Symbiopolyol, a VCAM-1 Inhibitor from a Symbiotic Dinoflagellate of the Jellyfish *Mastigias papua*

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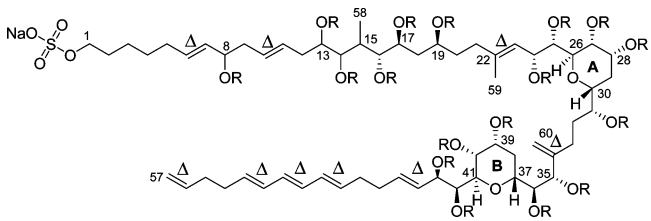
Received April 1, 2010

A super-carbon-chain compound, symbiopolyol (**1a**), was isolated from a symbiotic dinoflagellate of the jellyfish *Mastigias papua*. Although a direct comparison between symbiopolyol (**1a**) and lingshuiol B has not been completed, symbiopolyol (**1a**) is suggested to be the enantiomer of lingshuiol B. The structure of **1a**, including its partial relative configuration, was elucidated on the basis of interpretation of spectroscopic data and chemical transformations. This compound exhibited significant inhibitory activity against the expression of VCAM-1 in human umbilical vein endothelial cells (HUVEC).

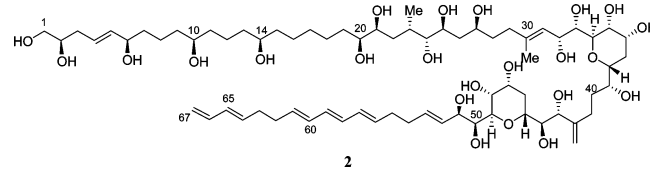
Inflammation is the response of an organism's immune system to damage caused to its cells and vascularized tissues by microbial pathogens such as viruses and bacteria, as well as by injurious chemicals or physical insult. It is characterized by the expression of a repertoire of inducible adhesion molecules that are closely regulated both spatially and temporally.[1] Potential adhesion molecules include vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin.[2,3] During the inflammation process, various inflammatory mediators including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and lipopolysaccharides induce the expression of adhesion molecules in endothelial cells.[2,4] VCAM-1 is of particular interest because it has been found to selectively bind to classes of leukocytes found predominantly during the early stages of inflammation.[1b,5] A recent study showed that VCAM-1, but not ICAM-1, plays a dominant role in the initiation of atherosclerotic lesions.[6] To date, a few natural products have been reported to inhibit the expression of VCAM-1, including halichlorine,[7] jolkinolide B,[8] HUN-7293,[9] and diarylheptanoids.[10] During functional screening to identify inhibitors of VCAM-1 from our symbiotic marine dinoflagellate library, and as part of our studies on the chemistry of symbiotic marine dinoflagellates,[7,8,11] we encountered an active water-soluble fraction from a symbiotic marine dinoflagellate of the jellyfish *Mastigias papua.* Preliminary observations suggested that the host, the Papuan jellyfish, *M. papua*, had a significant symbiotic relationship with the symbiont, a dinoflagellate that was required for its survival.[12] Purification of the active compound from the water layer using bioassay-guided fractionation gave a super-carbon-chain compound,[13] symbiopolyol (**1a**). This compound showed significant inhibitory activity against VCAM-1 cell adhesion. In this paper, we describe the isolation, structure, and biological activity of symbiopolyol (**1a**), a VCAM-1 inhibitor.



