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Six new polyacetylenic alcohols from the marine sponges Petrosia sp. and Halichondria sp.

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Summary
Six new polyacetylenic alcohols, termed strongylotriols A and B; pellynols J, K, and L; and isopellynol A, together with three known polyacetylenic alcohols, pellynols A, B, and C were isolated from the marine sponges Petrosia sp., and Halichondria sp. collected in Okinawa, Japan. Their planer structures were determined based on 2D-NMR and mass spectrometric analysis of the degraded products by RuCl$_3$ oxidation. The absolute stereochemistry of isolates was examined by their Mosher’s esters. The strongylotriols were found to be optically pure compounds, whereas the pellynols are diastereomeric mixtures at the C-6 position. Proliferation experiments using the HeLa and K562 cell lines suggested that the essential structural units for activity are the “hexa-2,4-diyn-1,6-diol” and “pent-1-en-4-yn-3-ol” on the termini.

Key words: polyacetylenic alcohol, marine sponge, Petrosia sp., Halichondria sp., strongylotriol, pellynol
INTRODUCTION

Polyacetylenic compounds are diverse groups of secondary metabolites that have been isolated from various sources, including plants, marine organisms, nudibranchs, hard corals and red algae. These compounds have been shown to possess various pharmacological activities, which including anti-tumor, anti-viral, anti-microbial and anti-inflammatory activities. In our continuing research on biologically active metabolites in marine invertebrates, we have isolated six new polyacetylenic alcohols from the marine sponges, Petrosia sp., and Halichondria sp. collected in Okinawa. This paper describes the isolation, structure and biological activity of acetylenic alcohols.

RESULTS and DISCUSSION

EtOH extracts prepared from 93 specimens of marine invertebrates collected at Iriomote, Okinawa, were screened for anti-proliferative activity. Among them, an extract of a Petrosia sp. and an extract of a Halichondria sp. were able to inhibit HeLa cell proliferation. These extracts were subjected to bioassay-guided separation. The Et$_2$O soluble fraction of the EtOH extract of Petrosia sp. was fractionated using a Sephadex LH-20 column (CHCl$_3$), and reverse-phase HPLC (5C$_{22}$, 91% MeOH/H$_2$O), yielding strongylotriol A (1) and B (2); and pellynols J (4), A (7), B (8) and C (9). The Et$_2$O soluble fraction of the EtOH extract of Halichondria sp. was fractionated using a silica-gel column, followed by reverse-phase HPLC (5C$_{30}$, 93% MeOH/H$_2$O or 5C$_{18}$, 95% MeOH/H$_2$O), yielding isopellynol A (3); pellynols J (4), K (5), L (6), A (7), B (8) and C (9).

Strongylotriol A (1) was found to be a white, amorphous solid, with ESITOF-MS showing a molecular formula of C$_{23}$H$_{40}$O$_3$. $^1$H- and $^{13}$C-NMR and HSQC spectroscopic data indicated the presence of one primary methyl, one oxygenated methylene, two oxygenated methines, three di-substituted alkynes, and 15 aliphatic methylenes, suggesting that 1 was an acyclic polyacetylenic alcohol. Its HMBC spectrum showed partial structures, including the terminal “hexa-2,4-diyn-1,6-diol” and the internal “prop-2-yn-1-ol” (Figure 2a). The location of the “prop-2-yn-1-ol” unit was determined by analysis of the degradation product of 1 resulting from RuCl$_3$ oxidation. Because the fragment ion peak observed at m/z 201.1039 (C$_{10}$H$_{17}$O$_4$, Δmnu - 9.3) was estimated to be a decanedioic acid, the “prop-2-yn-1-ol” unit was located at C-15 of 1, yielding the deduced planar structure shown in Figure 2b. The absolute stereochemistry of 1 was determined using a modified Mosher’s method. The proton chemical shifts of (R)- and
(S)-MTPA esters of 1 were assigned by COSY and TOCSY spectral data. The distribution of $\Delta \delta (\delta_S-\delta_R)$ values indicated that the absolute stereochemistry at C-6 and C-15 was 6$R$ and 15$S$, respectively (Figure 2c).

Figure 2a, 2b, and 2c

Strongylotriol B (2) was found to be a white, amorphous solid, with ESITOF-MS showing a molecular formula of $C_{26}H_{42}O_3$. $^1$H- and $^{13}$C-NMR spectra were similar to those of 1 except for the signals due to the terminal isopropyl moiety [$\delta_H$ 0.85 (6H, d), $\delta_C$ 22.6 (q), 27.2 (d)] (Tables 1 and 2). Mass spectrometric analysis of the degradation product of 2 resulting from RuCl$_3$ oxidation showed that its “prop-2-yn-1-ol” unit was located in the same position as in 1. The absolute stereochemistry of 2 was also examined using a modified Mosher’s method, and the absolute configurations at C-6 and C-15 were determined to be 6$R$ and 15$S$ respectively, as in 1.

