 t	63.9 t	63.9 t	63.9 t	63.9 t	63.9 t

[a] Measured in CD_3OD solutions at 125 MHz. Assignments were aided by DEPT, HMQC, and HMBC experiments. [b,c] Interchangeable signals.

Scheme 1



(i) H_2, Pd-C, MeOH; (ii) bromododecane, NaH, DMF; (iii) HCl, THF; (iv) Ac_2O, pyr.

spectroscopy. The spectral data for this compound were very similar to those obtained for **1**. The only difference in the NMR spectra was the appearance of signals for an additional methyl group. Because the two methyl groups in **2** gave identical chemical shifts in both the ^1H (δ 0.88) and ^{13}C (δ 23.1) NMR spectra, the location of this new methyl group was assigned to C-11 of the linear chain, and it was confirmed by HMBC experiments in which long-range correlations of the signals of H-12 (or -13) with C-10, C-11, and C-13 (or -12) were clearly observed.

The molecular formula for **3** was deduced as $C_{18}H_{30}O_3$ by HRFABMS data. Although the spectral data for this compound were very similar to those of other acetylenic ethers, the ^{13}C NMR spectra showed the presence of an additional methyl group at δ 19.7, which gave rise to a signal at δ 0.86 (3H, d, J = 6.8 Hz) in the ^1H NMR spectrum. The attachment of the new methyl group was assigned to C-12 of the linear chain based on the long-range correlations of proton signals at this carbon and the terminal methyl group at C-14 with the C-12 and C-13 signals.

Compound **4** was isolated as a colorless gum, its molecular formula established as $C_{15}H_{26}O_3$ by HRMS analysis. The NMR data for this compound resembled those obtained for other compounds in the presence of a glyceryl moiety, and a linear chain was readily recognized. However, analysis of the ^{13}C NMR spectra revealed the replacement of the double-bond signals by two upfield methylene signals in this compound, in agreement with a considerable shift of the UV absorption maxima from 275 and 290 nm of other analogues to 238 nm of **4**. A combination of ^1H COSY, HMQC, and HMBC experiments showed that the C-5 double bond of the acetylenic ether previously described appeared to be hydrogenated in **4** (Table 1). Thus, the structure of **4** was defined as a glyceryl enol ether containing an ene−yne functionality.

Two closely related derivatives, **5** and **6** were isolated as colorless gums. Both compounds had the same molecular formula, $C_{16}H_{28}O_3$, as indicated by HRMS and ^{13}C NMR spectrometry. The spectral data for these compounds were very similar to those of **4**. The only significant difference in the NMR spectra was the appearance of signals of an additional methyl group. Combined 2D NMR experiments allowed the location of the additional methyl group at C-10 and C-11 for **5** and **6**, respectively, making these compounds structurally comparable to **1** and **3** in the yne−dienic glycerol series, respectively.

Although acetylenic and polyacetylenic metabolites have been frequently isolated from sponges, in particular from animals of the genus *Petrosia*, the acetylenic enol ethers of glycerol belong to a relatively limited structural class. A literature survey revealed that, excepting **1**−**6** and raspailynes from *R. pumila* and *R. ramosa*, the only other metabolites containing the same functional groups are the diyne enol ethers from *P. hebes* and the petrosynes from an Okinawan specimen of the genus *Petrosia*.[16,17] A structurally related group of metabolites, hanishenols A and B from the sponge *Acanthella carteri* (= *A. aurantiaca*) possesses a glycerol enol ether functionality.[18]

In addition to the acetylenic enol ethers, a new acetylenic alcohol (**7**) was isolated as a colorless oil. The molecular formula for **7** was established as $C_{13}H_{20}O_3$ by combined HRMS and ^{13}C NMR analysis. The NMR spectra of this compound showed signals characteristic of the terminal yne-ol-ene functionality, which has been frequently found in several sponge-derived polyacetylenes.[1−3,5,6,11,12] In addition, the ^{13}C NMR spectra displayed signals for a carbonyl and a methoxy carbon at δ 174.2 (s) and 51.5 (q), respectively. The presence of a methyl ester in the structure of **7** was supported by the ^1H NMR signal at δ 3.65 (3H, s) and by the strong IR absorption at 1740 cm^{-1}.

