Note

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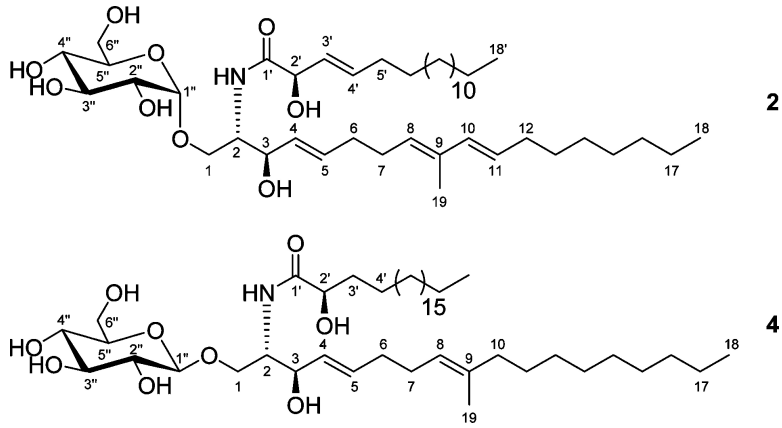
J. Nat. Prod., **2009**, 72 (3), 465-468• DOI: 10.1021/np800362g • Publication Date (Web): 12 November 2008



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Notes

Ceramide and Cerebrosides from the Octocoral *Sarcophyton ehrenbergi*[#]

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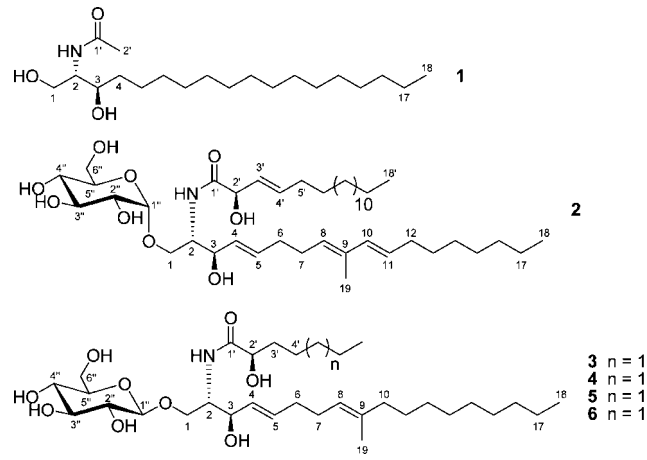
Received June 17, 2008

Chemical investigation of the octocoral *Sarcophyton ehrenbergi*, collected at the Dongsha Islands, Taiwan, has led to the isolation of a known ceramide (**1**) and two new cerebrosides, sarcoehrenosides A (**2**) and B (**4**), along with three known cerebrosides (**3**, **5**, and **6**). The structures of the new compounds were established by spectroscopic and chemical methods. Sarcoehrenoside A (**2**) differs from previously known marine cerebrosides in that it possesses a rare α-glucose moiety. Compounds **1**−**6** were evaluated for antimicrobial activity against a small panel of bacteria and for anti-inflammatory activity using RAW 264.7 macrophages.

Cerebrosides are a large group of molecules containing a ceramide unit and one or more sugars. The hydrophobic ceramide moiety includes a sphingoid base and an amide-linked fatty acid chain. Cerebroside derivatives have been isolated from various marine invertebrates, such as sea stars,[1] soft corals,[2] sponges,[2a,3] sea anemones,[4] and ascidians.[5] In recent years several different types of biological activities have been found for these compounds, including antifungal, antitumor, antiviral, cytotoxic, and immunomodulatory properties.[6]

In the course of a search for bioactive substances from marine sources,[7] chromatographic separation of an alcyonacean soft coral, *Sarcophyton ehrenbergi* Marenzeller (Octocorallia: Alcyonacea), collected at the Dongsha Islands, Taiwan, has afforded two new cerebrosides, sarcoehrenosides A (**2**) and B (**4**). Also obtained were a known ceramide (**1**)[8] and three known cerebrosides (**3**, **5**, and **6**),[3a,9,10] which were isolated from *S. ehrenbergi* for the first time. To our knowledge, sarcoehrenoside A (**2**), in possessing a rare α-D-glucose moiety at C-1 of the glycerol ether unit, differs from previous cerebrosides isolated from marine organisms. In this paper, we describe the isolation, structural elucidation, and evaluation of the antimicrobial and anti-inflammatory activity of these metabolites.

