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Corticatic Acids D and E, Polyacetylenic Geranylgeranyltransferase Type I Inhibitors, from the Marine Sponge *Petrosia corticata*

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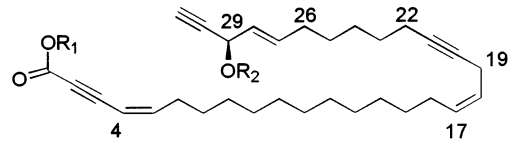
Two new polyacetylenic acids, corticatic acids D (**2**) and E (**3**), have been isolated from the marine sponge *Petrosia corticata* along with the known corticatic acid A (**1**) as geranylgeranyltransferase type I (GGTase I) inhibitors. Their structures were elucidated on the basis of spectroscopic and chemical methods. Compounds **1**−**3** inhibited GGTase I from *Candida albicans* with IC_{50} values of 1.9−7.3 μM.

Geranylgernyltransferase type I (GGTase I) catalyzes the post-translational attachment of the geranylgeranyl unit on the carboxy terminal cysteine residues of proteins, thus promoting membrane interaction and biological activities of these proteins.[1] Null GGTase mutant yeast is morphologically abnormal, which is due to dysfunction of Rho1p.[2] Rho1p, a regulatory subunit of 1,3-β-D-glucan synthesis and the key player in cell wall biosynthesis,[3] is known as one of targets of GGTase I and is essential for viability of *Saccharomyces cerevisiae*.[4] Since there is little sequence identity between the human and *Candida* GGTase I,[5] its inhibitors are expected to be selective antifungal agents.

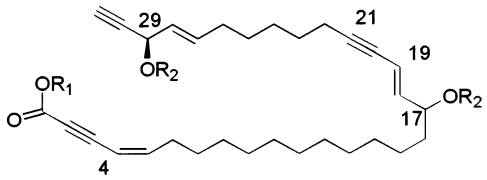
In the course of our continuous program for discovery of potential drug leads from Japanese marine invertebrates, we found potent selective activity against *Candida* GGTase I in the lipophilic extract of the marine sponge *Petrosia corticata* Wilson.[6] Bioassay-guided isolation resulted in the isolation of two new polyacetylenic acids, corticatic acids D and E. We describe the isolation, structure elucidation, and activity of these compounds.

The ether-soluble portion of the MeOH extract of the frozen sponge (300 g) was partitioned between 90% MeOH and *n*-hexane, and the 90% MeOH fraction was fractionated by ODS flash column chromatography, followed by gel filtration and ODS HPLC, to afford corticatic acids A (**1**), D (**2**), and E (**3**), in yields of 2.91×10^{-2}, 5.17×10^{-3}, and 6.33×10^{-4} % on the basis of wet weight, respectively.

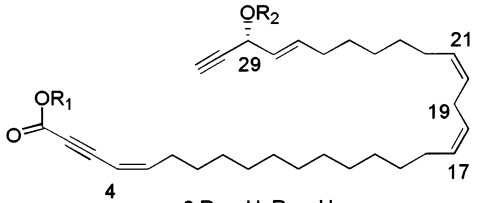
Corticatic acid A (**1**)[7] was readily identified by comparing NMR data with an authentic sample. Previously, we determined a five methylene unit between C-21 and C-27 on the basis of the two-step relayed-COSY data, in which a cross-peak between H_2-23 and H_2-26 was observed despite the presence of other overlapping methylene signals. To confirm this assignment, the methyl ester of **1** was esterified with racemic 2-methoxy-2-(1-naphthyl)propionyl acid (MαNP)[8,9] to obtain diastereomeric esters, which were separated to give MαNP ester (**4**). In the ^{1}H NMR spectrum of **4**, H_2-25 appeared as a well-separated signal (δ 1.09, 2H, m), which was correlated with H_2-23 at δ 1.41 (m) in the relayed COSY spectrum, thus confirming the proposed structure of corticatic acid A, which was also supported by FABMS/MS data (Figure 1a). The absolute stereochemistry of C-29 was determined to be *R* by application of the modified Mosher's method to the corticatic acid methyl ester.[10]



1 R_1 = H; R_2 = H
4 R_1 = Me; R_2 = MNP
5 R_1 = Me; R_2 = MTPA



2 R_1 = H; R_2 = H
6 R_1 = Me; R_2 = MTPA



3 R_1 = H; R_2 = H
7 R_1 = Me; R_2 = MTPA

Corticatic acid D (**2**) had a molecular formula of $C_{31}H_{43}O_4$, larger than **1** by one oxygen atom as established by HRFABMS. In fact, the ^{1}H NMR spectrum displayed an additional oxymethine proton at δ 4.09 (dt, J = 6.0, 6.0 Hz) and lacked methylene protons flanked by an acetylene and a vinyl group. COSY data led to the connectivities from C-17 to C-22, while other structural features were common.

