

α-Tocopherols from the Formosan Soft Coral *Lobophytum crassum*

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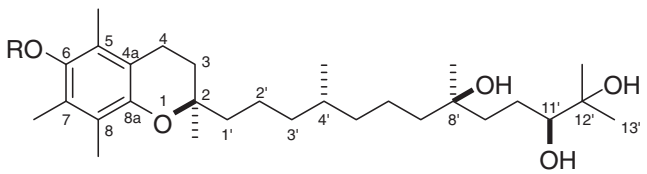
In our screening of marine organisms for bioactive metabolites we have obtained two new α-tocopherols, designated as crassumtocopherols A (**1**) and B (**2**), from the Formosan soft coral *Lobophytum crassum*. The structures of **1** and **2** were determined on the basis of comprehensive NMR and HR-ESI-MS analyses. The cytotoxicity of **1**, **2**, natural vitamin E, and synthetic vitamin E against cancer cell lines and antiviral activity against human cytomegalovirus were evaluated in vitro. Compounds **1** and **2** exhibited moderate cytotoxicity against P-388 (mouse lymphocytic leukemia) cell lines with IC_{50} of 3.2 and 2.7 μg mL^{-1}, respectively. In addition, compound **2** also displayed moderate cytotoxicity against HT-29 (human colon adenocarcinoma) with an IC_{50} of 3.9 μg mL^{-1}. However, natural vitamin E and synthetic vitamin E were not cytotoxic to the selected cancer cell lines and the results for inhibition of antiviral activity against human cytomegalovirus are all negative at a concentration of 1 μg mL^{-1}.

Soft corals belonging to the genus *Lobophytum* (Alcyoniidae) have been well recognized as a rich source of secondary metabolites[1–20] that have attracted much interest for synthetic and natural products chemists due to their structural complexity and remarkable bioactivities such as cytotoxicity,[2–9] antimicrobial activities,[10] and anti-inflammatory properties.[10–12] Our previous research of the Formosan soft coral *L. crassum* (von Marenzeller, 1886) resulted in the purification of two cytotoxic cembranoids, lobocrassolide,[4] and lobocrasol.[9] As part of our continuing investigation of this organism collected at Dongsha Atoll led to the discovery of two new α-tocopherols, designated as crassumtocopherols A (**1**) and B (**2**) (Figure 1).

Tocols, both tocopherols and tocotrienols, comprising naturally occurring α-, β-, γ-, and δ-homologs, are present in various oils in different proportions.[21] The main function of α-tocopherol (α-T) is to protect cell membranes against UV irradiation or oxidative damage.[22] It is believed to play a major role in the prevention of light-induced pathologies of human skin and eyes and degenerative disorders such as atherosclerosis, cardiovascular diseases, and cancer.[23] Freshwater microalgae *Euglena gracilis* yields a large amount of α-T.[24] The marine species *Dunaliella tertiolecta* widely used in aquaculture as feed for fish and mollusk larvae produces relatively high concentrations of α-T.[25] A marine-derived tocopherol (MDT) was first obtained from the lipophilic fraction of chum salmon eggs, and from the tissues of a variety of fish.[26] To the best of our knowledge, this is the first report on the occurrence of α-T derived from marine soft corals.



1 R= H
2 R= Ac

Figure 1. Structures of **1** and **2**.

Compounds **1** and **2** were isolated by cytotoxicity-guided fractionations and characterized by interpretation of detailed 1D and 2D NMR experiments, employing correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple bond correlation (HMBC), heteronuclear single quantum correlation (HSQC), and nuclear Overhauser effect spectroscopy (NOESY) experiments. Moreover, the cytotoxicity against A-549 (human lung epithelial carcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cancer cell lines, along with antiviral activity against human cytomegalovirus for **1**, **2**, natural vitamin E, and synthetic vitamin E were evaluated.

Results and Discussion

Conventional extraction procedures were used, and the acetone extract of the Formosan soft coral *L. crassum* was exhaustively partitioned between EtOAc and H_2O to afford the EtOAc-soluble fraction, which was evaporated under vacuum to yield a brown gum (20 g). The residue was successively subjected to fractionation with column chromatography and high-performance liquid chromatography, leading to the purification of crassumtocopherols A (**1**) and B (**2**) (see Experimental Section).

