Antiviral and Anti-inflammatory Diterpenoids from the Soft Coral *Sinularia gyrosa*

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Chemical investigation of the soft coral *Sinularia gyrosa* led to the purification of three new diterpenoids, designated as gyrosanols A−C (**1**−**3**). The structures of **1**−**3** were elucidated through extensive spectroscopic analyses. Compounds **1** and **2** exhibited antiviral activity against HCMV with IC_{50}'s of 2.6 and 3.7 μM, respectively. In addition, compounds **1** and **2** showed significant anti-inflammatory activity by reducing the levels of the COX-2 protein (19.6 ± 3.9% and 29.1 ± 9.6%, respectively) in RAW 264.7 macrophages.

Soft corals of the genus *Sinularia* (Alcyoniidae) have been well recognized as a rich source of sesquiterpenes, diterpenes, polyhydroxylated steroids, and polyamine metabolites.[1,2] A number of these isolated metabolites were shown to exhibit various biological activities such as cytotoxic, anti-inflammatory, and antimicrobial activities.[2] An unusual norcembrane-type diterpenoid, (4*R**,8*S**,11*R**,13*S**,14*R**)-14-hydroxy-4-isopropenyl-11-methyl-6,9-dioxo-8,11-epoxycyclotetradec-1-ene-1,13-carbolactone, was obtained from the soft coral *Sinularia gyrosa* (Klunzinger, 1877) by Schmitz's group.[3] In the course of our ongoing research to discover bioactive metabolites from marine organisms, chemical investigation of the soft coral *S. gyrosa* resulted in the isolation of three new diterpenoids, designated as gyrosanols A−C (**1**−**3**). The details of the isolation and structure elucidation of compounds **1**−**3** are discussed. Compounds **1**−**3** were evaluated in vitro for cytotoxicity against A-459 (human lung carcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cancer cell lines, antiviral activity against HCMV (human cytomegalovirus) cells, anti-inflammatory activity using RAW 264.7 macrophage cells, and antibacterial activity against five bacterial strains, *Enterobacter aerogenes*, *Salmonella enteritidis*, *Serratia marcescens*, *Shigella sonnei*, and *Yersinia enterocolitica*.

Gyrosanol A (**1**) was obtained as a colorless oil. The molecular formula was determined to be $C_{20}H_{32}O$, as deduced from its HRESIMS (*m*/*z* 311.2353, $[M + Na]^+$) and ^{13}C NMR data (Table 2), requiring five degrees of unsaturation. A broad IR absorption at 3433 cm^{-1} indicated the presence of a hydroxy group, which was further supported by the ^1H NMR signal (Table 1) at δ_H 3.55 (dd, J = 9.6, 6.4 Hz, H-6) and ^{13}C NMR signal (Table 2) at δ_C 76.4 (CH, C-6). The NMR data (Tables 1 and 2) showed resonances for two trisubstituted double bonds [δ_H 5.29 (d, J = 1.6 Hz, H-8) and 5.14 (t, J = 6.4 Hz, H-14); δ_C 119.4 (CH, C-8), 135.3 (C, C-9), 124.3 (CH, C-14), and 131.6 (C, C-15)] and a 1,1-disubstituted double bond [δ_H 4.76 (d, J = 1.2 Hz, H-18a) and 4.81 (s, H-18b); δ_C 107.3 (CH_2, C-18) and 154.6 (C, C-11)]. To satisfy the unsaturation number of five and taking into account the presence of three double bonds, it was assumed that **1** possesses a bicyclic structure.