With the aid of this information, the structure of **7** was determined by combined 2D NMR experiments. All of the proton-bearing carbons and their protons were matched precisely by the ^1H COSY and HMQC experiments. The HMBC correlations between signals of the methine and olefinic protons at δ 4.82, 5.59, and 5.88, respectively, with those of neighboring carbons confirmed the presence of the terminal yne−ol−ene functionality. In addition, the TOCSY experiment showed a correlation containing both signals of the α-olefinic protons at δ 2.05 and the α-carbonyl protons at δ 2.29. Thus, the structure of **7** was defined as a linear acetylenic alcohol containing a methyl ester group. Compound **7** contained an asymmetric carbon at C-3. The absolute configuration at this center was determined by the modified Mosher method.[19,20] The NMR spectra of the (*S*)- and (*R*)-MTPA esters, **7*S*** and **7*R***, were recorded, and based on the Δ (δ **7*S*** − δ **7*R***) values, the *R* configuration was assigned for the C-3 alcohol.

A structural similarity was found between this compound and the petrocortynes.[12] For an example, both **7** and petrocortyne A, the major metabolite from the specimens, had identical partial structures in the region of C-1 through C-11, including the stereochemistry at the C-3 alcohol. This is supporting evidence for the partial structure of petrocortyne A that was proposed by the results of a TOCSY experiment on a Eu(fod)$_3$-treated derivative.[12] In addition, the location of the ester at C-12 corresponded to the acetylene group in petrocortyne A, which could be easily transformed in the biogenetic process. Therefore, **7** might

be either a biogenetic degradation product or a precursor of the petrocortynes.

Sponge-derived acetylenes and related compounds are widely recognized for potent and diverse bioactivity.[1,2−5,7−9,12] In our measurement of bioactivities of glyceryl enol ether compounds, **1**−**3** of the yne−diene series exhibited weak cytotoxicity against the human leukemia cell-line K-562 (LC_{50} 9.2, 57, 29 μg/mL, for **1**−**3**, respectively), while **4**−**6**, possessing the yne−ene group, were not active (LC_{50} > 100 μg/mL).

Experimental Section

General Experimental Procedures. The optical rotations were measured on a JASCO digital polarimeter using a 5-cm cell. IR spectra were recorded on a Mattson Galaxy spectrophotometer. UV spectra were obtained in MeOH using a Milton−Roy spectrophotometer. NMR spectra were recorded in $CDCl_3$ and CD_3OD solutions on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal Me_4Si. MS spectra were provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside, and Korea Basic Science Institute, Taejeon, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. The specimens of *Petrosia* sp. (sample number 94K-13) were collected by hand using Scuba at a depth of 20−30 m in October 1994 and November 1995, along the shore of Keomun Island, Korea.[21] Morphologically this sponge is very similar to *P. corticata*, but differs in possessing only oxeas and no large strongylotes as spicules. Details of morphological characters were reported previously.[12]