The positive high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) of **1** exhibited a pseudomolecular ion peak at m/z 501.3553 $[M + Na]^+$, consistent with a molecular formula of $C_{29}H_{50}O_5$, requiring five degrees of unsaturation. Its NMR experiments acquired in C_5D_5N pro-

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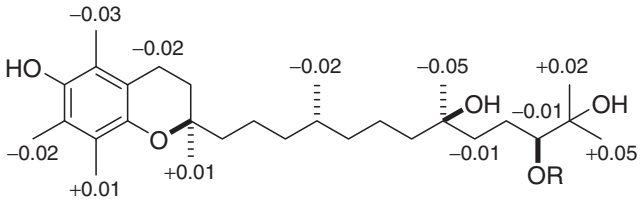
Figure 2. Selected ^{1}H–^{1}H COSY (—) and HMBC (→) correlations of **1**.



1a R= *S*-MTPA
1b R= *R*-MTPA

Figure 3. ^{1}H NMR chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) of the MTPA esters in $CDCl_3$.

vided better resolution than in $CDCl_3$, avoiding overlapped signals. An analysis of the NMR data coupled with ^{1}H–^{1}H COSY, HSQC, and HMBC correlations (Figure 2) were diagnostic in determining that the gross framework of crassumtocopherol A, possessing a tocopherol skeleton, was assigned as **1**. The above findings suggest that **1** has the identical benzopyranol (chromanol) structure of α-T and differs only by having three hydroxy groups at C-8′, C-11′, and C-12′ in the isoprenoid side chain.

The circular dichroism (CD) spectrum of **1** exhibited a negative Cotton effect at λ_{max} ($\Delta\varepsilon$) 289 nm (−0.98) due to the chromenol. The absolute configuration at C-2 was deduced to be *R* on the basis of its CD data comparable to that of the standard (2*R*)-D-α-T acetate.[27] The above assignment is also in good agreement with that of naturally occurring α-T, all of which have a (2*R*)-benzopyranol ring. The 4′*R* configuration in **1** could be deduced by comparison of the ^{13}C NMR spectroscopic data (measured in acetone-d_6, 100 MHz) of 4′-Me (δ_C 20.1) with those of (2*R*,4′*R*,8′*R*)-α-T (natural vitamin E) (δ_C 20.1) and (2*R*,4′*S*,8′*R*)-α-T (synthetic vitamin E) (δ_C 20.0).[28] Because of biogenetic considerations, the absolute configurations of C-2 and C-4′ of **1**, having a similar substitution pattern at each of the above carbons, are suggested to be the same as those of naturally occurring α-T. Furthermore, we determined the absolute configuration at C-11′ using a modified Mosher's esterification.[29] Analysis of $\Delta\delta_{S-R}$ values (Figure 3) for the protons neighboring C-11′ led to the assignment of the *S* configuration. The absolute configurations of C-8′ and C-11′ were also proposed on the basis of comparison of the NMR data with those of (8′*R*,11′*S*)-(+)-heptaol[30] and (8′*R*,11′*R*)-aurilol,[31] suggesting the 8′*R* and 11′*S* configurations. Accordingly, the structure of crassumtocopherol A (**1**) was established unambiguously.

The positive HR-ESI-MS of **2** showed a pseudomolecular ion peak at *m/z* 543.3659 $[M + Na]^+$, corresponding to a molecular formula of $C_{31}H_{52}O_6$ and six degrees of unsaturation. The NMR spectroscopic data of **2** were highly compatible with those obtained for **1**, except for the replacement of the hydroxy group by an acetoxy group. The presence of the acetoxy moiety was identified by the ^{1}H NMR signal at δ_H 2.29 (3H, s) and ^{13}C NMR signals at δ_C 169.6 (qC) and 20.4 (CH_3). Although there were no direct HMBC correlations available, the acetoxy group attached to C-6 was supported by the ^{13}C NMR data of the aromatic ring and IR absorption of the carbonyl at higher frequency (1758 cm^{-1}) due to the conjugation between single-bonded oxygen and the aromatic ring. The absolute configuration of **2** was elucidated as 2*R*,4′*R*,8′*R*,11′*S* after determination of the stereochemistry of **1** as they had the same sign of specific rotations and similar CD spectra. Moreover, compounds **1** and **2** were respectively acetylated with Ac_2O in pyridine at room temperature to afford the same product, further confirming the same stereochemistry between **1** and **2**. From the aforementioned observations, the structure of crassumtocopherol B (**2**) was proposed unambiguously.