From the COSY and long-range $^1H-^1H$ COSY correlations (Figure 1) of **1**, it was possible to establish two partial structures. COSY correlations were observed from H-10 to H_2-4 through H_2-1, H-2, and H_2-3; from H-6 to H-8 through H_2-7; and from H_2-12 to H-14 through H_2-13, while long-range $^1H-^1H$ COSY correlations established coupling relationships between H-2 and H_2-18, H_2-4 and H_3-19, H-8 and H_3-20, H_2-18 and H_2-12, H-14 and H_3-16, and H-14 and H_3-17. Moreover, the connectivities between C-9/C-10 and C-6/C-5 were confirmed by the crucial HMBC correlations from H_3-19 to C-4, C-5, C-6, and C-10; from H-10 to C-8; and from H_3-20 to C-8, C-9, and C-10. The key HMBC correlations from H_3-20 to C-8, C-9, and C-10; from H_2-18 to C-2, C-11, and C-12; and from H_3-16/H_3-17 to C-14 and C-15 helped locate the positions of the double bonds. In addition, the HMBC spectrum showed correlations from H_3-19 to C-6 and from H-6 to C-19, demonstrating the attachment of the hydroxy group at C-6 (Figure 1). Therefore, the planar structure of **1** with a decalin moiety was established.

The relative configuration of **1** was determined through inspection of the NOESY spectrum as well as a computer-generated lower energy conformation using MM2 force field calculations (Figure 2). The NOESY correlations between H-10/H-6, H-10/H-1β (δ_H 1.78), H-1β/H-2, H-6/H-4β (δ_H 1.13), and H-6/H-7β (δ_H 2.31) positioned the above protons on the same face of the decalin ring (β-orientation), while the NOESY correlations between H_3-19/H-1α (δ_H 1.24), H_3-19/H-3α (δ_H 1.48), H-4α (δ_H 1.91)/H-3α, H-18a (δ_H 4.81)/H-1α, and H-18a/H-3α suggested that H-1α, H-3α, H-4α, H_3-19, and the side chain attached at C-2 were orientated to the other face (α-orientation). The crucial NOESY correlations between H_3-19/H-1α and H-10/H-1β also indicated a *trans* ring fusion in **1**. Moreover, the absolute configurations of **1** was determined by a spectroscopic method according to Mosher's acylation.[4] The proton chemical shifts of relevant protons were assigned by analysis of the ^1H and $^1H-^1H$ COSY NMR spectra. Analysis of the $\Delta\delta_{S-R}$ values (Figure 3) according to the Mosher model pointed to the *S* configuration for C-6 of **1**, because H-2, H_2-3, and H_2-4 of (*S*)-MTPA ester **1a** were less shielded by the phenyl ring of the MTPA product, and subsequently established the 2*S* configuration based on their relative configurations. On the basis of the above-mentioned observations, the structure of gyrosanol A (**1**) was formulated as (2*S*,5*S*,6*S*,10*R*,8*Z*)-5,9-dimethyl-2-[15-methyl-11(18),14-heptadien-11-yl]-1,2,3,4,5,6,7,10-octahydro-7-naphthalen-6-ol.

The molecular formula of **2** was also assigned as $C_{20}H_{32}O$, as derived from its HRESIMS peak (*m*/*z* 311.2352 $[M + Na]^+$) and in agreement with the NMR data (Tables 1 and 2). Comparison of the NMR data of **2** with those of fuscol[5] revealed highly similar

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Table 1. ^{1}H NMR Spectroscopic Data (400 MHz) of **1**−**3**