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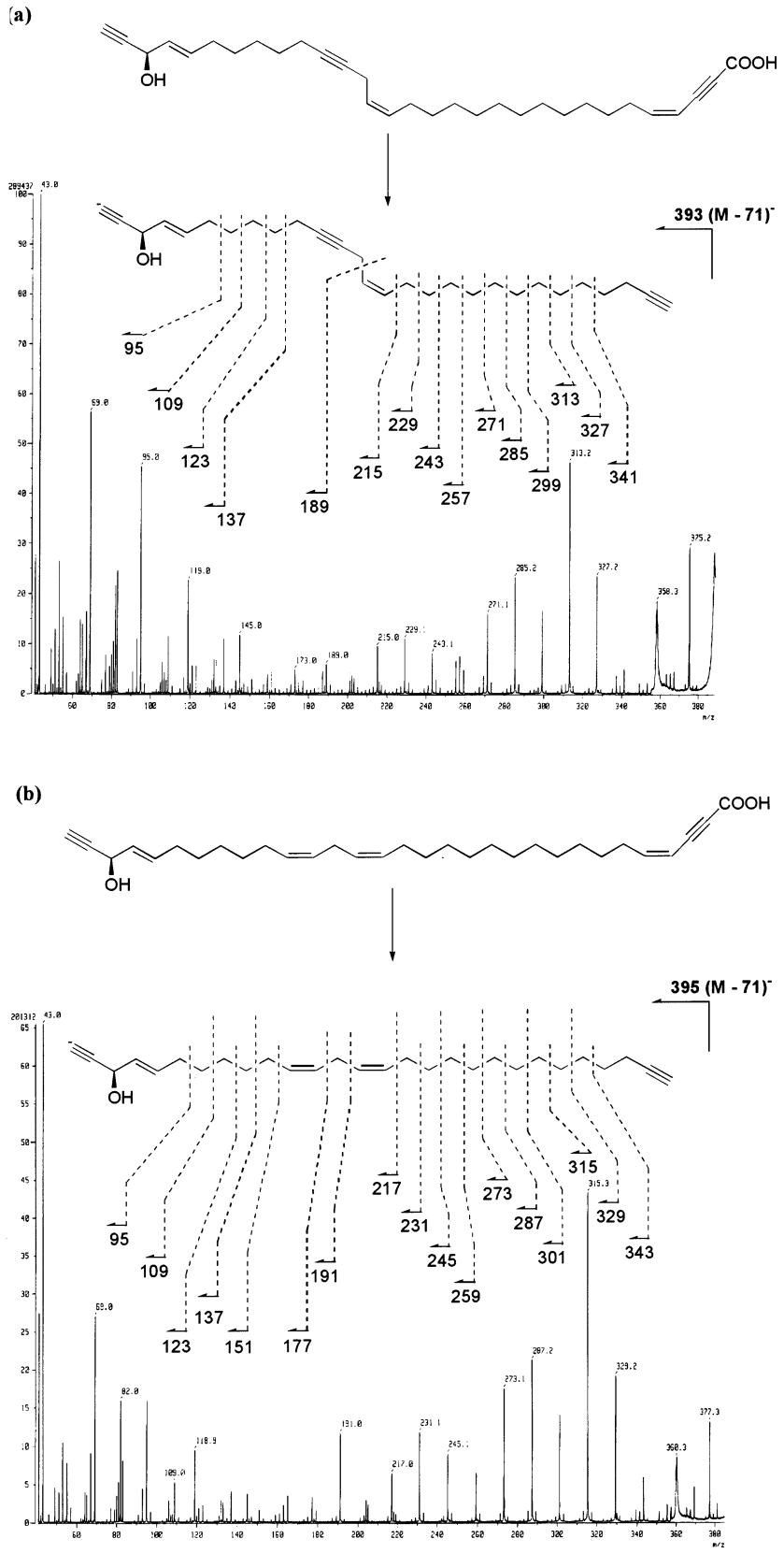

Figure 1. FABMS/MS analysis of compounds **1** (a) and **2** (b).