Figure 2a, 2b, and 2c

Isopellynol A (3) was isolated as a white, amorphous solid from the Et$_2$O extractive of Halichondria sp. ESITOF-MS showed that its molecular formula was $C_{33}H_{52}O_3$. $^1$H- and $^{13}$C-NMR and HSQC spectroscopic data indicated the presence of one oxygenated methylene, two oxygenated methines, two di-substituted alkenes, two di-substituted alkynes, one terminal acetylene and 20 aliphatic methylenes. The HMBC spectrum of 3 showed three partial structures, a terminal “hexa-2,4-diyn-1,6-diol”, a “pent-2-en-4-yn-1-ol” and an internal “Z-olefin” (Figure 3a). The olefin geometry was determined to be Z both from its coupling constant ($J$=10.8 Hz) and the $^{13}$C chemical shift of the allylic methylenes ($\delta_C$ 27.2). The location of the internal “Z-olefin” was determined by analysis of the degradation products of 3 resulting from RuCl$_3$ oxidation. The fragment ion peaks derived from tridecanedioic acid (C$_{13}$H$_{24}$O$_4$) and undecanedioic acid (C$_{11}$H$_{20}$O$_4$) were observed at $m/z$ 215.1305 (M-H)` and $m/z$ 243.1564 (M-H)` , respectively, resulting in the deduced planar structure shown in Figure 3b. The absolute stereochemistry of 3 was examined using its Mosher’s esters, and the distribution of $\Delta \delta (\delta_S-\delta_R)$ values closed to C-29 indicates that absolute stereochemistry at C-29 was in the $S$ configuration. However, the $^1$H-NMR spectra of (R)- and (S)-MTPA esters showed diastereomeric proton signals for H-6 in a ratio of 3/4.

Figure 3a, 3b, and 3c

Pellynol J (4) was obtained as a white, amorphous solid from the Et$_2$O extractives of
both Petrosia sp. and Halichondria sp. ESITOF-MS showed a molecular formula of C$_{31}$H$_{48}$O$_{5}$. $^1$H- and $^{13}$C-NMR and HSQC spectroscopic data indicated the presence of one oxygenated methylene, two oxygenated methines, two di-substituted alkenes, two di-substituted alkynes, one terminal acetylene and 18 aliphatic methylenes. Because, these spectral features corresponded to those of the known polyacetylenic alcohols, pellynol A (7; C$_{33}$H$_{42}$O$_{3}$) and B (8; C$_{32}$H$_{50}$O$_{3}$), pellynol J was regarded as a C$_{31}$ polyacetylenic alcohol possessing terminal "hexa-2,4-diyn-1,6-diol" and “pent-1-en-4-yn-3-ol” units and an internal “Z-olefin” unit (Tables 1 and 2). The position of “Z-olefin” at C16 was determined by the ESITOF-MS analysis of 4 with RuCl$_3$ oxidation. The absolute stereochemistry of C-29 was determined to be $R$ configuration, however the stereochemistry at C-6 was a diastereomeric mixture, as in isopellynol A (3).

Pellynol K (5) was obtained as a white, amorphous solid from the Et$_2$O extractive of Halichondria sp.. ESITOF-MS gave a molecular formula of C$_{34}$H$_{44}$O$_{3}$. $^1$H- and $^{13}$C-NMR and HSQC spectroscopic data were similar to those of pellynols A (7), B (8), and J (4), suggesting that pellynol K was a C$_{34}$ polyacetylenic alcohol possessing terminal "hexa-2,4-diyn-1,6-diol" and “pent-1-en-4-yn-3-ol” units and an internal “Z-olefin” unit. The position of “Z-olefin” at C-19 was determined by the ESITOF-MS analysis of 5 with RuCl$_3$ oxidation. A comparison of the specific rotation of 5 with those of 4, 7, and 8 indicated that the stereochemistry of 5 was 32$R$.

Pellynol L (6) was obtained as a white, amorphous solid from the Et$_2$O extractive of Halichondria sp. ESITOF-MS gave a molecular formula of C$_{35}$H$_{52}$O$_{2}$. $^1$H- and $^{13}$C-NMR and HSQC spectral data indicated the presence of one oxygenated methylene, one oxygenated methine, two di-substituted alkenes, three di-substituted alkynes, one terminal acetylene and 21 aliphatic methylenes. The HMBC spectrum of 6 yielded partial structures, terminal “pent-2,4-diyn-1-ol” and “pent-1-en-4-yn-3-ol” units and an internal “but-1-en-3-yn” unit (Figure 4a). These data suggested that 6 is a 6-deoxy-type derivative of pellynol C (9; C$_{33}$H$_{48}$O$_{3}$). The coupling constants of the olefins ($J=10.8$ Hz, 15.6 Hz) indicated that their geometries were Z and E, respectively. The location of the internal “but-1-en-3-yn” unit was determined by the degradation products of 6 with RuCl$_3$ oxidation followed by GC-MS analysis. Mass spectral data identified two dimethyl ester derivatives, dimethyl hexadecanedioate (C$_{19}$H$_{34}$O$_{4}$) and dimethyl nonanedioate (C$_{11}$H$_{20}$O$_{4}$) indicating that the “but-1-en-3-yn” unit was located at C-20 (Figure 4b). The absolute stereochemistry of 6 was also examined using a modified
Mosher’s method, with C-33 determined to have an absolute R configuration (Figure 4c).