As noted in the introduction, a large number of α-tocopherols and their analogs exhibited remarkable biological activities.[23] Crassumtocopherols A (**1**) and B (**2**) in the present study were evaluated in vitro for cytotoxicity against P-388, A-549, and HT-29 cancer cell lines using the MTT assay, as well as antiviral activity against human cytomegalovirus. Preliminary cytotoxic screening revealed that **1** and **2** displayed modest cytotoxicity against P-388 cell line with IC_{50} values of 3.2 and 2.7 μg mL^{-1}, respectively. Compound **2** exhibited modest cytotoxicity against HT-29 cell line with an IC_{50} value of 3.9 μg mL^{-1}. The anticancer agent mithramycin was used as the positive control and exhibited IC_{50} values of 0.06, 0.07, and 0.08 μg mL^{-1} against P-388, A-549, and HT-29 cells, respectively. With the exception of the above finding, the obtained negative result showed that compounds **1** and **2** (1 μg mL^{-1}) exhibited no discernible antiviral activity against human cytomegalovirus. Morever, natural vitamin E and synthetic vitamin E were not cytotoxic to P-388, A-549, and HT-29 cancer cell lines and the results for inhibition of antiviral activity against human cytomegalovirus are all negative at a concentration of 1 μg mL^{-1}.

Experimental

General Experimental Procedures. Optical rotations were determined with a JASCO P1020 digital polarimeter. CD, IR, and UV spectra were recorded on JASCO J-815, JASCO FT/IR-4100, and JASCO V-650 spectrophotometers, respectively. The NMR spectra were recorded on Bruker Avance 300 NMR and Varian MR 400 NMR spectrometers (300 and 400 MHz for ^{1}H, and 75 and 100 MHz for ^{13}C, respectively), using TMS as internal standard. Chemical shifts are given in δ (ppm) and coupling constants in Hz. ESI-MS were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, 230–400 mesh) and Sephadex LH-20 (Pharmacia) were used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for thin-layer chromatography (TLC) analysis. High-performance liquid chromatography (HPLC) was carried out using a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 220 nm and a preparative reversed-phase column (Merck, Hibar Licrospher RP-18e, 5 μm, 250 × 25 mm). *S*-(+)- and *R*-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride were obtained from ACROS Organics (Geel, Belgium).

Animal Material. The Formosan soft coral *L. crassum* was collected at Dongsha Atoll in April 2007, at a depth of 8 m, and was immediately frozen at −20 °C until further processed for extraction in the laboratory. Identification was kindly verified by Professor Chang-Feng Dai, Institute of Oceanography, National Taiwan University, Taiwan. A voucher specimen (TS-11) has been deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation. The frozen soft coral *L. crassum* was chopped into small pieces and extracted with acetone for 24 h at room temperature. The quantity of solvent used for each extraction (2.0 L) was at least three times the amount of the soft coral material used (1.5 kg). The combined extracts were concentrated in vacuo (under 35 °C) to obtain 25 g of dry extract, which was suspended in water and extracted with EtOAc. The EtOAc phase was evaporated to dryness in vacuo to give a dark brown residue (20 g). The resulting EtOAc residue was subjected to silica gel chromatography using a stepwise gradient mixture of *n*-hexane–EtOAc–MeOH for elution and separated into 40 fractions based on TLC and ^{1}H NMR analysis. Fraction 20 (223 mg) eluted with *n*-hexane/EtOAc (1:10) was submitted to repeated chromatography over silica gel using *n*-hexane–EtOAc mixtures of increasing polarity as eluent. Altogether, three subfractions were obtained, of which subfraction 20–3 (142 mg) was subjected column chromatography on RP-18 gel column eluting with 53% MeOH in H_2O to yield a mixture (72 mg). In turn, the mixture was further purified by RP-18 HPLC using an isocratic solvent system of 90% MeOH in H_2O to allow the isolation of **1** (26 mg) and **2** (4 mg), respectively.