position	**1**,[a] δ_H (J in Hz)	**2**,[a] δ_H (J in Hz)	**2**,[b] δ_H (J in Hz)	**3**,[a] δ_H (J in Hz)
1	α: 1.24, d (12.8)	α: 1.57, m	α: 1.59, m	1.38, m
	β: 1.78, m	β: 1.55 m	β: 1.67 m	
2	1.92, m	1.93, m	1.90, m	1.79, m
3	α: 1.48, dd (12.8, 3.6)	α: 1.46, m	α: 1.41, m	a: 1.73, m
	β: 1.68, m	β: 1.62, m	β: 1.57, m	b: 1.59, m
4	α: 1.91, m	1.45, m	1.38, m	3.90, br d (10.0)
	β: 1.13, td (12.8, 4.0)			
6	3.55, dd (9.6, 6.4)	5.82, dd (18.0, 10.4)	5.79, dd (17.6, 10.8)	2.18, m
7	α: 1.96, m; β: 2.31, m	a: 4.92, m; b: 4.88, m	a: 4.95, m; b: 4.92, m	a: 2.43, m; b: 2.18, m
8	5.29, d (1.6)	a: 4.82, br s	a: 4.92, br s	5.40, br s
		b: 4.59, br s	b: 4.69, d (0.8)	
10	1.93, m	2.00, dd (11.6, 4.8)	1.94, dd (12.8, 3.6)	a: 2.13, m; b: 1.97, m
12	2.09, m	2.78, d (6.8)	2.78, d (6.8)	a: 2.08, m; b: 2.01, m
13	2.12, m	5.64, m	5.66, dt (15.6, 6.8)	2.15, m
14	5.14, t (6.4)	5.64, m	5.55, dt (15.6, 1.2)	5.14, t (6.8)
16	1.70, s	1.33, s	1.18, s	1.73, s
17	1.63, s	1.33, s	1.18, s	1.66, s
18	a: 4.81, s	a: 4.81, br s	a: 4.91, br s	a: 4.82, s
	b: 4.76, d (1.2)	b: 4.74, d (1.2)	b: 4.89, d (1.6)	b: 4.77, s
19	0.79, s	1.00, s	1.00, s	a: 5.12, s; b: 5.09, s
20	1.61, s	1.71, s	1.79, s	1.70, br s

[a] Spectra were measured in $CDCl_3$. [b] Spectra were measured in C_6D_6.

Table 2. ^{13}C NMR Spectroscopic Data (100 MHz) of **1**−**3**

C#	**1**,[a] δ_C, mult.	**2**,[a] δ_C, mult.	**2**,[b] δ_C, mult.	**3**,[a] δ_C, mult.
1	28.9, CH_2	33.2, CH_2	33.7, CH_2	31.6, CH_2
2	44.9, CH	44.3, CH	44.7, CH	41.5, CH
3	26.9, CH_2	27.1, CH_2	27.5, CH_2	36.8, CH_2
4	35.2, CH_2	39.9, CH_2	40.2, CH_2	76.3, CH
5	37.5, C	39.8, C	40.0, C	149.2, C
6	76.4, CH	150.2, CH	150.4, CH	25.5, CH_2
7	32.3, CH_2	109.9, CH_2	110.1, CH_2	25.3, CH_2
8	119.4, CH	112.1, CH	112.6, CH	125.3, CH
9	135.3, C	147.7, C	147.7, C	134.0, C
10	46.8, CH	52.8, CH	53.0, CH	40.5, CH_2
11	154.6, C	153.1, C	153.2, C	153.4, C
12	34.8, CH_2	37.8, CH_2	38.2, CH_2	34.0, CH_2
13	26.9, CH_2	125.1, CH	124.8, CH	26.8, CH_2
14	124.3, CH	139.6, CH	140.5, CH	124.3, CH
15	131.6, C	70.7, C	70.1, C	131.6, C
16	25.7, CH_3	29.8, CH_3	30.1, CH_3	25.7, CH_3
17	17.7, CH_3	29.8, CH_3	30.1, CH_3	17.7, CH_3
18	107.3, CH_2	108.5, CH_2	108.9, CH_2	108.0, CH_2
19	9.6, CH_3	16.6, CH_3	16.8, CH_3	115.5, CH_2
20	20.9, CH_3	24.8, CH_3	25.1, CH_3	15.8, CH_3

[a] Spectra were measured in $CDCl_3$. [b] Spectra were measured in C_6D_6.

chemical shifts, except that the double bond at C-11/C-12 in fuscol was relocated to C-11/C-18 in **2**. This was confirmed by the crucial HMBC correlations from H_2-18 to C-2, C-11, and C-12 (Figure 1). The ^{1}H NMR spectrum acquired in C_6D_6 provided better resolution than in $CDCl_3$, avoiding overlapped signals of H-13 and H-14 (Table 1). The geometry of the disubstituted double bond at C-13 and C-14 was established to be *E* on the basis of the large coupling constant of $J_{13,14} = 15.6$ Hz. Furthermore, the NOESY correlations (Figure 4) indicated that **2** possessed the same configurations as those of reported lobane diterpenoids at the C-2, C-5, and C-10 stereocenters.[5−11] Moreover, the 2*S*,5*R*,10*R* configuration of **2** was proposed on the basis of comparison of its specific rotation ($[\alpha]^{25}_D$ +18) with that of fuscol ($[\alpha]_D$ +16), which was first isolated from the gorgonian *Eunicea fusca*.[5a,6] The chemical synthesis of (+)-fuscol has been achieved,[5b,c] firmly establishing its configuration. Hence, (2*S*,5*R*,10*R*,13*E*)-6,8,11(18),13-lobatetraen-15-ol was characterized as gyrosanol B.