The absolute stereochemistry of the known compounds, pellynols A (7) and C (9) was also assessed using the modified Mosher’s method yielding results identical to those of isopellynol A (3) and pellynol J (4) with the secondary alcohol at C-6 being a diastereomeric mixture (Figure 5).

The ability of the isolated compounds to inhibit the proliferation of HeLa and K562 cells were examined. Pellynols J (4), A (7), and B (8), all of which have the same structural units “hexa-2,4-diyn-1,6-diol” and ”pent-1-en-4-yn-3-ol”, on both ends, showed significant activity, with almost equivalent IC\textsubscript{50} values (Table 3). In contrast, strongylotriols A (1) and B (2) with structural unit “pent-1-en-4-yn-3-ol” on one-half, had nearly one-third the activity of pellynols J, A and B. Furthermore, the activities of isopellynol A (3) and pellynol L (6), with terminal “pent-2-en-4-yn-1-ol” and “pent-2,4-diyn-1-ol” units, respectively, were decreased slightly. These results were consistent with earlier findings.\textsuperscript{6), 11)}

In summary, six new polyacetylenic alcohols, strongylotriols A (1) and B (2); pellynols J (4), K (5), and L (6); and isopellynol A (3), were isolated from the marine sponges Petrosia sp. and Halichondria sp. Compounds 4, 7, and 8 were isolated from both sponges, despite the difference in appearance of these marine sponges. Furthermore, other abundant compounds differed in these species, in that meroditerpenoids such as strongylophorines were found only in Petrosia sp.,\textsuperscript{12) while polyacetylenic acids such as pellynic acids were found only in Halichondria sp. \textsuperscript{8) (Supplementary material). The isolated strongylotriols (1, 2) were optically pure, whereas the pellynols were diastereomeric mixtures at the C-6 position. The positions of the internal olefins differed in the pellynols, but their locations from the end terminal were identical to each other. Some symbionts may therefore biosynthesize these polyacetylenic compounds linking to two types of acetylene groups at this position.
EXPERIMENTAL

General Experimental Procedures
Optical rotations were measured with a Jasco Dip-370 digital polarimeter at 24°C. The NMR spectra were recorded on a Varian INOVA 600 operating at 600MHz for $^1$H and 150MHz for $^{13}$C in CDCl$_3$. $^1$H and $^{13}$C NMR chemical shifts is reported in ppm ($\delta$) and referenced to tetramethylsilane for $^1$H ($\delta_H = 0$) and the solvent signal for $^{13}$C (CDCl$_3$: $\delta_C = 77.2$). ESITOF-MS were measured with a Bruker microTOF mass spectrometer. GC-MS was performed on Gas Chromatography Mass Spetrometer-QP2010 using INERT Cap5MS/Sil, 30 m x 0.25 mm i.d., GL Science); column temp. 100-280°C, injection temp, 300°C, interface temp, 250°C. Chromatographic separations were carried out using Sephadex LH-20 (GE Helthcare), silica gel (Merck Silica gel 60), and reversed phase-HPLC (Cosmosil 5C22AR-II or 5C18AR-II, 250 x 4.6 mm i.d., nakalai tesque, Develosil C30 UG-3, 250 x 4.6 mm i.d., Nomura Chemicals) with a Jasco PU2089 gradient pump and PU2075 UV/VIS detector. Thin layer chromatography (TLC) analysis were carried out using Merck precoate TLC plates Silica gel 60 F$_{254}$ and RP-18F$_{254s}$.

Animal Material
Petrosia sp. and Halichondria sp. were collected by hand at a depth of 10-15m off the shore of Iriomote Island, Okinawa Prefecture, Japan, in Nobember 2009. Voucher specimens were deposited at the Graduate School of Pharmaceutical Sciences, Kyushu University under reg. no. 091120-36 for Petrosia sp., and no. 091120-35 for Halichondria sp..