This was also supported by HOHAHA, HMQC, and HMBC data. The absolute stereochemistry of the two secondary alcohols was analyzed by the modified Mosher's method, which disclosed that C-29 was *R*, while C-17 was racemic; both (+)- and (−)-MTPA esters exhibited two sets of signals with identical intensities (Figure 2).

Corticatic acid E (**3**) had a molecular formula of $C_{31}H_{45}O_3$, larger than **1** by two hydrogen atoms. The ^{1}H NMR spectrum exhibited two additional olefinic protons, and the other spectral features were almost identical to those of **1**, which was consistent with the idea that the C-20/C-21 acetylene unit was replaced by an olefinic unit. However,

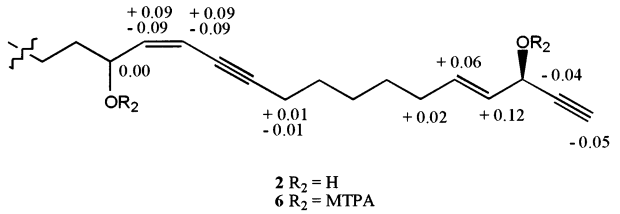

Figure 2. $\Delta\delta$ values obtained for the MTPA ester of **2**.

due to the nearly symmetrical nature of the portion from C-16 to C-22, four olefinic protons were overlapped. Location of these two olefins was overcome by a FABMS/MS experiment, thereby determining the $\Delta^{17,18}$ and $\Delta^{20,21}$ double bonds (Figure 1b). ^{13}C chemical shift values for C-16, C-19, and C-22 suggested *Z*-geometry of both olefins.[11] Absolute stereochemistry was determined to be 29*R* by the modified Mosher's method.

Corticatic acids A (**1**), D (**2**), and E (**3**) inhibited *Candida albicans* GGTase I with IC_{50} values of 1.9, 3.3, and 7.3 μM, respectively; corticatic acid A also inhibited the growth of *C. albicans* with an MIC value of 54 μM.

Polyacetylenic compounds isolated from marine invertebrates are reported to possess a variety of biological activities, such as antimicrobial,[12,13] cytotoxic,[14] and enzyme inhibitory[15] activities. This is the first report for their GGTase I inhibitory activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. NMR spectra were recorded on a JEOL alpha 600 NMR spectrometer. Chemical shifts were referenced to solvent peaks: δ_H 7.24 and δ_C 77.0 for $CDCl_3$. FAB mass spectra were obtained with a JEOL SX-102 mass spectrometer. Triethanolamine or NBA/NaCl was used as the matrix.

Collection, Extraction, and Isolation. Sponge samples were collected by hand using scuba at a depth of 15 m off Shikine-jima Island, 200 km south of Tokyo, immediately frozen, and kept frozen until needed. The frozen sample (300 g) was extracted three times with MeOH, and the combined extracts were partitioned between ether and water. The organic layer was further partitioned between 90% MeOH and *n*-hexane. The former fraction was separated on an ODS flash column with aqueous MeOH system to afford an active fraction (767.1 mg) eluted with 100% MeOH. This was gel filtered on Sephadex LH-20 with $CHCl_3$/MeOH (4:1). Active fractions were combined (217.4 mg) and purified by ODS HPLC on Cosmosil AR-II with 85% MeOH containing 100 mM $NaClO_4$ to furnish corticatic acids A (**1**, 87.2 mg), B (**2**, 15.5 mg), and C (**3**, 1.9 mg), in yields of 2.91×10^{-2}, 5.17×10^{-3}, and 6.33×10^{-4} % on the basis of wet weight, respectively.

Corticatic acid A (1): colorless oil; $[\alpha]^{20}_D$ −12.9° (*c* 0.067, $CDCl_3$); UV (MeOH) λ_{max} (ϵ) 246.0 (10 000), 260.0 (7100) nm; IR (KBr) ν_{max} 3600−2400, 3288, 2919, 2840, 2210, 1714, 1591, 1462, 1374 cm^{-1}; FABMS (negative) *m/z* 463 $(M - H)^-$ and 393 $(M - C_3H_2O_2 - H)^-$; HRFABMS (negative) *m/z* 463.3231 (calcd for $C_{31}H_{43}O_3$, 463.3213) and 393.3154 (calcd for $C_{28}H_{41}O$, 393.3157).