The HRESIMS spectrum of **3** exhibited a pseudomolecular ion peak at *m/z* 311.2352 $[M + Na]^+$ and again established a molecular formula of $C_{20}H_{32}O$, which was supported by the ^{13}C NMR data (Table 2). The ^{13}C NMR displayed 20 carbon signals, which were identified by the assistance of the DEPT spectrum as three methyls, nine methylenes, four methines, and four quaternary carbons. The ^{1}H NMR signal (Table 1) δ_H 3.90 (br d, $J = 10.0$ Hz, H-4) and an IR absorption at 3417 cm^{-1}, together with the observation of one oxygen-bearing carbon resonance δ_C 76.3 (CH, C-4) in the ^{13}C NMR spectrum, revealed the presence of a hydroxy group. Taking into account the five degrees of unsaturation inferred from the molecular formula and the four degrees of unsaturation corresponding to two trisubstituted double bonds, δ_C 125.3 (CH, C-8), 134.0 (C, C-9), 124.3 (CH, C-14), and 131.6 (C, C-15), and two 1,1-disubstituted double bonds, δ_C 108.0 (CH_2, C-18), 115.5 (CH_2, C-19), 153.4 (C, C-11), and 149.2 (C, C-5), the planar structure of **3** possesses a 10-membered monocarbocyclic ring.

From the COSY and HMBC correlations (Figure 1) as well as comparison of the NMR data (Tables 1 and 2) of **3** with those reported for a known diterpene, which was isolated from the aerial parts and the roots of *Helichrysum argyrophyllum*,[16] the structure of **3** was similar to the reported compound except for the absence of a trisubstituted double bond at C-5, the presence of 1,1-disubstituted double bond at C-5, and a hydroxy group at C-4. The NOESY correlations (Figure 5) observed between H-4/H-3b (δ_H 1.73) and H-4/H-19b (δ_H 5.09) indicated the β-orientation for the hydroxy group at C-4. Moreover, the NOESY correlations observed between H-7b (δ_H 2.18)/H-8, H-8/H-10b (δ_H 1.97), H-10b/H-2, and H_3-20/H-10a (δ_H 2.13) suggested the 2*R** configuration. However, the absolute configuration at C-4 of **3** remains to be determined by application of the modified Mosher's method because of the scarcity of material. In the present work the absolute configuration shown in **3** has been asssigned to the *R* configuration at C-2 because of a biosynthetic relationship to **1** and **2**. Therefore, the structure of gyrosanol C (**3**) was elucidated.

Preliminary cytotoxic screening revealed that compounds **1**−**3** were not cytotoxic against A-549 (human lung carcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cells. Compounds **1** and **2** showed antiviral activity against HCMV with IC_{50} values of 2.6 and 3.7 μM. The antibacterial assay showed that **1**−**3** (100 μg/disk) exhibited no discernible activities against *Enterobacter aerogenes* (ATCC13048), *Salmonella enteritidis* (ATCC13076), *Serratia marcescens* (ATCC25419), *Shigella sonnei* (ATCC11060), and *Yersinia enterocolitica* (ATCC23715). The primary anti-inflammatory activity of **1** and **2** (10 μM) was observed by suppression of COX-2 protein expression (16.9 ± 3.9% and 29.1 ± 9.6%), with no discernible activity against iNOS protein expression (118.5 ± 12.6% and 171.7 ± 15.1%) in RAW 264.7