Extraction and Isolation
Petrosia sp. (wet weight 405.89g) was homogenized and extracted with EtOH (3 x 0.5 L) and filtrated. The extract was evaporated in vacuo, and the resulting aqueous suspension was diluted H$_2$O (0.5 L) and extracted with Et$_2$O (3 x 0.2 L) and n-BuOH (3 x 0.2 L). The organic layers were evaporated to give an Et$_2$O extract (8.71 g) and n-BuOH extract (0.99 g). The part of Et$_2$O extract (4.99 g) was subjected to Sephadex LH-20 column chromatography with CHCl$_3$ to give twelve fractions, and fraction 8 (33.8 mg) was applied to reversed phase HPLC (Cosmosil 5C22 AR-II) with 91% MeOH/H$_2$O to give compounds 1 (2.0 mg), 2 (1.0 mg), 4 (2.4 mg), 7 (6.7 mg), and 8 (2.5 mg), respectively. Halichondria sp. (wet weight 100.0 g) was homogenized and extracted with EtOH (3 x 1.0 L) and filtrated. The extract was evaporated in vacuo, to give EtOH extract (5.0 g). The EtOH extract was partitioned between Et$_2$O, n-BuOH
and H₂O to give an Et₂O extract (2.73 g) and n-BuOH extract (1.06 g). The part of Et₂O extract (1.43 g) was subjected to silica gel column chromatography using n-hexane/EtOAc (1/9-0/1) to give seven fractions. Fraction 3 (92.9 mg) was subjected to silica gel column followed by reversed phase HPLC (Cosmosil 5C18 AR-II) with 95% MeOH/H₂O to give compound 6 (5.2 mg), and part of fraction 5 (165.8 mg) was applied to reversed phase HPLC (Develosil C30 UG-3) with 100% MeOH to give compounds 3 (2.8 mg), 4 (7.2 mg), 7 (71.2 mg), 8 (30.5 mg), and 9 (13.7 mg), respectively.

RuCl₃-NaIO₄ Oxidation
To a solution of each sample (ca. 1.0 mg) in CCl₄ (1.0 mL) was added NaIO₄ (10 eq.), RuCl₃ (0.04 mg), CH₃CN (1.0 mL), and H₂O (1.0 mL). The solution was stirred vigorously for 1 hr, then 1M HCl (2.0 mL) was added and extracted with Et₂O. Et₂O layer was analyzed ESITOF-MS (negative ion) directly or methylated with TMS-CH₂N₂ in 20% MeOH/benzene followed by GC-MS analysis.

Preparation of the (R)- or (S)-MTPA Esters
To a solution of each 6 (1.0 mg, 2 μmol) in dry CH₂Cl₂ (0.2 mL) stirring at room temperature was added (R)- or (S)-α-methoxy (trifluoromethyl) phenyl acetic acid (MTPA) (7.4 mg, 32.0 μmol), N, N'-dicyclohexyl carbodiimide DCC (6.5 mg, 32.0 μmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The solution was stirred at room temperature for 8 hr and filtered. The filtrate was dried with N₂ and subjected to silica gel column with n-hexane/EtOAc (9/1) to afford (R)-MTPA ester (1.9 mg) and (S)-MTPA ester (2.3 mg).

Strongylotriol A (1): amorphous solid; [α]D -8.3 (c=0.012, CHCl₃); ESITOF-MS m/z: 411.2900 [M+Na]⁺ (Calcd for C₂₅H₄₀NaO₃, 411.2870); ¹H- and ¹³C-NMR see Table 1 and 2. Oxidative product: decanedioic acid: m/z: 201.1039 [M-H]⁺ (Calcd for C₁₀H₁₇O₄, 201.1132).

(R)-MTPA ester of 1, ESITOF-MS m/z: 1059.4119 [M+Na]⁺ (Calcd for C₅₅H₆₁F₉NaO₉, 1059.4064); ¹H-NMR (CDCl₃) δ: 0.878(3H, t, H-25), 1.260(2H, m, H-12), 1.280(2H, m, H-8), 1.315(2H, m, H-20), 1.390(2H, m, H-13), 1.405(2H, m, H-19), 1.770(2H, t, H-7), 1.775(2H, m, H-14), 2.145(2H, t, H-18), 4.891(1H,d, H-1), 4.981(1H,d, H-1’), 5.484(1H, t, H-15), 5.563(1H, t, H-6), 3.544(3H, s, OMe), 3.568(6H, s, OMe), 7.3-7.7(15H, phenyl); (S)-MTPA ester of 1, ESITOF-MS m/z: 1059.4158 [M+Na]⁺ (Calcd for C₅₅H₆₁F₉NaO₉, 1059.4064); ¹H-NMR (CDCl₃) δ:0.878(3H, t, H-25),
Strongylotriol B (2): amorphous solid; [α]D -8.3 (c=0.013, CHCl3); ESITOF-MS m/z: 425.3009 [M+Na]+ (Calcd for C26H42NaO3, 425.3026); 1H- and 13C-NMR see Table 1 and 2. Oxidative product: decanedioic acid: m/z: 201.1035 [M-H]− (Calcd for C10H17O4, 201.1132).