Extraction and Isolation. The freshly collected samples were immediately frozen and kept at −25 °C until investigated chemically. The sponge (5.5 kg, wet wt) was defrosted, macerated, and extracted with MeOH (6 L × 2) and CH_2Cl_2 (6 L × 2). The combined crude extracts (353. 41 g) were partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer (100.61 g) was concentrated in vacuo, and the residue was repartitioned between *n*-hexane (71.20 g) and 15% aqueous MeOH (28.53 g). An aliquot (8.12 g) of the aqueous MeOH layer was dried and subjected to C_{18} reversed-phase vacuum flash chromatography using sequential mixtures of MeOH and H_2O as eluents (elution order: 30%, 20%, 10% aqueous MeOH, 100% MeOH, and EtOAc). The fraction (0.88 g) eluted with 20% aqueous MeOH was dried and separated by reversed-phase HPLC (YMC ODS-A column, 15% aqueous MeOH) to yield, in order of elution, **7**, **1**, **4**, **2**, raspailyne B2, **6**, **5**, and raspailyne B1. Final purification was made by reversed-phase HPLC (YMC ODS-A column, 30% aqueous MeCN) to afford 7.3, 9.4, 13.1, 10.4, 6.2, 4.2, 6.4, and 5.0 mg of raspailynes B1 and B2, **1**, **2**, and **4**−**7** as colorless gums, respectively.

The fraction (0.30 g) eluted with 10% aqueous MeOH from vacuum flash chromatography was dried and separated by reversed-phase HPLC (15% aqueous MeOH) to yield, in order of elution, isoraspailyne B1, raspailyne B2, **3**, and isoraspailyne B. Further purification was made by reversed-phase HPLC (30% aqueous MeCN) to afford 12.5, 6.0, 3.8, and 5.7 mg of pure raspailyne B2, isoraspailynes B and B1, and **3** as colorless gums, respectively.

Raspailyne B1: $[\alpha]^{25}_D$ −3.2° (*c* 0.08, MeOH); lit. value[14] −4.9° (*c* 0.47, $CHCl_3$); ^1H and ^{13}C NMR and HRMS data were comparable with reported ones.

Raspailyne B2: $[\alpha]^{25}_D$ −4.1° (*c* 0.40, MeOH); lit. value[14] −10.8° (*c* 0.065, $CHCl_3$) ^1H and ^{13}C NMR and HRMS data were comparable with reported ones.

Isoraspailyne B: $[\alpha]^{25}_D$ −2.3° (*c* 0.08, MeOH) ^1H and ^{13}C NMR and HRMS data comparable with reported ones.

Isoraspailyne B1: $[\alpha]^{25}_D$ +1.0° (*c* 0.10, MeOH) ^1H and ^{13}C NMR and HRMS data comparable with reported ones.

Petroraspailyne A1 (1): $[\alpha]^{25}_D$ −3.2° (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 276 (3.74), 291 (3.58) nm; IR (KBr) ν_{max} 3450 (br), 2925, 2860, 2160 (weak), 1630, 1560, 1260, 1100, 1050 cm^{-1}; ^1H NMR (CD_3OD) δ 6.45 (1H, d, *J* = 6.3 Hz, H-1), 5.82 (1H, dt, *J* = 10.8, 7.3 Hz, H-6), 5.55 (1H, br d, *J* = 10.8 Hz, H-5), 4.61 (1H, dd, *J* = 6.3, 2.4 Hz, H-2), 3.99 (1H, dd, *J* = 10.7, 4.6 Hz, H-1′), 3.91 (1H, dd, *J* = 10.7, 5.9 Hz, H-1′), 3.81 (1H, m, H-2′), 3.61 (1H, dd, *J* = 11.2, 5.4 Hz, H-3′), 3.55 (1H, dd, *J* = 11.2, 5.4 Hz, H-3′), 2.30 (2H, ddt, *J* = 7.3, 1.5, 7.3 Hz, H-7), 1.40 (2H, m, H-8), 1.31 (6H, m, H-9, -10, -11), 0.90 (3H, t, *J* = 7.1 Hz, H-12); ^{13}C NMR, see Table 1; HMBC correlations H-1/C-2, C-3, C-1′; H-2/C-1, C-4, C-5, C-6; H-5/C-3, C-7; H-6/C-4, C-5, C-7, C-8; H-7/C-5, C-6, C-8(9); H-12/C-10, C-11; H-1′/C-1, C-2′, C-3′; H-2′/C-1′, C-3′; H-3′/C-1′, C-2′; HRFABMS $[M + Na]^+$ *m/z* 275.1627 (calcd $C_{15}H_{24}O_3Na$ 275.1623).