Crassumtocopherol A (**1**): Colorless oil; $[\alpha]_D^{25} = -66$ (*c* 0.1, $CHCl_3$); CD (1.33×10^{-4} M, MeOH): λ_{max} ($\Delta\varepsilon$) 245 (+1.00), 289 nm (−0.98); UV (MeOH): λ_{max} (log ε) 245 (3.72), 289 nm (3.28); IR (KBr): ν_{max} 3394, 2973, 2933, 1640, 1558, 1459, 1377, 1258, 1216, 1163, 1081, 919, 755 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz): δ_H 1.75 (2H, m, H-3), 2.58 (2H, dd, $J = 9.6$, 6.4 Hz, H-4), 1.44 (1H, m, H-1′), 1.59 (1H, m, H-1′), 1.53 (1H, m, H-2′), 1.43 (1H, m, H-2′), 1.27 (1H, m, H-3′), 1.03 (1H, m, H-3′), 1.40 (1H, m, H-4′), 1.31 (1H, m, H-5′), 1.11 (1H, m, H-5′), 1.61 (2H, m, H-6′), 1.68 (2H, m, H-7′), 2.33 (1H, m, H-9′), 1.83 (1H, m, H-9′), 2.19 (1H, m, H-10′), 1.92 (1H, m, H-10′), 3.76 (1H, dd, $J = 9.6$, 6.8 Hz, H-11′), 1.49 (3H, s, H-13′), 1.25 (3H, s, 2-CH_3), 2.36 (3H, s, 5-CH_3), 2.42 (3H, s, 7-CH_3), 2.29 (3H, s, 8-CH_3), 0.84 (3H, d, $J = 6.4$ Hz, 4′-CH_3), 1.40 (3H, s, 8′-CH_3), 1.46 (3H, s, 12′-CH_3); ^{13}C NMR (C_5D_5N, 100 MHz): δ_C 74.6 (qC, C-2), 32.1 (CH_2, C-3), 21.2 (CH_2, C-4), 117.6 (qC, C-4a), 121.4 (qC, C-5), 146.9 (qC, C-6), 123.9 (qC, C-7), 122.3 (qC, C-8), 145.8 (qC, C-8a), 40.1 (CH_2, C-1′), 21.5 (CH_2, C-2′), 37.9 (CH_2, C-3′), 33.0 (CH, C-4′), 38.3 (CH_2, C-5′), 22.0 (CH_2, C-6′), 43.5 (CH_2, C-7′), 71.7 (qC, C-8′), 40.7 (CH_2, C-9′), 27.0 (CH_2, C-10′), 80.0 (CH, C-11′), 72.8 (qC, C-12′), 26.1 (CH_3, C-13′), 24.1 (CH_3, 2-Me), 12.6 (CH_3, 5-Me), 13.5 (CH_3, 7-Me), 12.4 (CH_3, 8-Me), 19.8 (CH_3, 4′-Me), 27.7 (CH_3, 8′-Me), 26.1 (CH_3, 12′-Me); ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.79 (2H, m, H-3), 2.60 (2H, t, $J = 6.4$ Hz, H-4), 1.45 (2H, m, H-1′), 1.43 (2H, m, H-2′), 1.08 (2H, m, H-3′), 1.39 (1H, m, H-4′), 1.24 (2H, m, H-5′), 1.35 (1H, m, H-6′), 1.25 (1H, m, H-6′), 1.37 (2H, m, H-7′), 1.71 (1H, m, H-9′), 1.55 (1H, m, H-9′), 1.57 (1H, m, H-10′), 1.44 (1H, m, H-10′), 3.34 (1H, dd, $J = 9.6$, 6.8 Hz, H-11′), 1.17 (3H, s, H-13′), 1.24 (3H, s, 2-CH_3), 2.11 (3H, s, 5-CH_3), 2.16 (3H, s, 7-CH_3), 2.11 (3H, s, 8-CH_3), 0.85 (3H, d, $J = 6.4$ Hz, 4′-CH_3), 1.15 (3H, s, 8′-CH_3), 1.21 (3H, s, 12′-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 74.4 (qC, C-2), 31.7 (CH_2, C-3), 20.7 (CH_2, C-4), 117.3 (qC, C-4a), 118.9 (qC, C-5), 145.5 (qC, C-6), 122.5 (qC, C-7), 121.4 (qC, C-8), 144.5 (qC, C-8a), 39.0 (CH_2, C-1′), 20.7 (CH_2, C-2′), 37.1 (CH_2, C-3′), 32.3 (CH, C-4′), 37.2 (CH_2, C-5′), 21.3 (CH_2, C-6′), 43.3 (CH_2, C-7′), 72.6 (qC, C-8′), 38.7 (CH_2, C-9′), 25.7 (CH_2, C-10′), 79.1 (CH, C-11′), 73.1 (qC, C-12′), 23.3 (CH_3, C-13′), 24.0 (CH_3, 2-Me), 11.8 (CH_3, 5-Me), 12.3 (CH_3, 7-Me), 11.3 (CH_3, 8-Me), 19.6 (CH_3, 4′-Me), 26.3 (CH_3, 8′-Me), 26.5 (CH_3, 12′-Me); ^{1}H NMR (acetone-d_6, 400 MHz): δ_H 1.78 (2H, m, H-3), 2.58 (2H, t, $J = 6.4$ Hz, H-4), 1.51 (2H, m, H-1′), 1.48 (2H, m, H-2′), 1.08 (2H, m, H-3′), 1.43 (1H, m, H-4′), 1.29 (2H, m, H-5′), 1.32 (2H, m, H-6′), 1.39 (2H, m, H-7′), 1.76 (1H, m, H-9′), 1.44 (1H, m, H-9′), 1.65 (1H, m, H-10′), 1.36 (1H, m, H-10′), 3.25 (1H, br d, $J = 10.0$ Hz, H-11′), 1.10 (3H, s, H-13′), 1.21 (3H, s, 2-CH_3), 2.04 (3H, s, 5-CH_3), 2.12 (3H, s, 7-CH_3), 2.08 (3H, s, 8-CH_3), 0.86 (3H, d, $J = 6.4$ Hz, 4′-CH_3), 1.10 (3H, s, 8′-CH_3), 1.10 (3H, s, 12′-CH_3); ^{13}C NMR (acetone-d_6, 100 MHz): δ_C 75.0 (qC, C-2), 32.6 (CH_2, C-3), 21.5 (CH_2, C-4), 117.9 (qC, C-4a), 120.7 (qC, C-5), 146.4 (qC, C-6), 123.1 (qC, C-7), 122.5 (qC, C-8), 146.1 (qC, C-8a), 40.1 (CH_2, C-1′), 21.7 (CH_2, C-2′), 38.3 (CH_2, C-3′), 33.4 (CH, C-4′), 38.5 (CH_2, C-5′), 22.1 (CH_2, C-6′), 43.5 (CH_2, C-7′), 72.2 (qC, C-8′), 40.2 (CH_2, C-9′), 26.8 (CH_2, C-10′), 80.1 (CH, C-11′), 73.0 (qC, C-12′), 25.1 (CH_3, C-13′), 24.3 (CH_3, 2-Me), 12.1 (CH_3, 5-Me), 12.9 (CH_3, 7-Me), 11.9 (CH_3, 8-Me), 20.1 (CH_3, 4′-Me), 27.5 (CH_3, 8′-Me), 26.1 (CH_3, 12′-Me); ESI-MS *m/z* 501 $[M + Na]^+$; HR-ESI-MS *m/z* 501.3553 $[M + Na]^+$ (Calcd for $C_{29}H_{50}O_5Na$, 501.3556).