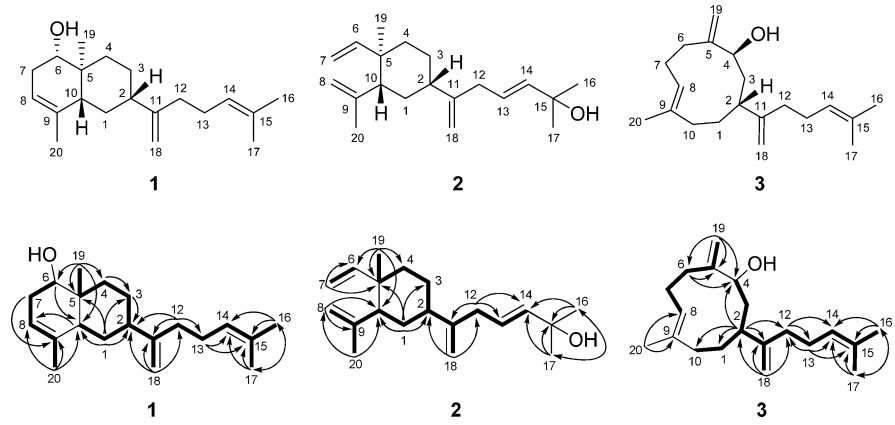

Figure 1. Selected ^{1}H−^{1}H COSY (−) and HMBC (→) correlations of **1**−**3**.

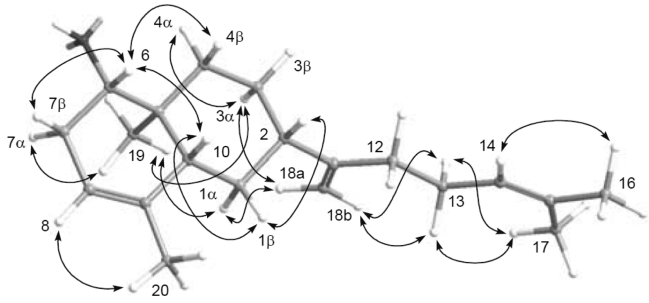

Figure 2. Key NOESY correlations for **1**.

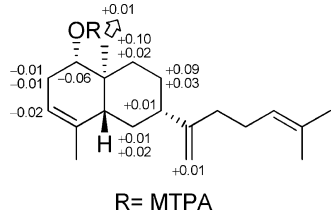

Figure 3. Selected ^{1}H NMR $\Delta\delta_{S-R}$ values in ppm for the (*S*)- and (*R*)-MTPA esters of **1** in $CDCl_3$.

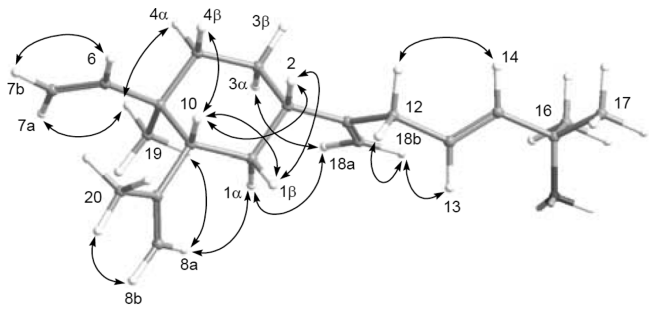

Figure 4. Key NOESY correlations for **2**.

macrophages. The above findings are being employed to make our continuing drug discovery efforts more effective.

Gyrosanol A (**1**) belongs to the prenyleudesmane class of diterpenes. It is worthwhile to mention that the 2*S*,5*S*,10*R* configuration of **1** is distinct from the 2*R*,5*S*,10*S* configuration of dysokusone A, which were obtained from the plant *Dysoxylum kuskusense*.[13a] Gyrosanol A is also closely related to the deoxygenation product of another soft coral metabolite, 2′-[(*R**,4a′*S**,8a′*R**)-4a′,8′-dimethyl-1′,2′,3′,4′,4a′,5′,6′,8a′-octahydronaphthelen-2′-yl]-5,6-epoxy-6-methylhept-1-ene isolated from a *Nephthea* sp. in 1982.[13b] Compound **2** is a lobane-type diterpenoid related to the fuscol class, while **3** belongs to the dilophol class. Fuscol and