Petroraspailyne A2 (2): $[\alpha]^{25}_D$ −3.2° (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ϵ) 275 (3.82), 291 (3.64) nm; IR (KBr) ν_{max} 3400 (br), 2925, 2860, 2180, 1630, 1460, 1370, 1160, 1050 cm^{-1}; ^1H NMR (CD_3OD) δ 6.45 (1H, d, *J* = 6.8 Hz, H-1), 5.82 (1H, dt, *J* = 10.7, 7.6 Hz, H-6), 5.56 (1H, brd, *J* = 10.7 Hz, H-5), 4.62 (1H, dd, *J* = 6.8, 2.5 Hz, H-2), 3.99 (1H, dd, *J* = 10.7, 4.9 Hz, H-1′), 3.91 (1H, dd, *J* = 10.7, 6.4 Hz, H-1′), 3.81 (1H, m, H-2′), 3.61 (1H, dd, *J* = 11.2, 5.4 Hz, H-3′), 3.55 (1H, dd, *J* = 11.2, 5.9 Hz, H-3′), 2.30 (2H, ddt, *J* = 7.6, 1.5, 7.3 Hz, H-7), 1.52 (1H, m, H-11), 1.37 (2H, m, H-8), 1.33 (2H, m, H-9), 1.20 (2H, m, H-10), 0.88 (6H, d, *J* = 6.4 Hz, H-12, -13); ^{13}C NMR, see Table 1; HMBC correlations H-1/C-2, C-3, C-1′; H-2/C-4; H-7/C-5, C-6, C-8; H-12(13)/C-10, C-11, C-13(12); H-1′/C-1, C-2′, C-3′; H-2′/C-3′; H-3′/C-1′, C-2′; HREIMS $[M]^+$ *m/z* 266.1879 (calcd $C_{16}H_{26}O_3$ 266.1882).

Petroraspailyne A3 (3): $[\alpha]^{25}_D$ +2.6° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 276 (3.92), 290 (3.74) nm; IR (KBr) ν_{max} 3400 (br), 2960, 2925, 2855, 2180, 1630, 1460, 1375, 1270, 1090, 1050 cm^{-1}; ^1H NMR (CD_3OD) δ 6.45 (1H, d, *J* = 6.4 Hz, H-1), 5.82 (1H, dt, *J* = 10.8, 7.3 Hz, H-6), 5.56 (1H, br d, *J* = 10.8 Hz, H-5), 4.61 (1H, dd, *J* = 6.4, 2.4 Hz, H-2), 3.99 (1H, dd, *J* = 11.2, 4.9 Hz, H-1′), 3.91 (1H, dd, *J* = 11.2, 5.9 Hz, H-1′), 3.81 (1H, m, H-2′), 3.61 (1H, dd, *J* = 11.2, 5.4 Hz, H-3′), 3.55 (1H, dd, *J* = 11.2, 5.4 Hz, H-3′), 2.30 (2H, ddt, *J* = 7.3, 1.5, 7.3 Hz, H-7), 1.41 (2H, m, H-8), 1.36 (2H, m, H-13), 1.33 (1H, m, H-11), 1.31 (5H, m, H-9, -10, -12), 1.12 (1H, m, H-11), 0.87 (3H, t, *J* = 7.3 Hz, H-14), 0.86 (3H, d, *J* = 6.8 Hz, H-15); ^{13}C NMR, see Table 1; HMBC correlations H-1/C-2, C-3; H-2/C-1, C-4; H-5/C-3, C-6, C-7; H-6/C-4, C-5, C-7; H-7/C-5, C-6, C-8; H-14/C-12, C-13; H-15/C-11, C-12, C-13; H-1′/C-1, C-2′, C-3′; H-2′/C-1′, C-3′; H-3′/C-1′, C-2′; HRFABMS $[M+Na]^+$ *m/z* 317.2096 (calcd $C_{18}H_{30}O_3Na$ 317.2093).