1a R= H
1b R=Ac
1c R=H, Δ saturated

The dinoflagellate *Amphidinium* sp. was isolated from the tentacles of the Papuan jellyfish, *M. papua.* The cells were cultured unialgally in sterilized seawater enriched with 2% Provasoli's Erd-Schreiber (ES) supplement for 60 days under a 14 h/10 h light/dark cycle at 20−25 °C. The 80% aqueous EtOH extract of the cultivated dinoflagellate (195.40 g wet wt) was partitioned between EtOAc and H_2O. The H_2O layer was further separated using a polystyrene gel column (H_2O−EtOH) to give five fractions. The active fraction (50% EtOH eluate) was purified by ODS HPLC (MeCN−H_2O) and then by Luna phenyl-hexyl HPLC (MeCN−H_2O) to give symbiopolyol (**1a**, 7.0 mg, 0.036% wet weight) as an active compound. Symbiopolyol (**1a**) was isolated as a pale yellow, amorphous solid with a molecular formula of $C_{60}H_{99}O_{23}SNa$ by HRESIMS ([M + Na]$^+$ *m/z* 1265.6107). The 1D and 2D NMR data as well as mass spectrometric data were similar to those of lingshuiol B,[14] which indicated that the planar structure of **1a** is the same as that of lingshuiol B. The presence of hydroxy groups in **1a** was confirmed by acetylation to give a 17-acetylated product (**1b**) (negative ESIMS *m/z* 1933.7 [M ($C_{94}H_{133}O_{40}S$) − Na]$^-$). Furthermore, reduction of the double bonds in **1a** using Pd/C in H_2 atmosphere confirmed the presence of nine double bonds as in **1c** (negative ESIMS *m/z* 1237.7 [M ($C_{60}H_{117}O_{23}S$) − Na]$^-$). The UV absorption spectrum confirmed the presence of a conjugated triene chromophore.[15] The existence of a sulfate ester was evidenced by the results of a MS/MS analysis of negative-ion MALDI-TOF measurement [*m/z* 80 (SO_3^-) and 97 (HSO_4^-)]. The pattern of mass fragmentation of **1a** in the MS/MS MALDI-TOF spectra was close to that of luteophanol A,[16] which was also isolated in this work, except for the polyene section (from C-42 to C-57 in the structure of luteophanol A). Only the specific rotation of symbiopolyol (**1a**) was different from that of lingshuiol B. The specific rotation of symbiopolyol (**1a**) was $[\alpha]_D^{23.6}$ −5.9 (0.43, MeOH), while that of lingshuiol B was $[\alpha]_D^{25}$ +2.5 (0.5, MeOH),[14] which suggests that compound **1a** may be the antipode of lingshuiol B.[17] Further cultivation, extraction, and separation of *Amphidinium* sp. were performed, and the negative specific rotation for symbiopolyol (**1a**) was confirmed as $[\alpha]_D^{22.3}$ −4.9 (0.30, MeOH).

The partial relative configuration of **1a**, which was not reported for lingshuiol B, was deduced by an investigation of coupling constants, ROE/ROESY correlations, and phase-sensitive HMBC spectra and by comparison to those of AM-3 (**2**)[18] for certain segments (C-16−C-17, C-31−C-37, C-42−C-43, and the two tetrahydropyran rings).



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Pyran ring A was suggested to have a chair conformation with the pairs H-28 and H-30, H-30 and H-25, and H-25 and H-28 in 1,3 diaxial

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10.1021/np100221k
Published on Web 06/17/2010

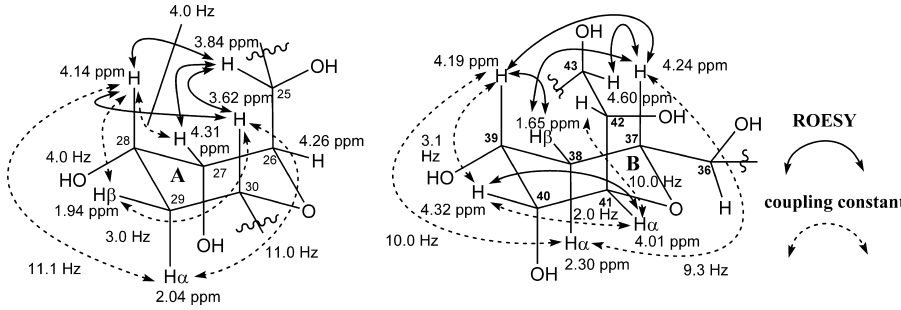

Figure 1. Analysis of coupling constant and ROESY data in the two pyran rings (A and B) of symbiopolyol (**1a**).

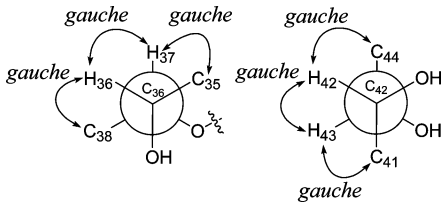

Figure 2. Newman projections for the C-36−C-37 and C-42−C-43 portions of **1a**.