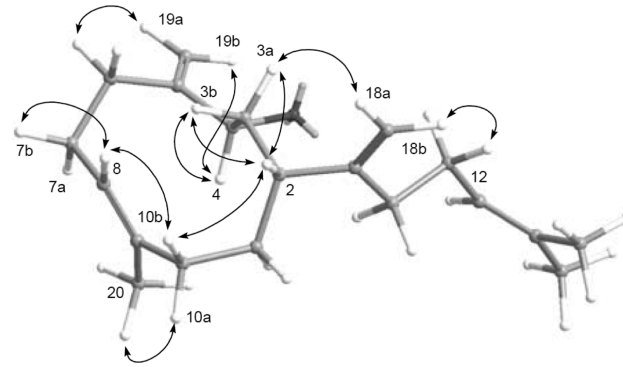

Figure 5. Key NOESY correlations for **3**.

its analogues have previously been identified in the gorgonian *E. fusca*[5a,6] and the soft corals of the genera *Lobophytum* and *Sinularia*.[7−11] Dilophol derivatives were previously isolated from marine organisms, including several brown marine algae of the family Dictyotacea,[12,14,15] and gorgonian species of the genus *Eunicea*.[16,17] Moreover, we have proposed a plausible biosynthetic pathway for the formation of these compounds (see Supporting Information). Gyrosanols A−C (**1**−**3**) are isomers presumed to originate from **3** through double-bond migration, cyclization, Cope rearrangement, and oxidation processes by the soft coral.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO P1020 polarimeter. IR spectra were measured on a JASCO FT/IR-4100 spectrophotometer. The NMR spectra were recorded on a Varian 400 MR NMR spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively. Chemical shifts are expressed in δ (ppm) referring to the solvent peaks δ_H 7.15 and δ_C 128.5 for C_6D_6 and δ_H 7.27 and δ_C 77.0 for $CDCl_3$, respectively, and coupling constants are expressed in Hz. ESIMS spectra were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography, and precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis. C_{18} reversed-phase silica gel (230−400 mesh, Merck) was also used for column chromatography. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 220 nm and an ODS column (Merck, Hibar Purospher RP-18e, 5 μm, 250 × 10 mm). *S*-(+)- and *R*-(−)-α-Methoxy-α-trifluoromethyl phenylacetyl chloride were obtained from ACROS Organics.

Animal Material. The soft coral *Sinularia gyrosa* was collected by hand using scuba at the Dongsha Atoll off Taiwan in April 2007, at a depth of 8−10 m, and was stored in a freezer for two months until extraction. This soft coral was identified by one of the authors (C.-F.D.). A voucher specimen (TS-19) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation. The frozen specimen of *S. gyrosa* (2.0 kg) was chopped into small pieces and extracted with fresh 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. The combined acetone extracts were concentrated to a brown gum, which was partitioned between H_2O and EtOAc. The resulting EtOAc partition (30.0 g) was subjected to column chromatography on silica gel using *n*-hexane−EtOAc and EtOAc−MeOH mixtures of increasing polarity for elution to furnish 40 fractions. Fraction 16 (0.6 g), eluted with *n*-hexane−EtOAc (1:2), was further subjected to RP-18 gravity column chromatography by eluting with 80% MeOH in H_2O, 90% MeOH in H_2O, and 100% MeOH, respectively. Altogether, 12 subfractions were obtained, of which subfraction 5 (88 mg) was further purified by RP-18 HPLC (90% MeOH in H_2O, flow rate 5.0 mL/min) to afford **2** (3 mg) and a mixture. In turn, the mixture was further separated by silica gel column chromatography using *n*-hexane−EtOAc (20:1) to give **1** (2 mg) and **3** (1 mg), respectively.

Gyrosanol A (1): colorless oil; $[\alpha]^{25}_D$ −70 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3433, 3068, 2924, 2859, 1639, 1441, 1376, 1343, 1091, 1012, 886, 803 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 311 $[M + Na]^+$; HRESIMS *m/z* 311.2353 $[M + Na]^+$ (calcd for $C_{20}H_{32}ONa$, 311.2351).