Petroraspailyne B1 (4): $[\alpha]^{25}_D$ +0.5° (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ϵ) 237 (3.57) nm; IR (KBr) ν_{max} 3400 (br), 2930, 2860, 1635, 1460, 1375, 1280, 1160, 1090 cm^{-1}; ^1H NMR (CD_3OD) δ 6.36 (1H, d, *J* = 6.8 Hz, H-1), 4.44 (1H, dt, *J* = 6.8, 2.2 Hz, H-2), 3.96 (1H, dd, *J* = 10.7, 4.9 Hz, H-1′), 3.87 (1H, dd, *J* = 10.7, 5.9 Hz, H-1′), 3.80 (1H, m, H-2′), 3.61 (1H, dd, *J* = 11.2, 5.4 Hz, H-3′), 3.55 (1H, dd, *J* = 11.2, 5.4 Hz, H-3′), 2.29 (2H, dt, *J* = 2.2, 7.0 Hz, H-5), 1.51 (2H, quin, *J* = 7.0 Hz, H-6), 1.42 (2H, m, H-7), 1.31 (8H, m, H-8, -9, -10, -11), 0.90 (3H, t, *J* = 6.8 Hz, H-12); ^{13}C NMR, see Table 1; HMBC correlations H-1/C-2, C-3, C-1′; H-2/C-1, C-4; H-5/C-1, C-2, C-3, C-4, C-6(7); H-6/C-4, C-5; H-12/C-10, C-11; H-1′/C-1, C-2′, C-3′; H-2′/C-1′, C-3′; H-3′/C-1′, C-2′; HRFABMS $[M + Na]^+$ *m/z* 277.1794 (calcd $C_{15}H_{26}O_3Na$ 277.1780).

Petroraspailyne B2 (5): $[\alpha]^{25}_D$ +5.5° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (3.51) nm; IR (KBr) ν_{max} 3400 (br), 2930, 2860, 1635, 1460, 1400, 1160, 1090 cm^{-1}; ^1H NMR (CD_3OD) δ 6.35 (1H, d, *J* = 6.4 Hz, H-1), 4.44 (1H, dt, *J* = 6.4, 2.4 Hz, H-2), 3.96 (1H, dd, *J* = 10.7, 4.9 Hz, H-1′), 3.87 (1H, dd, *J* = 10.7, 5.9 Hz, H-1′), 3.81 (1H, m, H-2′), 3.61 (1H, dd, *J* = 11.2, 5.4 Hz, H-3′), 3.55 (1H, dd, *J* = 11.2, 5.9 Hz, H-3′), 2.29 (2H, dt, *J* = 2.4, 6.8 Hz, H-5), 1.51 (2H, quin., *J* = 6.8 Hz, H-6), 1.40 (2H, m, H-7), 1.32 (6H, m, H-8, -9, -10, -11), 1.14 (1H, m, H-9), 0.88 (3H, t, *J* = 7.3 Hz, H-12), 0.87 (3H, d, *J* = 6.8 Hz, H-13); ^{13}C NMR, see Table 1; HMBC correlations H-1/C-2, C-3; H-5/C-1, C-2, C-3, C-4; H-6/C-4, C-5; H-12/C-10, C-11; H-13/C-9, C-10, C-11; H-1′/C-1, C-2′, C-3′; H-3′/C-1′, C-2′; HRFABMS $[M + Na]^+$ *m/z* 291.1942 (calcd $C_{16}H_{28}O_3Na$ 291.1936).