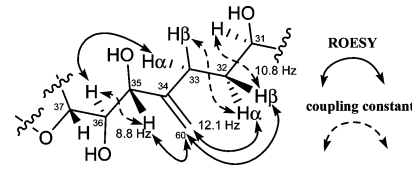

Figure 3. ROESY correlations and coupling constants for the C-31−C-36 segment of **1a**.

orientations based on the prominent positive ROESY correlations (H-28/H-30, H-30/H-25, H-25/H-28) and the small magnitudes of $^3J_{H28-H27}$ (4.0 Hz), $^3J_{H28-H29\beta}$ (4.0 Hz), and $^3J_{H29\beta\text{-}H30}$ (3.0 Hz). These signal assignments were also supported by large coupling constants [$^3J_{H28-H29\alpha}$ (11.1 Hz), $^3J_{H29\alpha\text{-}H30}$ (11.0 Hz)], as depicted in Figure 1. Tetrahydropyran ring B was also assigned a chair conformation since ROESY correlations were observed between H-37/H-39, H-37/H-43, H-37/H-38β, and H-39/H-38β. This was confirmed by the large coupling constants for $^3J_{H39-H38\alpha}$ (10.0 Hz) and $^3J_{H37-H38\alpha}$ (9.3 Hz) and the small coupling constants for $^3J_{H39-H40}$ (3.0 Hz) and $^3J_{H40-H41}$ (2.0 Hz) (Figure 1). These results suggested that H-37 and H-39 were oriented axially and H-40 was equatorial. The configurations of the two ether rings A and B of symbiopolyol (**1a**) are the same as those reported for amphidinols 1,[15] 3, 4,[19] 7,[20] and 12[21] and luteophanols A,[16] B, and C.[22]

A bond-rotation analysis of C-36−C-37 and C-42−C-43 was performed by *J*-based configuration analysis.[24] In the BIRD PS-HMBC of **1a**, correlations between H-37 and C-36 and between H-36 and C-37 were observed as very small cross-peaks, and those for H-36 and C-38, and for H-37 and C-35, were missing, indicating their small $^3J_{C,H}$'s and $^2J_{C,H}$'s (0−2 Hz), while the small coupling constant observed for $^3J_{H-36-H-37}$ (1.4 Hz) suggested the C-36−C-37 portion has a *gauche* conformation. With regard to the C-42−C-43 portion, the weak and missing intensities were observed in the BIRD PS-HMBC spectrum between H-42/C-44, H-42/C-43, and H-43/C-41, while a small coupling constant was observed for $^3J_{H-42-H-43}$ (3.4 Hz), indicating their small $^{2,3}J_{C,H}$'s. Those observations were consistent with the idea that C-44/H-42, H-42/H-43, and H-43/C-41 have a *gauche* conformation. The relative configuration for C-36−C-37 and C-42−C-43 was assigned as depicted in Figure 2.

A configurational analysis of C-31 to C-36 was performed on the basis of the characteristics of $^3J_{H,H}$ and $^2J_{C,H}$ and ROESY observations. An *anti* configuration of H-31−H-33 was shown by the large $^3J_{H-31-H-32\beta}$ (10.8 Hz), which was supported by a very weak intensity of $^2J_{C-31-H-32\alpha}$ and a large coupling constant of $^3J_{H-32\alpha-H-33\beta}$ (12.1 Hz). A prominent ROESY between H-60α (δ 5.00) and H_2-32 (δ 1.65 and 2.10) as well as H-60β (δ 5.13) and H-35 (δ 4.38) disclosed that H-60 was *syn* to C-32 and C-35. The stereochemical relationship between H-33β (δ 2.61) and H-36 (δ 3.50) was determined from the ROESY spectrum. The $^3J_{H,H}$ of H-35 and H-36 showed a large coupling constant of 8.8 Hz and a very small intensity of $^2J_{C,H}$ of H-37−C-36 (Figure 3). The values of $^3J_{H,H}$ and $^2J_{C,H}$ and ROESY data of the C-31−C-36 segment were consistent with those of the corresponding segment of AM-3 (**2**).[18a]

An analysis of *vic*-coupling constants showed that the C-16−C-17, C-25−C-26, C-35−C-36, and C-41−C-42 axes shared an *anti* configuration (coupling constants ranging from 8 to 10 Hz), which in turn provided two possible conformers: either *erythro* or *threo*. As a consequence, the analysis of $^{2,3}J_{C,H}$ would not be fruitful since two conformers could be distinguished on the basis of observation of the ROESY spectrum to determine their 1,4-ROESY relationship.[18a] No ROESY cross-peak was observed at H-24 (δ 4.74)−H-27 (δ 4.31) for the C-25−C-26 bond, H-15 (δ 2.59)−H-18 (δ 1.63/2.08) for the C-16−C-17 bond, H-35 (δ 4.38)−H-38 (δ 1.65/2.30) for the C-36−C-37 bond, or H-40 (δ 4.31) and H-43 (δ 4.60) for the C-41−C-42 bond, which indicated that they have C/C *anti* orientations (*erythro* conformer). In addition to this assignment, ROESY cross-peaks could be observed between H-24 and H-30 as well as H-37 and H-43.