(R)-MTPA ester of 2, ESITOF-MS m/z: 1073.4332 [M+Na]+ (Calcd for C56H63F9NaO9, 1073.4221); 1H-NMR (CDCl3) δ: 0.853(6H,d, H-25, 26), 1.260(2H, m, H-12), 1.280(2H, m, H-8), 1.315(2H, m, H-20), 1.390(2H, m, H-13), 1.405(2H, m, H-19), 1.770(2H, t, H-7), 1.775(2H, m, H-14), 2.145(2H, t, H-18), 4.891(1H,d, H-1), 4.981(1H,d, H-1’), 5.484(1H, t, H-15), 5.563(1H, t, H-6), 3.544(3H, s, OMe), 3.568(6H, s, OMe), 7.3-7.7(15H, phenyl); (S)-MTPA ester of 2, ESITOF-MS m/z: 1073.4302 [M+Na]+ (Calcd for C56H63F9NaO9, 1073.4221); 1H-NMR (CDCl3) δ: 0.850(3H, t, H-25), 1.210(2H, m, H-12), 1.400(2H, m, H-8), 1.335(2H, m, H-20), 1.280(2H, m, H-13), 1.465(2H, m, H-19), 1.830(2H, t, H-7), 1.730(2H, m, H-14), 2.180(2H, t, H-18), 4.880(1H,d, H-1), 4.982(1H,d, H-1’), 5.517(1H, t, H-15), 5.527(1H, t, H-6), 3.536(3H, s, OMe), 3.565(3H, s, OMe), 3.586(3H, s, OMe) 7.3-7.7(15H, phenyl).

Isopellynol A (3): amorphous solid; [α]D -15.0 (c=0.222, CHCl3); ESITOF-MS m/z: 519.3854 [M+Na]+ (Calcd for C33H52NaO3, 519.3817); 1H- and 13C-NMR see Table 1 and 2. Oxidative product: undecanedioic acid: m/z: 215.1305 [M-H]− (Calcd for C11H15O4, 215.1289), tridecanedioic acid: m/z: 243.1564 [M-H]− (Calcd for C13H21O4, 243.1602).

(R)-MTPA ester of 3, ESITOF-MS m/z:1167.5015 [M+Na]+ (Calcd for C63H73F9NaO9, 1167.5003); 1H-NMR (CDCl3) δ: 1.765(2H,m, H-28), 1.779(1H, m, H-7), 1.839(1H, m, H-7), 1.998-2.023(4H, brd, H-17,20), 3.220(1H, s, H-33), 4.872-4.983(2H, ddd, H-1), 5.328(2H, brdt, H-18,19), 5.529(0.5H, t, H-6), 5.565(0.5H, t, H-6), 5.611(1H, d, H-31), 5.802(1H, dd, H-30), 5.925(1H, q, H-29), 3.524(3H, s, OMe), 3.532(3H, s, OMe), 3.553(3H, s, OMe), 7.380-7.500(15H, phenyl); (S)-MTPA ester of 3, ESITOF-MS m/z: 1167.5007 [M+Na]+ (Calcd for C63H73F9NaO9, 1167.5003); 1H-NMR (CDCl3) δ: 1.721(2H,m, H-28), 1.778(1H, m, H-7), 1.839(1H, m, H-7), 1.994(4H, brd, H-17,20), 3.227(1H, s, H-33), 4.873-4.985(2H, ddd, H-1), 5.330(2H, brdt, H-18,19), 5.529(0.5H, t, H-6), 5.566(0.5H, t, H-6), 5.642(1H, d, H-31), 5.944(1H, dd, H-30), 5.971(1H, q, H-29),
Pellynol J (4): amorphous solid; [α]D -9.6 (c = 0.016, CHCl3); ESITOF-MS m/z: 491.3541 [M+Na]+ (Calcd for C21H48NaO3, 491.3503); 1H- and 13C-NMR see Table 1 and 2. Oxidative product: undecanedioic acid: m/z: 215.1305 [M-H]- (Calcd for C11H10O4, 215.1289).

(R)-MTPA ester of 4, ESITOF-MS m/z: 1139.4659 [M+Na]+ (Calcd for C61H68F8NaO9, 1139.4690); 1H-NMR (CDCl3) δ: 1.798(2H, m, H-26), 1.805(1H, m, H-7), 1.857(1H, m, H-7), 2.012-2.034(4H, brd, H-15,18), 2.620(1H, s, H-31), 4.884-4.999(2H, ddd, H-1), 5.343(2H, brd, H-16,17), 5.495(1H, dd, H-28), 5.546(0.5H, t, H-6), 5.583(0.5H, t, H-6), 6.006(1H, m, H-27), 6.030(1H, d, H-29), 3.540(3H, s, OMe), 3.570(3H, s, OMe), 3.587(3H, s, OMe), 7.392-7.516(15H, phenyl); (S)-MTPA ester of 4, ESITOF-MS m/z: 1139.4697 [M+Na]+ (Calcd for C61H68F8NaO9, 1139.4690); 1H-NMR (CDCl3) δ: 1.793(1H, m, H-7), 1.856(1H, m, H-7), 1.843(2H, m, H-26), 2.007-2.074(4H, brd, H-15,18), 2.579(1H, s, H-31), 4.884-4.998(2H, ddd, H-1), 5.343(2H, brd, H-16,17), 5.546(0.5H, t, H-6), 5.582(0.5H, t, H-6), 5.600(1H, dd, H-28), 6.009(1H, d, H-29), 6.067(1H, m, H-27), 3.541(3H, s, OMe), 3.547(3H, s, OMe), 3.568(3H, s, OMe), 7.398-7.519(15H, phenyl).