Crassumtocopherol B (**2**): Colorless oil; $[\alpha]_D^{25} = -50$ (*c* 0.1, $CHCl_3$); CD (1.23×10^{-4} M, MeOH): λ_{max} ($\Delta\varepsilon$) 237 (+2.66), 285 nm (−1.51); UV (MeOH): λ_{max} (log ε) 237 (3.80), 285 nm (3.16); IR (KBr): ν_{max} 3408, 2970, 2933, 1758, 1648, 1575, 1456, 1372, 1212, 1162, 1110, 1077, 923, 755 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz): δ_H 1.63 (2H, m, H-3), 2.47 (2H, dd, $J = 9.6$, 6.4 Hz, H-4), 1.43 (2H, m, H-1′), 1.48 (1H, m, H-2′), 1.38 (1H, m, H-2′), 1.24 (1H, m, H-3′), 1.02 (1H, m, H-3′), 1.40 (1H, m, H-4′), 1.31 (1H, m, H-5′), 1.11 (1H, m, H-5′), 1.59 (2H, m, H-6′), 1.68 (2H, m, H-7′), 2.36 (1H, m, H-9′), 1.84 (1H, m, H-9′), 2.20 (1H, m, H-10′), 1.92 (1H, m, H-10′), 3.76 (1H, dd, $J = 9.6$, 6.8 Hz, H-11′), 1.49 (3H, s, H-13′), 1.18 (3H, s, 2-CH_3), 2.01 (3H, s, 5-CH_3), 2.05 (3H, s, 7-CH_3), 2.15 (3H, s, 8-CH_3), 0.84 (3H, d, $J = 6.4$ Hz, 4′-CH_3), 1.40 (3H, s, 8′-CH_3), 1.46 (3H, s, 12′-CH_3), 2.29 (3H, s, 6-OAc); ^{13}C NMR (C_5D_5N, 100 MHz): δ_C 75.4 (qC, C-2), 31.2 (CH_2, C-3), 20.8 (CH_2, C-4), 118.0 (qC, C-4a), 125.7 (qC, C-5), 141.5 (qC, C-6), 127.3 (qC, C-7), 123.0 (qC, C-8), 140.2 (qC, C-8a), 40.2 (CH_2, C-1′), 21.4 (CH_2, C-2′), 37.8 (CH_2, C-3′), 33.0 (CH, C-4′), 38.3 (CH_2, C-5′), 22.0 (CH_2, C-6′), 43.6 (CH_2, C-7′), 71.7 (qC, C-8′), 40.8 (CH_2, C-9′), 27.0 (CH_2, C-10′), 80.0 (CH, C-11′), 72.8 (qC, C-12′), 26.1 (CH_3, C-13′), 24.0 (CH_3, 2-Me), 12.4 (CH_3, 5-Me), 13.2 (CH_3, 7-Me), 12.1 (CH_3, 8-Me), 19.8 (CH_3, 4′-Me), 27.7 (CH_3, 8′-Me), 26.1 (CH_3, 12′-Me), 169.6 (qC, 6-OAc), 20.4 (CH_3, 6-OAc); ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.78 (2H, m, H-3), 2.59 (2H, t, $J = 6.4$ Hz, H-4), 1.55 (2H, m,