The *E* geometry of Δ^6, Δ^{10}, Δ^{22}, Δ^{44}, Δ^{48}, Δ^{50}, and Δ^{52} was determined by ROESY observation (H-6/H-8, H-59/H-24) and coupling constants. The ^{1}H NMR spectrum and 2D homonuclear *J*-coupling spectroscopy of **1a** showed large vicinal coupling constants in the range J = 15−16 Hz for H-6/H-7, H-10/H-11, H-44/H-45, H-48/H-49, H-50/H-51, and H-52/H-53. The configuration of the triene system (Δ^{48}, Δ^{50}, and Δ^{52}) was also established to be *E, E, E* on the basis of a comparison with a model triene in the literature.[24] Support for an *E* geometry for the conjugated triene system came from the ^{1}H NMR chemical shifts for H-49 (δ 6.00) and H-52 (δ 6.01), respectively, since δ 6.6−6.8 should be observed for an *E, Z, E* configuration.[25]

The ^{13}C NMR data for the central segment of symbiopolyol (**1a**) (C-15 to C-43) were essentially identical to those for AM-3 (**2**)[18a] (C-24−C-51) in CD_3OD/C_5D_5N (2:1), because $\Delta\delta_{C\ symbiopolyol\text{-}AM\text{-}3}$ was ±0.6 ppm. Therefore, this result indicated that the partial stereostructure of symbiopolyol (**1a**) shared the same configuration as AM-3 (**2**).

Symbiopolyol (**1a**) showed VCAM-1 inhibitory activity. The addition of **1a** prior to TNF-α stimulation markedly decreased VCAM-1 expression in human umbilical vein endothelial cells (HUVEC) with an IC_{50} value of 8.23 μg/mL (6.62 μM) (Figure 4). An independent assessment of **1a** by an external laboratory (CEREP, France) showed that **1a** at 10 μg/mL (8 μM) inhibited TNF-α/IL-4-induced cell adhesion between HUVEC and Ramos cells by 77% (Figure 5). Since Ramos cells highly express VLA-4, which is the ligand of VCAM-1, the adhesion between Ramos

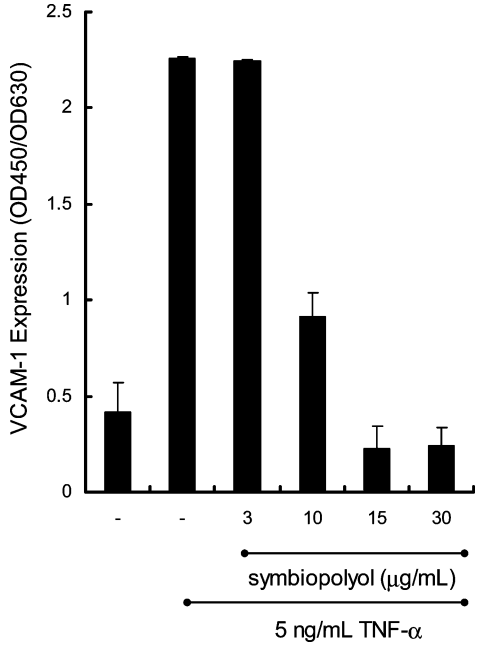

Figure 4. Inhibitory effects of symbiopolyol (**1a**) on VCAM-1 expression. HUVEC were treated with the indicated concentrations of symbiopolyol (**1a**) for 30 min and then stimulated with 5 ng/mL of TNF-α for 18 h. The expression of VCAM-1 on the cell surface was analyzed by enzyme-linked immunosorbent assay (ELISA) using anti-VCAM-1 antibody. Values are the means ± SD of triplicate experiments.

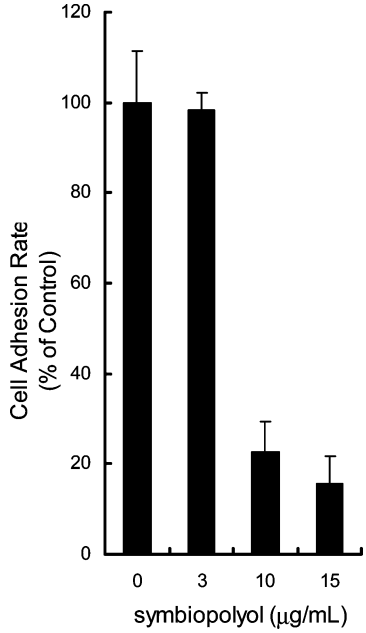

Figure 5. Inhibition of lymphocyte adhesion to TNF-α/IL-4-stimulated HUVEC by symbiopolyol (**1a**). HUVEC were stimulated by addition of 1 ng/mL TNF-α and 500 U/mL IL-4 in the presence of the indicated concentrations of **1a** for 20 h. After washing, Ramos cells labeled with a fluorescent probe (BCECF) were added to the HUVEC. After 10 min, the adhesion of Ramos cells to HUVEC was determined by measuring the fluorescence of adherent cells. Values are the means ± SD of triplicate experiments.