Pellynol K (5): amorphous solid; [α]D -6.3 (c = 0.027, CHCl3); ESITOF-MS m/z: 533.3950 [M+Na]+ (Calcd for C34H54NaO3, 533.3971); 1H- and 13C-NMR see Table 1 and 2. Oxidative product: undecanedioic acid: m/z: 215.1281 [M-H]- (Calcd for C11H10O4, 215.1289), tetradecanedioic acid: m/z: 257.1782 [M-H]- (Calcd for C14H25O4, 257.1758).

Pellynol L (6): amorphous solid; [α]D -2.6 (c = 0.019, CHCl3); ESITOF-MS m/z: 527.3964 [M+Na]+ (Calcd for C35H52NaO2, 527.3865); 1H- and 13C-NMR see Table 1 and 2. Oxidative product: dimethyl nonanedioate (C11H20O4), tR=10.04 min., m/z: 185(M-31)+, 152, 111, 83, 55(base peak), dimethyl hexadecanedioate (C16H34O4), tR=17.66 min., m/z: 283(M-31)+, 241, 112, 98 (base peak).

(R)-MTPA ester of 6, ESITOF-MS m/z: 959.4668 [M+Na]+ (Calcd for C55H66F8NaO6, 959.4656); 1H-NMR (CDCl3) δ: 1.798(2H, m, H-30), 2.276(2H, q, H-19), 2.343(2H, t, H-24), 2.556(1H, s, H-35), 4.886(2H, d, H-1), 5.433(1H, d, H-21), 5.497(1H, dd, H-32), 5.796(1H, q, H-20), 6.015(1H, m, H-31), 6.036(1H, d, H-33), 3.552(3H, s, OMe), 3.569(3H, s, OMe), 3.587(3H, s, OMe), 7.394-7.516(15H, phenyl); (S)-MTPA ester of 6,
ESITOF-MS \( m/z: 959.4727 [M+Na]^+ \) (Calcd for \( \text{C}_{53}\text{H}_{66}\text{F}_9\text{NaO}_9 \), 959.4656); \(^1\)H-NMR (CDCl\(_3\)) \( \delta: 1.778(2\text{H}, \text{m, H-30}), 2.306(2\text{H}, \text{q, H-19}), 2.373(2\text{H}, \text{t, H-24}), 2.516(1\text{H}, \text{s, H-35}), 4.906(2\text{H}, \text{d, H-1}), 5.453(1\text{H}, \text{d, H-21}), 5.597(1\text{H}, \text{dd, H-32}), 5.816(1\text{H}, \text{q, H-20}), 6.065(1\text{H}, \text{m, H-31}), 6.006(1\text{H}, \text{d, H-33}), 3.550(3\text{H}, \text{s, OMe}), 3.569(3\text{H}, \text{s, OMe}), 3.587(3\text{H}, \text{s, OMe}), 7.394-7.516(15\text{H}, \text{phenyl}).

Pellonyl A (7): amorphous solid; \([\alpha]_D\) -5.0 (\(c=0.024, \text{CHCl}_3\)), ref \(^9\)[\([\alpha]_D\) -8.5 (\(c=1.0, \text{CHCl}_3\)); ESITOF-MS \( m/z: 519.3864 [M+Na]^+ \) (Calcd for \( \text{C}_{33}\text{H}_2\text{NaO}_3 \), 519.3817); \(^1\)H- and \(^13\)C-NMR see Table 1 and 2. Oxidative product: undecanedioic acid: \( m/z: 215.1281 [\text{M}-\text{H}]^- \) (Calcd for \( \text{C}_{11}\text{H}_{19}\text{O}_4 \), 215.1289), tridecanedioic acid: \( m/z: 243.1585 [\text{M}-\text{H}]^- \) (Calcd for \( \text{C}_{14}\text{H}_{22}\text{O}_4 \), 243.1602).

(R)-MTPA ester of 7, ESITOF-MS \( m/z: 1167.4934 [\text{M}+\text{Na}]^+ \) (Calcd for \( \text{C}_{66}\text{H}_{73}\text{F}_9\text{NaO}_9 \), 1167.5003); \(^1\)H-NMR (CDCl\(_3\)) \( \delta: 1.727(1\text{H}, \text{m, H-7}), 1.787(1\text{H}, \text{m, H-7}), 1.925(2\text{H}, \text{m, H-28}), 1.966-1.978(4\text{H}, \text{brd, H-17,20}), 2.550(1\text{H}, \text{s, H-33}), 4.814-4.944(2\text{H}, \text{ddd, H-1}), 5.276(2\text{H}, \text{brdt, H-18,19}), 5.427(1\text{H}, \text{dd, H-30}), 5.478(0.5\text{H}, \text{t, H-6}), 5.514(0.5\text{H}, \text{t, H-6}), 5.938(1\text{H}, \text{m, H-29}), 5.957(1\text{H}, \text{d, H-31}), 3.472(3\text{H}, \text{s, OMe}), 3.500(3\text{H}, \text{s, OMe}), 3.519(3\text{H}, \text{s, OMe}), 7.322-7.335(15\text{H}, \text{phenyl}); (S)-MTPA ester of 2, ESITOF-MS \( m/z: 1167.4978 [\text{M}+\text{Na}]^+ \) (Calcd for \( \text{C}_{66}\text{H}_{73}\text{F}_9\text{NaO}_9 \), 1167.5003); \(^1\)H-NMR (CDCl\(_3\)) \( \delta: 1.726(1\text{H}, \text{m, H-7}), 1.787(1\text{H}, \text{m, H-7}), 1.966(2\text{H}, \text{m, H-28}), 1.966-1.978(4\text{H}, \text{brd, H-17,20}), 2.512(1\text{H}, \text{s, H-33}), 4.894-4.944(2\text{H}, \text{ddd, H-1}), 5.277(2\text{H}, \text{brdt, H-18,19}), 5.528(1\text{H}, \text{dd, H-30}), 5.476(0.5\text{H}, \text{t, H-6}), 5.514(0.5\text{H}, \text{t, H-6}), 5.999(1\text{H}, \text{m, H-29}), 5.767(1\text{H}, \text{d, H-31}), 3.473(3\text{H}, \text{s, OMe}), 3.502(3\text{H}, \text{s, OMe}), 3.519(3\text{H}, \text{s, OMe}), 7.322-7.335(15\text{H}, \text{phenyl}).