Gyrosanol B (2): colorless oil; $[\alpha]^{25}_D$ +18 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3398, 3079, 2965, 2928, 1639, 1456, 1373, 1259, 1095, 1034, 889, 799 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 311 $[M + Na]^+$; HRESIMS *m/z* 311.2352 $[M + Na]^+$ (calcd for $C_{20}H_{32}ONa$, 311.2351).

Gyrosanol C (3): colorless oil; $[\alpha]^{25}_D$ −11 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3417, 2917, 1575, 1558, 1541, 1456, 1417, 1375, 1110, 874 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 311 $[M + Na]^+$; HRESIMS *m/z* 311.2352 $[M + Na]^+$ (calcd for $C_{20}H_{32}ONa$, 311.2351).

Preparation of (*R*)- and (*S*)-MTPA Esters of 1. Duplicate (0.5 mg) samples of **1** were prepared for both (*R*)- and (*S*)-MTPA chloride acylation reactions. In separate vials, the samples 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 layers were combined, dried over anhydrous $MgSO_4$, and evaporated. The residue resulting from (*R*)-MTPA chloride was subjected to a short silica gel column eluting with *n*-hexane−EtOAc (10:1) to yield the (*S*)-MTPA ester (**1a**, 0.5 mg). The (*R*)-MTPA ester (**1b**, 0.5 mg) was prepared with (*S*)-MTPA chloride according to the same procedure. Selected ^1H NMR ($CDCl_3$, 400 MHz) data of **1a**: δ_H 7.39−7.52 (5H, m, Ph), 5.27 (1H, br s, H-8), 5.13 (1H, t, J = 6.4 Hz, H-14), 4.95 (1H, dd, J = 9.6, 6.4 Hz, H-6), 4.79 (1H, s, H-18a), 4.75 (1H, d, J = 1.2 Hz, H-18b), 3.53 (3H, s, OCH_3), 2.54 (1H, m, H-7β), 2.12 (1H, m, H-2), 2.07 (1H, m, H-10), 2.03 (1H, m, H-7α), 1.93 (1H, m, H-4α), 1.81 (1H, m, H-1β), 1.71 (1H, m, H-3β), 1.70 (3H, s, Me-16), 1.62 (3H, s, Me-17), 1.56 (3H, s, Me-20), 1.43 (1H, dd, J = 12.8, 3.6 Hz, H-3α), 1.24 (1H, d, J = 12.8 Hz, H-1α), 1.19 (1H, dd, J = 12.8, 4.0 Hz, H-4β), 0.85 (3H, s, Me-19). Selected ^1H NMR ($CDCl_3$, 400 MHz) data of **1b**: δ_H 7.39−7.55 (5H, m, Ph), 5.29 (1H, br s, H-8), 5.13 (1H, t, J = 6.4 Hz, H-14), 5.01 (1H, dd, J = 9.6, 6.4 Hz, H-6), 4.78 (1H, s, H-18a), 4.75 (1H, br s, H-18b), 3.56 (3H, s, OCH_3), 2.55 (1H, m, H-7β), 2.11 (1H, m, H-2), 2.07 (1H, m, H-10), 2.04 (1H, m, H-7α), 1.91 (1H, m, H-4α), 1.80 (1H, m, H-1β), 1.70 (3H, s, Me-16), 1.62 (1H, m, H-3β), 1.62 (3H, s, Me-17), 1.56 (3H, s, Me-20), 1.40 (1H, dd, J = 12.8, 3.6 Hz, H-3α), 1.22 (1H, d, J = 12.8 Hz, H-1α), 1.09 (1H, dd, J = 12.8, 4.0 Hz, H-4β), 0.84 (3H, s, Me-19).

Biological Assays. The in vitro cytotoxicity, anti-inflammatory activity, HCMV activity, and antibacterial assays were carried out according to the procedures described previously.[18−20]

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Supporting Information Available: A plausible biosynthetic pathway and relationship for **1**−**3** and the ^1H and ^{13}C NMR spectra for **1**−**3** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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