H-1′), 1.42 (2H, m, H-2′), 1.12 (2H, m, H-3′), 1.41 (1H, m, H-4′), 1.29 (2H, m, H-5′), 1.30 (2H, m, H-6′), 1.44 (2H, m, H-7′), 1.74 (1H, m, H-9′), 1.58 (1H, m, H-9′), 1.58 (1H, m, H-10′), 1.45 (1H, m, H-10′), 3.35 (1H, dd, $J = 9.6$, 6.8 Hz, H-11′), 1.17 (3H, s, H-13′), 1.22 (3H, s, 2-CH_3), 1.98 (3H, s, 5-CH_3), 2.02 (3H, s, 7-CH_3), 2.09 (3H, s, 8-CH_3), 0.87 (3H, d, $J = 7.2$ Hz, 4′-CH_3), 1.18 (3H, s, 8′-CH_3), 1.22 (3H, s, 12′-CH_3), 2.33 (3H, s, 6-OAc); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 75.0 (qC, C-2), 31.0 (CH_2, C-3), 20.6 (CH_2, C-4), 117.3 (qC, C-4a), 124.9 (qC, C-5), 140.5 (qC, C-6), 126.6 (qC, C-7), 123.0 (qC, C-8), 149.4 (qC, C-8a), 39.6 (CH_2, C-1′), 20.9 (CH_2, C-2′), 37.3 (CH_2, C-3′), 32.5 (CH, C-4′), 37.5 (CH_2, C-5′), 21.4 (CH_2, C-6′), 43.2 (CH_2, C-7′), 72.7 (qC, C-8′), 38.7 (CH_2, C-9′), 25.8 (CH_2, C-10′), 79.1 (CH, C-11′), 73.1 (qC, C-12′), 23.3 (CH_3, C-13′), 24.5 (CH_3, 2-Me), 12.1 (CH_3, 5-Me), 12.9 (CH_3, 7-Me), 11.8 (CH_3, 8-Me), 19.6 (CH_3, 4′-Me), 26.6 (CH_3, 8′-Me), 26.5 (CH_3, 12′-Me), 169.8 (qC, 6-OAc), 20.6 (CH_3, 6-OAc); ESI-MS m/z 543 $[M + Na]^+$; HR-ESI-MS m/z 543.3659 $[M + Na]^+$ (Calcd for $C_{31}H_{52}O_6Na$, 543.3661).