Pellonyl B (8): amorphous solid; \([\alpha]_D\) -8.8 (\(c=0.019, \text{CHCl}_3\)), ref \(^9\)[\([\alpha]_D\) -7.6 (\(c=0.28, \text{CHCl}_3\)); ESITOF-MS \( m/z: 505.3771 [\text{M}+\text{Na}]^+ \) (Calcd for \( \text{C}_{32}\text{H}_{50}\text{NaO}_3 \), 505.3660); \(^1\)H- and \(^13\)C-NMR see Table 1 and 2. Oxidative product: undecanedioic acid: \( m/z: 215.1281 [\text{M}-\text{H}]^- \) (Calcd for \( \text{C}_{11}\text{H}_{19}\text{O}_4 \), 215.1289), dodecanedioic acid: \( m/z: 229.1393 [\text{M}-\text{H}]^- \) (Calcd for \( \text{C}_{12}\text{H}_{21}\text{O}_4 \), 229.1445).

Pellonyl C (9): amorphous solid; \([\alpha]_D\) -8.7 (\(c=0.031, \text{CHCl}_3\)), ref \(^9\)[\([\alpha]_D\) -11.2 (\(c=2.38, \text{CHCl}_3\)); ESITOF-MS \( m/z: 515.3496 [\text{M}+\text{Na}]^+ \) (Calcd for \( \text{C}_{33}\text{H}_{48}\text{NaO}_3 \), 515.3503); \(^1\)H- and \(^13\)C-NMR see Table 1 and 2. Oxidative product: dimethyl nonanedioate (\( \text{C}_{11}\text{H}_{20}\text{O}_4 \)), \( t_R=10.08 \text{ min.}, m/z: 185(\text{M-31})^+ \), 152, 111, 83, 55(base peak), dimethyl tridecanedioate (\( \text{C}_{15}\text{H}_{28}\text{O}_4 \)), \( t_R=14.74 \text{ min.}, m/z: 241(\text{M-31})^+ \), 199, 167, 112, 98 (base peak).
(R)-MTPA ester of 9, ESITOF-MS m/z: 1163.5035 [M+Na]^+ (Calcd for C_{63}H_{69}F_9NaO_9, 1163.4690); ^1H-NMR (CDCl_3) δ: 1.797(2H, m, H-28), 1.806(1H, m, H-7), 1.857(1H, m, H-7), 2.274(2H, q, H-17), 2.342(2H, t, H-22), 2.557(1H, s, H-33), 4.886-4.998(2H, ddd, H-1), 5.432(1H, d, H-19), 5.498(1H, dd, H-30), 5.548(0.5H, t, H-6), 5.573(0.5H, t, H-6), 5.794(1H, q, H-18), 6.013(1H, m, H-29), 6.033(1H, d, H-31), 3.542(3H, s, OMe), 3.568(3H, s, OMe), 3.577(3H, s, OMe), 7.392-7.516(15H, phenyl); (S)-MTPA ester of 9, ESITOF-MS m/z: 1163.4941 [M+Na]^+ (Calcd for C_{63}H_{69}F_9NaO_9, 1163.4690); ^1H-NMR (CDCl_3) δ: 1.837(2H, m, H-28), 1.793(1H, m, H-7), 1.857(1H, m, H-7), 2.274(2H, q, H-17), 2.343(2H, t, H-22), 2.517(1H, s, H-33), 4.886-4.997(2H, ddd, H-1), 5.432(1H, d, H-19), 5.597(1H, dd, H-30), 5.54(0.5H, t, H-6), 5.573(0.5H, t, H-6), 5.795(1H, q, H-18), 6.064(1H, m, H-29), 5.853(1H, d, H-31), 3.542(3H, s, OMe), 3.568(3H, s, OMe), 3.577(3H, s, OMe), 7.392-7.516(15H, phenyl).