Corticatic acid D (2): colorless oil; $[\alpha]^{20}_D$ −19.7° (*c* 0.067, $CDCl_3$); UV (MeOH) λ_{max} (ϵ) 244.4 (13 000), 259.4 (7987.2) nm; IR (KBr) ν_{max} 3600−2400, 3308, 2924, 2853, 2210, 1682, 1589, 1455, 1362 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; FABMS (negative) *m/z* 479 $(M - H)^-$ and 309; HRFABMS (negative) *m/z* 479.3151 (calcd for $C_{31}H_{43}O_4$, 479.3162).

Corticatic acid E (3): colorless oil; $[\alpha]^{20}_D$ −3.1° (*c* 0.10, $CDCl_3$); UV (MeOH) λmax (ϵ) 250.0 (6800) nm; IR (KBr) ν_{max} 3600−2400, 3289, 2925, 2853, 2209, 1714, 1455, 1347, 1257 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.15 (1H, dt, J = 10.8, 7.2 Hz, H-5), 5.87 (1H, dt, J = 15.6, 6.6 Hz, H-27), 5.59 (1H, dd, J = 15.6, 6.6 Hz, H-28), 5.52 (1H, d, J = 10.8 Hz, H-4), 5.33−5.29 (4H, m, H-17, 18, 20, and 21), 4.82 (1H, d, J = 5.4 Hz, H-29), 2.74 (2H, dd, J = 6.0, 6.0 Hz, H_2-19), 2.51 (1H, d, J = 1.8 Hz, H-31), 2.34 (2H, dt, J = 7.8, 7.2 Hz, H_2-6), 2.02 (6H, m, H_2-16, 22, and 26), 1.40−1.23 (24H, H_2-7−15 and 23−25); ^{13}C NMR (determined by HMQC and HMBC data) δ 149.9 (d, C-5), 134.0 (d, C-27), 130.1 (2C, d, C-17 and -21), 128.5 (d, C-28), 127.8 (2C, d, C-18 and -20), 107.3 (d, C-4), 86.9 (s, C-2), 83.8 (s, C-31), 82.1 (s, C-3), 73.4 (s, C-30), 62.3 (s, C-29), 32.4−27.3 (14C, C-6−15 and -23−26), 27.1 (2C, t, C-16 and -22), 25.4 (t, C-19), chemical shift of the carboxyl carbon was not observed.; FABMS (negative) *m/z* 465 $(M - H)^-$ and 395 $(M - C_3H_2O_2 - H)^-$; HRFABMS (negative) *m/z* 465.3397 (calcd for $C_{31}H_{45}O_3$, 465.3371) and 395.3292 (calcd for $C_{28}H_{43}O$, 395.3313).

MαNP Ester of 1. To a stirred solution of **1** (2.0 mg) in a 2:7 mixture of MeOH and toluene (0.9 mL) was added 30 μL of 2.0 M $TMSCHN_2$ in *n*-hexane and 20 μL of 3 N HCl at room temperature. The mixture was stirred for 30 min at room temperature and evaporated to dryness in vacuo. The residue was dissolved in CH_2Cl_2 (0.3 mL), to this solution was added DCC (7.0 mg), DMAP (5.0 mg), and MαNP acid (15 mg), and the mixture was left standing at room temperature for 3 days. The reaction mixture was dried in vacuo, diluted with 1 mL of 60% MeOH, and extracted with $CHCl_3$ (1 mL × 3). The organic phase was dried over $MgSO_4$ and evaporated. The residue was dissolved in hexane/ether (4:1) and separated by normal-phase HPLC with hexane/ether (4:1) to yield **4** (0.8 mg): ^1H NMR ($CDCl_3$) δ 8.35 (1H, m), 7.82 (2H, m), 7.58 (1H, d, J = 6.6 Hz), 7.45−7.41 (3H, m), 6.23 (1H, dt, J = 10.8, 7.2 Hz, H-5), 5.84 (1H, d, J = 6.0 Hz, H-29), 5.52 (1H, d, J = 10.8 Hz, H-4), 5.51 (1H, dt, J = 15.0, 6.6 Hz, H-27), 5.40 (2H, m, H-17 and 18), 5.11 (1H, dd, J = 15.0, 6.0 Hz, H-28), 3.77 (3H, s, COOMe), 3.07 (3H, s, OMe), 2.89 (2H, brm, H_2-19), 2.46 (1H, d, J = 1.8 Hz, H-31), 2.34 (2H, dt, J = 7.2, 7.2 Hz, H_2-6), 2.11 (2H, brt, J = 7.2 Hz, H_2-22), 2.00 (2H, dt, J = 6.6, 6.6 Hz, H_2-16), 1.99 (3H, s, Me), 1.76 (2H, dt, J = 6.6, 6.6 Hz, H_2-26), 1.41 (4H, m, H_2-7 and 23), 1.30 (2H, m, H_2-15), 1.28−1.23 (16H, H_2-8−14 and -24), 1.09 (2H, m, H_2-25); FABMS (positive) *m/z* 713 $(M + Na)^+$.