cells and HUVEC is largely mediated by VCAM-1.[25] On the other hand, **1a** did not have any cytotoxic effect against HUVEC at up to 15 μg/mL (12 μM). To the best of our knowledge, this is the first report of a high-molecular-weight polyol compound (MW > 1000) with strong reducing activity against the expression of VCAM-1 in HUVEC. As a result, symbiopolyol (**1a**) may be a potential anti-inflammatory agent. In summary, symbiopolyol (**1a**), the enantiomer of lingshuiol B, has been isolated from symbiotic dinoflagellates of the Papuan jellyfish, *M. papua*, as an inhibitor of VCAM-1. The activity of symbiopolyol (**1a**) may be associated with the symbiotic relationship between the host animal and the symbiont and may protect the jellyfish from predators.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 or JASCO P-1020 polarimeter. The maximum UV absorption was measured using a V-570 JASCO spectrophotometer. NMR spectra were recorded on JEOL JNM-ECA800 (800 MHz for ^{1}H NMR and 201 MHz for ^{13}C), JEOL JNM-ECA600, and JEOL A-600 (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C) spectrometers. Chemical shifts are reported in parts per million (ppm) with coupling constants (J) in hertz relative to solvent peaks in $CD_3OD-C_5D_5N$ (2:1). The center peaks of the ^{1}H and ^{13}C values of the mixed solvent $CD_3OD-C_5D_5N$ (2:1) were at δ_H 3.31 and δ_C 49.0, respectively. Multiplicities of ^{13}C NMR data were determined by DEPT and HSQC experiments. MALDI-TOF mass spectra were recorded on a VOYAGER DE-Pro spectrometer (Applied Biosystems) or Bruker Ultraflex III using α-cyano-4-hydroxycinnamic acid as a matrix. MS/MS spectra were recorded with a Bruker Ultraflex III MALDI-TOF and TOF/TOF MS. ESIMS were measured on an ESI-TOFMS QSTAR Pulsar mass spectrometer (PE Biosystems). HPLC separations were carried out on a JASCO PU-980 Intelligent pump equipped with a UV-970 Intelligent UV−vis detector. Columns used for HPLC were reversed-phase silica gel (250 × 20 mm, 250 × 10 mm Develosil, Nomura Chemical Co.) and phenyl-hexyl (250 × 4.6 mm, Luna 5 μ phenyl-hexyl, Phenomenex). Analytical TLC was performed on commercial silica gel 60 F_{254} plates and monitored with anisaldehyde and a UV lamp at 254 nm. Assay material MCDB 131 medium, 3,3′,5,5′-tetramethylbenzidine (TMB) liquid substrate system for ELISA, and 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. HUVEC were purchased from Dainippon, Osaka, Japan. Mouse (monoclonal) anti-human VCAM-1 capture antibody was purchased from Invitrogen. Peroxidase-monoclonal rat anti-mouse IgG_1 was from Amersham Biosciences. PBS − is made from 8 g/L NaCl, 0.2 g/L KCl, 1.15 g/L Na_2HPO_4, and 0.2 g/L KH_2PO_4, while PBS + is made from PBS − and addition of 0.9 mM $CaCl_2$ and 0.33 mM $MgCl_2$. The 96-well collagen-coated plates were purchased from Iwaki, Tokyo.

Microalgal Material. The symbiotic dinoflagellate of the Papuan jellyfish was identified as *Amphidinium* sp. (strain KD-056) by Dr. Takeo Hiroguchi of Hokkaido University. This *Amphidinium* sp. strain is deposited at Keio University. It was initially isolated from the tentacles of the jellyfish *M. papua* collected from Kochi Prefecture, Japan. The unialgal culture was grown in a 50 mL sterilized flask containing sterilized seawater and 2% ES supplement at 25 °C under a 14 h/10 h light/dark cycle. After 60 days of culture, the volume of dinoflagellate was increased to as high as 400 L of cultured cells containing sterilized seawater and 2% ES medium.