Petroraspailyne B3 (**6**): $[\alpha]^{25}_D$ +3.7° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (3.57) nm; IR (KBr) ν_{max} 3400 (br), 2930, 2860, 2200 (weak), 1635, 1460, 1325, 1160, 1090, 1050 cm^{-1}; ^1H NMR (CD_3OD) δ 6.36 (1H, d, J = 6.4 Hz, H-1), 4.44 (1H, dt, J = 6.4, 2.4 Hz, H-2), 3.97 (1H, dd, J = 10.7, 4.9 Hz, H-1′), 3.88 (1H, dd, J = 10.7, 5.9 Hz, H-1′), 3.81 (1H, m, H-2′), 3.62 (1H, dd, J = 11.2, 5.4 Hz, H-3′), 3.56 (1H, dd, J = 11.2, 5.4 Hz, H-3′), 2.30 (2H, dt, J = 2.0, 6.8 Hz, H-5), 1.53 (1H, m, H-11), 1.51 (2H, m, H-6), 1.32 (6H, m, H-7, -8, -9), 1.20 (2H, dt, J = 7.8, 7.8 Hz, H-10), 0.89 (6H, d, J = 6.8 Hz, H-12, -13); ^{13}C NMR, see Table 1; HMBC correlations H-1/C-2, C-3, C-1′; H-2/C-1, C-4; H-5/C-3, C-4, C-6(7); H-6/C-5, C-7(8); H-11/C-12(13); H-12(13)/C-10, C-11; H-1′/C-2′, C-3′; H-2′/C-1′, C-3′; H-3′/C-1′, C-2′; HRFABMS $[M + Na]^+$ *m*/*z* 291.1950 (calcd $C_{16}H_{28}O_3Na$ 291.1936).

Petrynol (**7**): $[\alpha]^{25}_D$ +15.3° (*c* 0.08, $CHCl_3$); IR (KBr) ν_{max} 3350 (br), 2925, 2870, 1740, 1620, 1440, 1205, 1010 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.88 (1H, dt, J = 15.1, 6.8 Hz, H-5), 5.59 (1H, dd, J = 15.1, 6.1 Hz, H-4), 4.82 (1H, m, H-3), 3.65 (3H, s, OMe), 2.54 (1H, d, J = 2.0 Hz, H-1), 2.29 (2H, t, J = 7.6 Hz, H-11), 2.05 (2H, dt, J = 6.8, 6.8 Hz, H-6), 1.84 (1H, br s, OH), 1.59 (2H, m, H-10), 1.39 (2H, tt, J = 7.3, 6.8 Hz, H-7), 1.30 (4H, m, H-8, H-9); ^{13}C NMR ($CDCl_3$) δ 174.2 (C, C-12), 134.3 (CH, C-5), 128.5 (CH, C-4), 83.3 (C, C-2), 74.0 (CH, C-1), 62.8 (CH, C-3), 51.5 (CH_3, OMe), 34.1 (CH_2, C-11), 31.8 (CH_2, C-6), 28.9 (CH_2, C-8), 28.7 (CH_2, C-9), 28.5 (CH_2, C-7), 24.9 (CH_2, C-10); HMBC correlations H-1/C-3; H-3/C-1, C-2, C-4, C-5; H-4/C-2, C-3, C-6; H-5/C-3, C-6, C-7; H-6/C-4, C-5, C-7; H-11/C-9, C-10, C-12; OMe/C-12; HRCIMS $[M + NH_4]^+$ *m*/*z* 242.1762 (calcd $C_{13}H_{24}NO_3$ 242.1756).