MTPA Esters. To a stirred solution of **1** (2.0 mg) in 0.9 mL of MeOH/toluene (2:7) was added 40 μL of 2.0 M $TMSCHN_2$ in *n*-hexane and 10 μL of 3 N HCl at room temperature. The mixture was stirred for 30 min at room temperature and evaporated to dryness in vacuo. The residue was dissolved in dry pyridine (2 drops), to this solution was added (*R*)-(−)-MTPACl (5 mg in 50 μL of dry toluene), and the mixture was

Table 1. ^1H and ^{13}C NMR Spectral Data for **2**

no.	^1H (mult., J in Hz)	^{13}C (mult.)	HMBC data
1		[b]	
2		86.5[c]	
3		82.0[c]	
4	5.53 (d, 10.8)	107.4	C-2, 3, 5
5	6.14 (dt, 10.8, 7.8)	149.9	C-3, 4
6	2.34 (dt, 7.8, 7.2)	30.6	C-4, 5, 6
7	1.38[a]	27.5−31.8	C-5
8−15	1.22−1.28	27.5−31.8	
16	1.49 (m)	36.9[c]	C-17, 18
17	4.09 (dt, 6.0, 6.0)	72.5	C-16, 18, 19
18	6.01 (dd, 15.6, 6.0)	144.2	C-16, 17, 20
19	5.64 (d, 15.6)	110.1	C-17, 21
20		78.3[c]	
21		91.0[c]	
22	2.26 (t, 7.8)	19.2	C-18, 19, 20, 21
23	1.49 (m)	27.7[c]	C-21, 22
24	1.28[a]	27.5−31.8	
25	1.38[a]	27.5−31.8	C-27
26	2.05 (dt, 7.2, 6.6)	31.8	C-27, 28
27	5.87 (dt, 15.0, 6.6)	134.0	C-26, 29
28	5.59 (dd, 15.0, 6.0)	128.2	C-26, 29, 30
29	4.82 (d, 4.8)	62.8	C-27, 28, 30, 31
30		84.3[c]	
31	2.52 (d, 1.8)	74.5	C-29, 30

[a] J_{H-H} couplings were not assigned due to their overlapped signals. [b] ^{13}C chemical shift could not be assigned. [c] ^{13}C chemical shifts were assigned by HMQC and HMBC data.

left standing at room temperature for 10 min. The reaction mixture was dried in vacuo, diluted with water, and extracted with EtOAc. The organic layer was evaporated and submitted to spectral analysis. Other MTPA esters were prepared in the same manner.

(*S*)-(−)-MTPA ester of 1: ^{1}H NMR ($CDCl_3$) δ 7.51 (3H, m), 7.36 (2H, m), 6.23 (1H, dt, J = 10.8, 7.8 Hz, H-5), 6.05 (1H, dt, J = 15.6, 7.2 Hz, H-27), 5.99 (1H, d, J = 6.0 Hz, H-29), 5.58 (1H, dd, J = 15.6, 6.0 Hz, H-28), 5.52 (1H, d, J = 10.8 Hz, H-4), 5.40 (2H, m, H-17 and -18), 3.77 (3H, s, COOMe), 3.53 (3H, OMe), 2.87 (2H, brd, m, H_2-19), 2.57 (1H, brs, H-31), 2.34 (2H, dt, J = 7.2, 7.2 Hz, H_2-6), 2.11 (2H, brt, J = 6.6 Hz, H_2-22), 2.07 (2H, dt, J = 7.2, 7.2 Hz, H_2-26), 2.00 (2H, dt, J = 6.6, 6.6 Hz, H_2-16), 1.23−1.46 (24H, H_2-7−15 and -23−25); FABMS (positive) m/z 718 $(M + Na)^+$.