Extraction and Isolation. The harvested cells (195.40 g from 400 L of culture) were extracted with 80% aqueous EtOH (3 × 1 L) for three days and extracted with MeOH (1 L) for one day. The whole extract was concentrated, and the residue was partitioned between H_2O (500 mL) and EtOAc (5 × 500 mL). The aqueous layer was loaded on a TSK G-3000S polystyrene gel column (ϕ 50 × 60 mm, Tosoh Co), eluted with 0, 25, 50, 75, and 100% aqueous EtOH (1 L each), and finally washed with 100% MeOH. The 50% aqueous EtOH (0.3 g) active fraction in the VCAM-1-mediated assay, which showed *m*/*z* 1219.6 in the negative ESIMS and MALDI-TOF MS, was separated on a ODS HG Develosil reversed-phase column (20 × 250 mm) using gradient elution from 20% to 80% aqueous MeCN for 60 min and an additional 5 min with 80% aqueous MeCN to 100% MeCN at flow rate of 8 mL/min using UV detection at 215 nm. The third fraction (90.0 mg), which contained *m*/*z* 1219.6 in the negative ESIMS and was active in a VCAM-1-mediated assay, was purified by semipreparative HPLC with ODS HG Develosil (10 × 250 mm) using gradient elution with 30% aqueous MeCN to 100% MeCN for 40 min to give eight subfractions. The fifth subfraction (17.2 mg), which showed *m*/*z* 1219.6 (in the negative mode of HRESIMS), was subsequently purified using a hexyl-phenyl column (4.6 × 250 mm) with gradient elution

with 20% aqueous MeCN to 80% aqueous MeCN for 80 min and an additional 5 min with 80% aqueous MeCN to 100% MeCN at a flow rate of 1 mL/min, UV detection at 215 nm, to give 7.0 mg of pure symbiopolyol (**1a**) (t_R = 16.99 min) as an active compound.