Hydrogenation of 1. To a stirred solution of 4.8 mg of **1** in 1 mL of dry MeOH, 4 mg of 10% palladium charcoal was added. The mixture was stirred under a H_2 atmosphere for 15 h at room temperature. After removing the catalyst by filtration through a glass filter, evaporation under vacuum gave 4.6 mg of pure **8** as a colorless oil; $[\alpha]^{25}_D$ −0.5° (*c* 0.09, MeOH); ^1H NMR (CD_3OD) δ 3.73 (1H, m, H-2′), 3.57 (1H, dd, J = 11.2, 4.9 Hz, H-3′), 3.50 (1H, dd, J = 11.2, 5.9 Hz, H-3′), 3.47 (1H, dd, J = 10.0, 4.9 Hz, H-1′), 3.45 (2H, t, J = 6.4 Hz, H-1), 3.40 (1H, dd, J = 10.0, 6.1 Hz, H-1′), 1.56 (2H, m, H-2), 1.33 (2H, m, H-3), 1.28 (16H, m), 0.89 (3H, t, J = 6.8 Hz, H-12); ^{13}C NMR (CD_3OD) δ 73.2 (CH_2, C-1′), 72.6 (CH_2, C-1), 72.3 (CH, C-2′), 64.6 (CH_2, C-3′), 33.1 (CH_2, C-10), 30.8 (CH_2 × 2), 30.7 (CH_2 × 3), 30.6 (CH_2), 30.5 (CH_2), 27.2 (CH_2, C-2), 23.8 (CH_2, C-11), 14.5 (CH_3, C-12); HREIMS $[M]^+$ *m*/*z* 260.2356 (calcd $C_{15}H_{32}O_3$ 260.2351).

Acetylation of 8. To a stirred solution of 3.5 mg of **8** in 0.3 mL of dry pyridine, 0.2 mL of Ac_2O was added. After stirring the mixture for 3 h at room temperature, the solvent and excess reactant were removed by blowing with N_2. Separation by reversed-phase HPLC (YMC ODS column, 100% MeCN) gave 2.7 mg of pure **9** as a colorless solid; mp 45−47°; $[\alpha]^{25}_D$ −11.6° (*c* 0.16, MeOH); ^1H NMR (CD_3OD) δ 5.15 (1H, m, H-2′), 4.32 (1H, dd, J = 11.8, 3.8 Hz, H-3′), 4.12 (1H, dd, J = 11.8, 6.8 Hz, H-3′), 3.57 (1H, dd, J = 10.7, 5.9 Hz, H-1′), 3.54 (1H, dd, J = 10.7, 4.9 Hz, H-1′), 3.47 (1H, dt, J = 9.5, 6.6 Hz, H-1), 3.43 (1H, dt, J = 9.5, 6.6 Hz, H-1), 2.04 (3H, s, OAc), 2.03 (3H, s, OAc), 1.56 (2H, m, H-2), 1.33 (2H, m, H-3), 1.28 (16H, m), 0.89 (3H, t, J = 7.1 Hz, H-12).

Synthesis of (*S*)-1,2-Diacetoxy-3-dodecyloxypropane-1,2-diol (**9**). To a stirred solution of 116 mg of (*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol in 10 mL of DMF was added 23 mg of NaH. After refluxing the mixture for 1 h, 0.3 mL of bromododecane was added by syringe. The mixture was refluxed for 3 h, and the solvent removed under vacuum and the residue partitioned between Et_2O and H_2O. The ether layer was dried, and the residue was redissolved in 30 mL of THF. On the addition of 5 mL of 10% HCl, the mixture was stirred overnight at room temperature. After removing the solvent under vacuum, separation by silica column chromatography (1 × 17 cm, 50% EtOAc in hexane) gave pure (*S*)-3-dodecyloxypropane-1,2-diol (**8**): $[\alpha]^{25}_D$ −0.1° (*c* 1.1, MeOH). Acetylation of this compound was performed by using the same method as for **8**. From 21.0 mg of (*S*)-3-dodecyloxypropane-1,2-diol was obtained 17.2 mg of (*S*)-1,2-diacetoxy-3-dodecyloxypropane-1,2-diol (**9**). The ^1H NMR data of this compound were identical with those of **9**: $[\alpha]^{25}_D$ −9.6° (*c* 1.15, MeOH).