(*R*)-(+)-MTPA ester of 1: ^{1}H NMR ($CDCl_3$) δ 7.50 (3H, m), 7.36 (2H, m), 6.23 (1H, dt, J = 10.8, 7.8 Hz, H-5), 6.00 (1H, d, J = 6.0 Hz, H-29), 5.98 (1H, dt, J = 15.0, 6.0 Hz, H-27), 5.52 (1H, d, J = 10.8 Hz, H-4), 5.48 (1H, dd, J = 15.6, 6.6 Hz, H-28), 5.40 (2H, m, H-17 and -18), 3.77 (3H, s, COOMe), 3.53 (3H, s, OMe), 2.87 (2H, brd, m, H_2-19), 2.62 (1H, brs, H-31), 2.34 (2H, dt, J = 7.2, 7.2 Hz, H_2-6), 2.11 (2H, brt, J = 6.6 Hz, H_2-22), 2.00 (2H, dt, J = 7.2, 7.2 Hz, H_2-26), 2.00 (2H, dt, J = 6.6, 6.6 Hz, H_2-16), 1.23−1.46 (24H, H_2-7−15 and -23−25); FABMS (positive) m/z 718 $(M + Na)^+$.

$\Delta\delta$ Values for MTPA esters of 1: +0.07 (H_2-26), +0.07 (H-27), +0.10 (H-28), −0.01 (H-29), −0.05 (H-31).

Bis[(*S*)-(−)-MTPA] ester of 2: ^{1}H NMR ($CDCl_3$) δ 7.52 (6H, m), 7.35 (4H, m), 6.22 (1H, dt, J = 10.8, 7.8 Hz, H-5), 6.03 (1H, dt, J = 15.6, 7.2 Hz, H-27), 5.96 (1H, d, J = 6.0 Hz, H-29), 5.92 (0.5H, dd, J = 14.4, 6.6 Hz, H-18), 5.83 (0.5H, dd, J = 14.4, 6.6 Hz, H-18), 5.75 (0.5H, d, J = 15.0 Hz, H-19), 5.66 (0.5H, d, J = 15.0 Hz, H-19), 5.56 (1H, dd, J = 15.6, 6.0 Hz, H-28), 5.52 (1H, d, J = 10.8 Hz, H-4), 5.40 (1H, m, H-17), 3.76 (3H, s, COOMe), 3.53 (3H, s, OMe), 2.60 (1H, brs, H-31), 2.34 (2H, dt, J = 7.2, 7.2 Hz, H_2-6), 2.25 (1H, m, H_2-22), 2.08 (1H, dt, J = 7.2, 7.2 Hz, H_2-26), 1.62 (2H, m, H_2-16), 1.45−1.21 (24H, H_2-7−15 and -23−25); FABMS (positive) m/z 949 $(M + Na)^+$.

Bis[(*R*)-(+)-MTPA] ester of 2: ^{1}H NMR ($CDCl_3$) δ 7.52 (6H, m), 7.36 (4H, m), 6.22 (1H, dt, J = 10.8, 7.8 Hz, H-5), 6.00 (1H, d, J = 6.0 Hz, H-29), 5.97 (1H, dt, J = 15.6, 7.2 Hz, H-27), 5.92 (0.5H, dd, J = 14.4, 6.6 Hz, H-18), 5.83 (0.5H, dd, J = 15.0, 6.6 Hz, H-18), 5.75 (0.5H, d, J = 15.0 Hz, H-19), 5.66 (0.5H, d, J = 15.0 Hz, H-19), 5.52 (1H, d, J = 10.8 Hz, H-4), 5.48 (1H, dd, J = 15.6, 6.0 Hz, H-28), 5.40 (1H, m, H-17), 3.76 (3H, s, COOH), 3.53 (3H, s, OMe), 2.65 (1H, brs, H-31), 2.34 (1H, dt, J = 7.2, 7.2 Hz, H_2-6), 2.25 (1H, m, H_2-22), 2.06 (1H, dt, J = 7.2, 7.2 Hz, H_2-26), 1.62 (2H, m, H_2-16), 1.45−1.21 (24H, H_2-7−15 and -23−25); FABMS (positive) m/z 949 $(M + Na)^+$.