Preparation of Mosher's Esters of 1. In separate vials, duplicate (1.0 mg) samples of **1** were dissolved in 0.5 mL of dry pyridine and allowed to react overnight at room temperature with (*R*)- and (*S*)-MTPA chloride (one drop), respectively. The reaction was quenched by the addition of 1.0 mL of H_2O, followed by extraction with EtOAc (3 × 1.0 mL). The EtOAc-soluble extracts were combined and evaporated. The resulting residue was subjected to a short silica gel column eluting with *n*-hexane–EtOAc (2:1) to yield (*S*)-MTPA ester **1a** (0.6 mg). In the same manner, the (*R*)-MTPA ester **1b** (0.5 mg) was prepared with (*S*)-MTPA chloride according to the same procedure as described above. Selected ^{1}H NMR ($CDCl_3$, 300 MHz) of **1a**: δ_H 7.41–7.61 (5H, m, Ph), 4.98 (1H, br d, $J = 8.8$ Hz, H-11′), 3.58 (3H, s, OCH_3), 2.59 (2H, t, $J = 6.2$ Hz, H-4), 1.79 (1H, m, H-9′), 1.24 (3H, s, H-13′), 1.24 (3H, s, 2-CH_3), 2.08 (3H, s, 5-CH_3), 2.09 (3H, s, 7-CH_3), 2.17 (3H, s, 8-CH_3), 0.85 (3H, d, $J = 6.2$ Hz, 4′-CH_3), 1.07 (3H, s, 8′-CH_3), 1.18 (3H, s, 12′-CH_3); Selected ^{1}H NMR ($CDCl_3$, 300 MHz) of **1b**: δ_H 7.40–7.61 (10H, m, Ph), 4.99 (1H, br d, $J = 8.8$ Hz, H-11′), 3.56 (3H, s, OCH_3), 2.61 (2H, t, $J = 6.2$ Hz, H-4), 1.78 (1H, m, H-9′), 1.19 (3H, s, H-13′), 1.23 (3H, s, 2-CH_3), 2.11 (3H, s, 5-CH_3), 2.11 (3H, s, 7-CH_3), 2.16 (3H, s, 8-CH_3), 0.87 (3H, d, $J = 6.2$ Hz, 4′-CH_3), 1.12 (3H, s, 8′-CH_3), 1.16 (3H, s, 12′-CH_3).