Symbiopolyol (1a): pale yellow, amorphous solid; $[\alpha]_D^{23.6}$ −5.9 (0.43, MeOH); UV (MeOH) λ_{max} (log ε) 259 (4.51), 268 (4.63), 279 (4.51) nm; ^{1}H NMR (CD_3OD/C_5D_5N, 2:1, 600 MHz) δ 4.07 (H_2-1, t, J = 6.7); 1.59 (H_2-2, m); 1.32 (H_2-3, m); 1.28 (H_2-4, m); 1.91 (H_2-5); 5.57 (H-6, m, J = 15.5); 5.47 (H-7, dd, J = 15.3, 6.7); 4.06 (H-8, m); 2.26 (H_2-9, m); 5.6 (H-10, m, J = 15.5); 5.7 (H-11, m, J = 15.0); 2.25 (H-12α, m); 2.61 (H-12β, m); 3.67 (H-13, m); 3.71 (H-14, m); 2.59 (H-15, m); 3.72 (H-16, m); 3.88 (H-17, dt, J = 8.1, 2.4); 1.63 (H-18α, m); 2.08 (H-18β, m); 3.97 (H-19, m); 1.66 (H-20α, m); 1.70 (H-20β, m); 2.24 (H-21α, m); 2.08 (H-21β, m); 5.64 (H-23, m); 4.74 (H-24, dd, J = 8.9, 2.1); 3.84 (H-25, dd, J = 9.3, 2.0); 4.26 (H-26, brd, J = 9.3); 4.31 (H-27, d, J = 2.4); 4.14 (H-28, td, J = 4.0, 4.0, 11.1); 2.04 (H-29α, m); 1.94 (H-29β, m); 3.62 (H-30, ddd, J = 3.0, 6.0, 11.0); 3.72 (H-31, m); 1.65 (H-32α, m); 2.10 (H-32β, m); 2.24 (H-33α, m); 2.61 (H-33β, m); 4.38 (H-35, d, J = 8.9); 3.50 (H-36, dd, J = 8.8, 1.4); 4.24 (H-37, dt, J = 2.0, 9.3, 9.3); 2.30 (H-38α, m); 1.65 (H-38β, m); 4.19 (H-39, dd, J = 3.1, 10); 4.32 (H-40, d, J = 3.0); 4.01 (H-41, dd, J = 2.0, 10.0); 4.20 (H-42, m); 4.60 (H-43, dd, J = 3.4, 7.1); 5.77 (H-44, m); 5.81 (H-45, m); 2.05 (H_2-46, m); 2.05 (H-47, m); 5.61 (H-48); 6.00 (H-49); 6.06 (H-50); 6.00 (H-51); 6.01 (H-52, m); 5.60 (H-53, m); 2.06 (H_2-54, m); 2.04 (H_2-55, m); 5.78 (H-56, m); 4.89 (H-57α, d, J = 10.8); 4.95 (H-57β, dd, J = 1.9, 17.1); 1.10 (H-58, d J = 7.2); 1.71 (H-59, d, J = 1.0); 5.00 (H-60α, s); 5.13 (H-60β, s); ^{13}C NMR (CD_3OD/C_5D_5N, 2:1, 150 MHz) δ 68.6 (C-1, CH_2); 30.3 (C-2, CH_2); 26.3 (C-3, CH_2); 29.9 (C-4, CH_2); 33.1 (C-5, CH_2); 131.8 (C-6, CH); 134.4 (C-7, CH); 73.3 (C-8, CH); 42.2 (C-9, CH_2); 130.0 (C-10, CH); 130.8 (C-11, CH); 38.3 (C-12, CH_2); 72.5 (C-13, CH); 79.5 (C-14, CH); 35.1 (C-15, CH); 80.3 (C-16, CH); 72.4 (C-17, CH); 41.3 (C-18, CH_2); 71.6 (C-19, CH); 36.9 (C-20, CH_2); 36.7 (C-21, CH_2); 138.7 (C-22, C); 126.8 (C-23, CH); 67.8 (C-24, CH); 72.5 (C-25, CH); 79.2 (C-26, CH); 68.9 (C-27, CH); 67.4 (C-28, CH); 30.8 (C-29, CH_2); 75.8 (C-30, CH); 74.5 (C-31, CH); 32.6 (C-32, CH_2); 28.0 (C-33, CH_2); 152.0 (C-34, C); 76.7 (C-35, CH); 75.2 (C-36, CH); 70.6 (C-37, CH); 31.9 (C-38, CH_2); 67.3 (C-39, CH); 68.7 (C-40, CH); 80.6 (C-41, CH); 72.2 (C-42, CH); 74.1 (C-43, CH); 129.8 (C-44, CH); 134.2 (C-45, CH); 33.5 (C-46,CH_2); 33.5 (C-47, CH_2); 134.5 (C-48, CH); 132.0 (C-49, CH); 132.1 (C-50, CH); 132.3 (C-51, CH); 132.2 (C-52, CH); 134.2 (C-53, CH); 33.2 (C-54, CH_2); 34.5 (C-55, CH_2); 139.2 (C-56, CH); 115.4 (C-57, CH_2); 7.0 (C-58, CH_3); 17.4 (C-59, CH_3); 112.8 (C-60, CH_2); HRESIMS $[M + Na]^+$ m/z 1265.6107 (calcd for $C_{60}H_{99}O_{23}SNa_2$, 1265.6093) and $[M - Na]^-$ m/z 1219.6278 (calcd for $C_{60}H_{99}O_{23}S$, 1219.6298).

Acetylation of 1a. A sample of **1a** (0.2 mg) was treated with acetic anhydride (0.2 mL) and pyridine (0.4 mL) for 24 h at room temperature. The solution was then concentrated *in vacuo*. The concentrated-peracetylated product **1b** showed a molecular ion at m/z 1933.7 [M ($C_{94}H_{133}O_{40}S$) − $Na]^-$ in the negative-ion mode of ESIMS, which corresponded to an increase of 17 acetyl groups in symbiopolyol (**1a**).

Reduction of 1a. A solution of **1a** (0.2 mg) in MeOH (0.5 mL) was treated with H_2 and Pd/C (2 mg) at room temperature for 5 h to afford **1c**. The product **1c** showed a molecular ion peak at m/z 1237.7 [M ($C_{60}H_{117}O_{23}S$) − $Na]^-$) in the negative-ion mode ESIMS. The molecular ion peak was consistent with a loss of nine double bonds, as in **1c**.