(*S*)-MTPA Ester of 7. To a stirred solution of 1.7 mg of **7** in 0.3 mL of dry pyridine was added 20 μL of (−)-MTPA chloride. After stirring the mixture under N_2 at room temperature for 1 h, the solvent was removed by blowing with N_2. The residue was redissolved in 2 mL of 30% EtOAc−hexane and filtered through a Sep-Pak silica column. After removing the solvent under vacuum, the residue was separated by reversed-phase HPLC (YMC ODS column, 100% MeCN) to yield 1.1 mg of **7S** as a colorless gum: ^1H NMR ($CDCl_3$) δ 7.54 (2H, m, Ar), 7.43−7.41 (3H, m, Ar), 6.07 (1H, ddt, J = 15.1, 1.0, 6.8 Hz, H-5), 6.01 (1H, br dd, J = 6.8, 2.0 Hz, H-3), 5.60 (1H, ddt, J = 15.1, 6.8, 1.5, H-4), 3.66 (3H, s, OMe), 3.56 (3H, s, OMe), 2.59 (1H, d, J = 2.0 Hz, H-1), 2.30 (2H, t, J = 7.3 Hz, H-11), 2.09 (2H, ddt, J = 6.8, 1.5, 7.3 Hz, H-6), 1.61 (2H, quin, J = 7.3 Hz, H-10), 1.40 (2H, quin, J = 7.3 Hz, H-7), 1.31−1.29 (4H, m, H-8, -9); HRFABMS $[M + Na]^+$ *m*/*z* 463.1731 (calcd $C_{23}H_{27}F_3O_5Na$ 463.1708).

(*R*)-MTPA Ester of 7. Prepared as described for **7*S***. From 1.1 mg of **7** and 20 μL of (+)-MTPA chloride was obtained 0.9 mg of **7*R*** as a colorless gum: ^1H NMR ($CDCl_3$) δ 7.54 (2H, m, Ar), 7.44−7.38 (3H, m, Ar), 6.03 (1H, br d, J = 6.8 Hz, H-3), 6.00 (1H, ddt, J = 15.1, 1.0, 6.8 Hz, H-5), 5.50 (1H, ddt, J = 15.1, 6.8, 1.5 Hz, H-4), 3.66 (3H, s, OMe), 3.59 (3H, s, OMe), 2.63 (1H, d, J = 2.0 Hz, H-1), 2.30 (2H, t, J = 7.3 Hz, H-11), 2.05 (2H, ddt, J = 6.8, 1.5, 7.3 Hz, H-6), 1.60 (2H, quin, J = 7.3 Hz, H-10), 1.37 (2H, quin, J = 7.3 Hz, H-7), 1.29 (4H, m, H-8, -9); Δ (δ**7*S*** − δ**7*R***) H-1, -20.5 Hz; H-3, -8.8 Hz; H-4, +52.7 Hz; H-5, +32.3 Hz; H-6, +19.8 Hz; H-7, +16.1 Hz; H-10, +2.9 Hz; H-11, +1.5 Hz.

Cytotoxicity Assay.[22] The human leukemia cell (K-562) was cultivated in a medium (RPMI 1640 including 10% FBS and 20 μg/mL of kanamycin) at 37 °C under 5% CO_2 atmosphere. The cells were harvested by centrifugation at 1500 rpm for 5 min and washed with PBS buffer to remove the medium. Using a hemocytometer under an inverted microscope, the cells were counted and suspended (2 × 10^4 cells/mL) in a new medium of the same constitutents. The newly suspended cells were distributed into a 96-well microplate (0.1 mL each), and samples dissolved in DMSO (series of sequential ten-fold dilution, 0.1 mL each) were added. The suspensions were incubated at 37 °C under 5% CO_2 for 5 days. On the addition of MTT solution (1.1 mg/mL of stock solution, 50 μL each), the suspensions were incubated for 4 h under the same condition. After removing the supernatant with microplate washer, 0.15 mL of DMSO was added to dissolve formazan. The absorbance was measured at 540 nm with a microplate reader (Bio-Rad Model 3550) and IC_{50} value was calculated.

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