Acetylation of 1 and 2. A mixture of **1** (2.0 mg), Ac_2O (a drop), and pyridine (1.0 mL) stood at room temperature overnight and then was diluted with 1.0 mL H_2O. The crude residue was suspended in H_2O and extracted with EtOAc. The EtOAc extract was purified by a short silica gel column using *n*-hexane–EtOAc (5:1) to give an acetylated product (1.9 mg). The ^{1}H NMR data of the product were in good accordance with those of the product acetylated from **2** according to the same procedure as described above. ^{1}H NMR (C_5D_5N, 400 MHz): δ_H 1.63 (2H, m, H-3), 2.47 (2H, dd, $J = 9.6$, 6.4 Hz, H-4), 1.42 (2H, m, H-1′), 1.45 (1H, m, H-2′), 1.34 (1H, m, H-2′), 1.22 (1H, m, H-3′), 1.04 (1H, m, H-3′), 1.37 (1H, m, H-4′), 1.32 (1H, m, H-5′), 1.12 (1H, m, H-5′), 1.55 (2H, m, H-6′), 1.67 (2H, m, H-7′), 2.33 (1H, m, H-9′), 1.82 (1H, m, H-9′), 2.10 (1H, m, H-10′), 1.92 (1H, m, H-10′), 5.32 (1H, dd, $J = 9.6$, 6.8 Hz, H-11′), 1.39 (3H, s, H-13′), 1.18 (3H, s, 2-CH_3), 2.01 (3H, s, 5-CH_3), 2.04 (3H, s, 7-CH_3), 2.15 (3H, s, 8-CH_3), 0.84 (3H, d, $J = 6.8$ Hz, 4′-CH_3), 1.34 (3H, s, 8′-CH_3), 1.39 (3H, s, 12′-CH_3), 2.28 (3H, s, 6-OAc), 2.01 (3H, s, 11′-OAc); ^{13}C NMR (C_5D_5N, 100 MHz): δ_C 75.4 (qC, C-2), 31.4 (CH_2, C-3), 20.9 (CH_2, C-4), 118.1 (qC, C-4a), 125.7 (qC, C-5), 141.6 (qC, C-6), 127.4 (qC, C-7), 123.1 (qC, C-8), 150.3 (qC, C-8a), 40.4 (CH_2, C-1′), 21.4 (CH_2, C-2′), 37.9 (CH_2, C-3′), 33.0 (CH, C-4′), 38.3 (CH_2, C-5′), 22.0 (CH_2, C-6′), 43.1 (CH_2, C-7′), 71.4 (qC, C-8′), 39.8 (CH_2, C-9′), 24.9 (CH_2, C-10′), 81.3 (CH, C-11′), 71.6 (qC, C-12′), 27.0 (CH_3, C-13′), 24.2 (CH_3, 2-Me), 12.2 (CH_3, 5-Me), 13.2 (CH_3, 7-Me), 12.4 (CH_3, 8-Me), 19.8 (CH_3, 4′-Me), 27.6 (CH_3, 8′-Me), 26.1 (CH_3, 12′-Me), 169.7 (qC, 6-OAc), 20.5 (CH_3, 6-OAc), 171.1 (qC, 11′-OAc), 21.2 (CH_3, 11′-OAc).

Cytotoxicity Assay. To measure the cytotoxicity of natural product against P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma), and A-549 (human lung epithelial carcinoma), each cell line was initiated at 1500, 750, 750 cells/well, respectively, in 96-well microplates. Three to eight concentrations encompassing an 8- to 128-fold range were on each cell line. P-388, A-549, and HT-29 cells were enumerated using MTT after the exposure to test samples for 3, 6, and 6 days, respectively. Fifty μL of 1 mg mL^{-1} MTT were added to each well, and plates were incubated at 37 °C for 5 h. Supernatant was aspirated. Formazan crystals were redissolved in DMSO for 10 min with shaking, and the plate was read immediately on a microplate reader at a wavelength of 540 nm.[32]

Anticytomegalovirus Assay. To determine the effects of natural product upon human cytomegalovirus (HCMV) cytopathic effect (CPE), confluent human embryonic lung (HEL) cells grown in 24-well plates were incubated for 1 h in the presence or absence of various concentrations of tested natural product. Then, cells were infected with HCMV at an input of 1000 pfu (plaque forming units) per well of 24-well dish. Antiviral activity is expressed as IC_{50} (50% inhibitory concentration), or compound concentration required to reduce virus induced CPE by 50% after 7 days as compared with the untreated control. To monitor the cell growth upon treating with natural products, an MTT-colorimetric assay was employed.[32]

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Supporting Information

^{1}H NMR and ^{13}C NMR spectra of compounds **1** and **2**. This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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