**Cell Culture and Cell Proliferation Assay**

Human malignant epithelial cells (HeLa) were cultured in Eagle’s Minimum Essential medium (EMEM) supplemented with 10% FBS kept in an incubator at 37 °C in a humidified air containing 5% CO_2. FBS was purchased from Nichirei Bioscience Inc. (Tokyo, Japan). Human immortalized myelogenous leukemia cells (K562) were cultured in RPMI-1640 medium supplemented with 10% FBS kept in an incubator at 37 °C in a humidified air containing 5% CO_2. FBS was purchased from Nichirei Bioscience Inc. Cell viability was determined by a Cell-Titer 96 AQueous Non-Radioactive Cell Proliferation (MTS) Assay (Promega, WI) according to the manufacturer’s protocol. HeLa and K562 cells (3 × 10^4 cells/well) were seeded in 96 well plates and incubated for 24 hr, subsequently grown with compounds for additional 48 hr, and then cell proliferation assay was performed.
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CONFLICT OF INTEREST
The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL
The online version of this article contains supplementary material.
REFERENCES

1) Jung H. J., Im S. K., Bae H. B., “Biomaterials from Aquatic and Terrestrial Organisms, Chap. 12, Bioactive Polyacetylenic Compounds from Marine Sponges”, ed. by Fingerman M. and Nagabhushanam R., Science Publishers, New Hampshire, 2006.

2) Bohlmann F., Lam J., Breteler H., Arnason T., Hansen L., “Chemistry and Biology of Naturally Occurring Acetylenes and Related Compounds”, Elsevier, Amsterdam, 1998.

3) Bohlmann F., Burkhardt T., Zdero C., “Naturally Occuring Acetylenes”, Academic Press, London and New York, 1973.

4) Perry N. B., Span E. M., Zidorn C., Tetrahedron Lett., 42, 1170-1177 (2001).

5) Allen E. H., Thomas C. A., Phytochemistry, 10, 1579-1582 (1971).

6) Shirouzu T., Watari K., Ono M., Koizumi K., Saiki I., Tanaka C., van Soest R. W. M., Miyamoto T., J. Nat. Prod., 76, 1337-1342 (2013).

7) The genus of Petrosia was classified to Strongylophora and Halichondria was classified to Pellina until 2002. De Weerdt W. H., “Family Chalinidae Gary. 1867 in Systema Porifera, a Guide to the Classification of Sponges”, ed. by Hooper J. N. A. and Van Soest R.W.M, Kluwer Academic/Plenum Publishers, New York. 2002.

8) Fu X., Abbas S. A., Schmitz F. J., Vidavsky I., Gross M. L., Laney M., Schatzman R. C., Cabuslay R. D., Tetrahedron, 53, 799-814 (1997).

9) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., J. Am Chem. Soc., 113, 4092-4096 (1991).

10) Fusetani N., Yasumuro K., Matsunaga S., Hirota H., Tetrahedron Lett., 30, 6891-6894 (1989).

11) Zhou G.-X., Molinski T. F., Marine Drugs, 1, 46-53 (2003).

12) Braekman J. C., Daloze D., Hulot G., Tursch B, Declercq J. P., Germain G., Van Meerssche M., Bull. Soc. Chim. Belg., 87, 917-926 (1987).
FIGURE LEGENDS

Figure 1: Structures of Compounds 1-9

Figure 2a: HMBC correlations and partial structure of 1

Figure 2b: RuCl₃ oxidation and its degradation product of 1

Figure 2c: Δδ values of (R)- and (S)-MTPA esters of 1

Figure 3a: HMBC and COSY correlations and partial structure of 3

Figure 3b: RuCl₃ oxidation and its degradation products of 3

Figure 3c: ¹H-NMR chemical shifts at H-6 and H₂-7, and Δδ values of (R)- and (S)-MTPA esters of 3

Figure 4a: HMBC and COSY correlations and partial structure of 6

Figure 4b: RuCl₃ oxidation and its degradation products of 6

Figure 4c: Δδ values of (R)- and (S)-MTPA esters of 6

Figure 5: ¹H-NMR spectra of (R)-MTPA esters of 7 and 1
Figure 1 Structures of Compounds 1-9
Figure 2a HMBC correlations and partial structure of 1

Figure 2b RuCl₃ oxidation and its degradation product of 1

Figure 2c Δδ values of (R)- and (S)-MTPA esters of 1
Figure 3a HMBC and COSY correlations and partial structure of 3

Figure 3b RuCl₃ oxidation and its degradation products of 3

Figure 3c ¹H-NMR chemical shifts at H-6 and H₂-7, and Δδ values of (R)- or (S)-MTPA esters of 3
Figure 4a HMBC and COSY correlations and partial structure of 6

Figure 4b RuCl₃ oxidation and its degradation products of 6

Figure 4c Δδ values of (R)- and (S)-MTPA esters of 6
Figure 5 $^1$H-NMR spectra of (R)-MTPA esters of 7 and 1