(*S*)-(−)-MTPA ester of 3: ^{1}H NMR ($CDCl_3$) δ 7.53 (3H, m), 7.37 (2H, m), 6.24 (1H, dt, J = 10.8, 7.8 Hz, H-5), 6.05 (1H, dt, J = 15.6, 7.2 Hz, H-27), 5.99 (1H, d, J = 6.0 Hz, H-29), 5.58 (1H, dd, J = 15.6, 6.0 Hz, H-28), 5.52 (1H, d, J = 10.8 Hz, H-4), 5.34 (4H, m, H-17, -18, -20, and -21), 3.77 (3H, s, COOMe), 3.54 (3H, s, OMe), 2.74 (2H, dd, J = 6.0, 6.0 Hz, H_2-19), 2.58 (1H, brs, H-31), 2.35 (2H, dt, J = 7.2, 7.2 Hz, H_2-6), 2.05 (6H, m, H_2-16, -22, and -26), 1.23−1.46 (24H, H_2-7−15 and -23−25); FABMS (positive) m/z 720 $(M + Na)^+$.

(*R*)-(+)-MTPA ester of 3: ^{1}H NMR ($CDCl_3$) δ 7.53 (3H, m), 7.37 (2H, m), 6.23 (1H, dt, J = 10.8, 7.8 Hz, H-5), 6.01 (1H, d, J = 6.0 Hz, H-29), 5.99 (1H, dt, J = 15.0, 6.0 Hz, H-27), 5.53 (1H, d, J = 10.8 Hz, H-4), 5.48 (1H, dd, J = 15.6, 6.6 Hz, H-28), 5.34 (4H, m, H-17, -18, -20, and -21), 3.77 (3H, s, COOMe), 3.57 (3H, s, OMe), 2.74 (2H, dd, J = 6.0, 6.0 Hz, H_2-19), 2.61 (1H, brs, H-31), 2.34 (2H, dt, J = 7.2, 7.2 Hz, H_2-6), 2.02 (6H, m, H_2-16, -22, and -26), 1.23−1.46 (24H, H_2-7−15 and -23−25); FABMS (positive) m/z 718 $(M + Na)^+$.

$\Delta\delta$ Values for the MTPA esters of 3: +0.03 (H_2-26), +0.06 (H-27), +0.10 (H-28), −0.02 (H-29), −0.03 (H-31).

Enzyme Inhibition Assay. GGTase I inhibition assay was performed in a streptavidin scintillation proximity assay (SPA) format in which biotinylated peptide is captured by streptavidin-labeled SPA beads.[16] Crude GGTase I from *C. albicans* (ATCC 10231) was prepared as described.[17] Synthetic biotinylated peptide substrate (sequence TEKKKKKCVLL) was dissolved in 50 mM HEPES buffer, pH 7.5, containing 25 mM Na_2HPO_4, 20 mM KCl, 5 mM DTT, and 0.01% Triton X-100 at a concentration of 5 μM. [^{3}H] GGPP was dissolved in 50 mM HEPES buffer, pH 7.5, containing 30 mM $MgCl_2$, 20 mM KCl, 5 mM DTT, and 0.01% Triton X-100 at a concentration of 0.1 μM. Stock solution of the enzyme was diluted with 50 mM HEPES buffer, pH 7.5, containing 20 μM $ZnCl_2$, 1 mM DTT, and 10% glycerol immediately before use. To a reaction mixture containing 10 μL of biotinylated peptide solution, 1 μL of test sample in DMSO, and 15 μL of [^{3}H] GGPP solution was added 25 μL of enzyme solution, and the mixture was incubated at 30 °C for 2 h. The reaction was terminated by the addition of 75 μL of 280 mM H_3PO_4 buffer containing 0.7% BSA, 0.1% tween 20, and 1.67 mg/mL of SPA beads. Radioactivity of the captured geranylgeranylated peptide was determined using a liquid scintillation counter (Packard TopCount).

References and Notes

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