Enzyme-Linked Immunosorbent Assay (ELISA) for VCAM-1. HUVEC were cultured in MCDB 131 medium containing 10% heat-inactivated fetal calf serum (FCS) and 10 ng/mL basic fibroblast growth factor (bFGF). Cells were placed in 96-well collagen-coated plates and incubated at 37 °C overnight under an atmosphere of 5% CO_2. Symbiopolyol (**1a**) was dissolved in MeOH before use. Each solution was diluted to the desired concentration in the corresponding 96-well plates (1.5×10^4 cells/well) to give final concentrations of 0, 3, 10, 15, and 30 μg/mL **1a**. The control medium contained MeOH in quantities equal to those used for the experiment. After the addition of **1a**, 5 ng/mL TNF-α was added to the corresponding wells, and the cells were then incubated for 18 h. After incubation, the wells were immediately washed twice with PBS (+) (2×300 μL), and fixative solution (0.3% H_2O_2 in MeOH) (100 μL/well) was added. The wells were then incubated for 30 min at room temperature. The wells were then washed twice with PBS (+) (2×300 μL), and 100 μL of diluted first antibody, 4 μg/mL mouse (monoclonal) anti-human VCAM-1 capture antibody, was added. This mixed solution of antibody was incubated for 30 min. After incubation, the wells were immediately washed twice with PBS (+) (each 300 μL), and 100 μL of the diluted second antibody, peroxidase-monoclonal rat anti-mouse IgG_1 (×1000-fold), was put into the corresponding wells. The wells were then incubated for 30 min at room temperature. After 30 min, the wells were immediately washed three times with PBS (+) (3×300 μL). TMB substrate solution (100 μL) was then applied to the cells. When adequate color had developed (30 min), an equal volume of 1 N H_2SO_4 was added to stop the reaction. Spectroscopic readings were immediately taken at wavelengths of 450 and 630 nm by a plate reader. The viability of cells was evaluated using HUVEC, which were seeded in MCDB 131 medium containing 10% FCS and 10 ng/mL bFGF. Cells were then exposed to graded concentrations of **1a** (0, 1, 5, 10, 15 μg/mL) at 37 °C for 72 h in triplicate. After the addition of **1a**, 5 ng/mL TNF-α was added, and the wells were incubated for 72 h. Cytotoxicity was measured by an MTT colorimetric assay at OD 550/630 nm.

VCAM-1-Mediated Cell Adhesion Assay. A VCAM-1-mediated cell adhesion assay was performed by CEREP (Paris, France) according to the method reported previously.[26] Confluent HUVEC in culture plates were stimulated by the addition of 1 ng/mL TNF-α and 500 U/mL IL-4 in a culture medium (MED 199) supplemented with 10% FCS, 10% human serum, 1% antibiotics, and 1% L-glutamine, in the absence (control) or presence of symbiopolyol (**1a**). After an incubation period of 20 h at 37 °C under a 5% CO_2 air atmosphere, the medium was removed and the plates washed with a buffer containing 10 mM Hepes/Tris, 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 0.5 mM Na_2HPO_4, and 5 mM glucose (pH 7.4). Thereafter, Ramos cells labeled with a fluorescent probe (BCECF) were added at a density of 3.5×10^5 cells/well and allowed to adhere onto the stimulated HUVEC for 10 min at room temperature under a 5% CO_2 air atmosphere, still in the absence (control) or presence of **1a**. Following incubation, the plates were washed three times and the cells were lysed with 0.1 M NaOH. The number of adherent Ramos cells was determined by measuring the fluorescence intensity at λ_{ex} = 485 nm and λ_{em} = 530 nm using a spectrofluorimeter. The standard inhibitory reference compound was cycloheximide, which was tested in each experiment at several concentrations to obtain a concentration−response curve from which its IC_{50} value was calculated.

Acknowledgment. We thank Dr. T. Horiguchi (Hokkaido University) for identifying the dinoflagellate. This work was supported in part by Grants-in-Aid for Scientific Research (16GS0206, 21221009 to D.U. and 20611006 to O.O.) and the Global COE program in Chemistry at Nagoya University (Grant No. B-021) from the MEXT, Japan. We are also indebted to FANCL Co. Ltd. for their financial support. N.H. gratefully acknowledges a student fellowship from the Watanuki Foundation.

Supporting Information Available: NMR spectra for symbiopolyol (**1a**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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(17) While the opposite sign of the specific rotation may suggest that symbiopolyol is the enantiomer of lingshuiol B, a direct comparison of the two compounds was not possible because a sample of lingshuiol B was not available. Therefore, the exact structural relationship between these two molecules cannot be established. Symbiopolyol may be the enantiomer, or it could be a diastereomer. Although the $\Delta\delta_{\text{C-8 symbiopolyol-lingshuiol B}}$ (in CD_3OD/C_5D_5N, 2:1) was −0.2 ppm, the C-8 epimer would not be expected to have significant spectroscopic differences from lingshuiol B but could have a different sign of rotation. There was a misassignment for carbon chemical shifts at C-43−C-45, C-47−C-52, and C-53 of lingshuiol B; therefore, we could not compare all of the carbon chemical shifts of lingshuiol B and symbiopolyol. Because of the structural uncertainties, the new name symbiopolyol is proposed.
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NP100221K