Sarcoehrenoside A (**2**) was isolated as a white, amorphous powder and exhibited a $[M + Na]^+$ peak at *m/z* 774.5490, corresponding to a molecular formula of $C_{43}H_{77}NO_9$ in the positive HRESIMS. The IR absorptions at 1638 and 1547 cm^{-1} and ^{13}C NMR signals at δ 54.6 (C-2) and 175.4 (C-1′) suggested a secondary amide in the molecule. In the ^1H and ^{13}C NMR spectra of **2**, the characteristic signals of an amide linkage (a nitrogenated methane proton at δ 3.93 and an amide carbonyl carbon at δ 175.4), a long chain (terminal methyl protons at δ 0.90 and methylene protons at δ 1.29−1.38), and a sugar (an anomeric proton at δ 4.87) were observed, strongly suggesting the glycosphingolipid nature of **2**. The α-glucopyranose moiety was indicated by the anomeric proton at δ 4.87 (1H, d, J = 4.5 Hz, H-1″) and the chemical shifts in the ^1H and ^{13}C NMR spectra (Table 1). The anomeric proton of the α-glucose unit exhibited a long-range $^1H-^{13}C$ correlation with C-1 (Figure 1), demonstrating attachment of the glucose moiety at C-1. The relative configuration of the α-glucopyranose moiety was determined by a NOESY experiment (Figure 2) and from the ^1H NMR J values. In the ^1H NMR spectrum, a small coupling constant (J = 4.5 Hz)[11] was observed for H-1″, which required an equatorial-axial relationship between the anomeric proton (H-1″) and H-2″. The large coupling constants (J = 7.5 Hz) between H-2″ and H-3″, H-3″ and H-4″, and H-4″ and H-5″ as well as the NOESY correlations of H-2″/H-1″, H-4″ and H-3″/H-5″ suggested that H-2″, H-3″, H-4″, and H-5″ are all axial. Thus, the relative configuration of the α-glucose moiety at the C-1 glycerol ether could be established. Methanolysis of **2** gave a mixture of α- and β-methyl glucopyranoside. The specific rotation of the methyl glucoside mixture, $[\alpha]^{24}_D$ +74.3 (*c* 0.2, MeOH), was close to that of an



[#] Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work on bioactive natural products.

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10.1021/np800362g CCC: $40.75
Published on Web 11/12/2008

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **2** and **4**[a]

	2			**4**		
C/H	^{13}C		^{1}H	^{13}C		^{1}H
	lipid base unit			lipid base unit		
1	68.8 t[b]	a	3.88 dd (10.5, 4.5)[c]	69.9 t[b]	a	4.12 dd (10.5, 3.5)[c]
		b	3.83 dd (10.5, 4.5)		b	3.71 dd (10.5, 3.5)
2	54.6 d		3.93 dd (8.0, 4.5)	54.7 d		3.99 dd (7.5, 3.5)
3	72.9 d		4.14 t (8.0)	73.0 d		4.13 t (7.5)
4	131.6 d		5.47 dd (15.5, 8.0)	131.3 d		5.48 dd (15.5, 7.5)
5	134.3 d		5.72 dd (15.5, 6.5)	134.8 d		5.74 dd (15.5, 6.0)
6	33.7 t		2.08 td (7.5, 6.5)	34.0 t		2.06 m
7	29.0 t		2.20 td (7.5, 7.0)	28.8 t		2.07 m
8	130.6 d		5.35 t (7.0)	125.0 d		5.14 t (6.5)
9	135.3 s			136.9 s		
10	136.3 d		6.02 d (15.5)	40.9 t		1.97 t (7.5)
11	128.7 d		5.55 dt (15.5, 7.0)	29.3 t		1.39 m
12	34.1 t		2.08 td (7.5, 7.0)			
13	30.4 t		1.38 m			
12−15				30.5−30.9 t		1.29 m
14, 15	30.4−31.1 t		1.29 m			
16	33.2 t		1.29 m	33.2 t		1.29 m
17	23.9 t		1.29 m	23.9 t		1.29 m
18	14.6 q		0.90 t (6.0)	14.6 q		0.90 t (6.5)
19	12.9 q		1.71 s	16.3 q		1.59 s
	N-acyl unit			*N*-acyl unit		
1′	175.4 s			177.4 s		
2′	74.3 d		4.45 d (6.0)	73.2 d		3.99 dd (8.0, 4.0)
3′	129.1 d		5.50 dd (15.5, 6.0)	36.0 t		1.70 m; 1.55 m
4′	135.0 d		5.83 dd (15.5, 6.5)	26.3 t		1.41 m
5′	33.6 t		2.03 td (7.5, 6.5)			
5′−19′				30.5−30.9 t		1.29 m
6′	30.5 t		1.38 m			
7′−15′	30.4−31.1 t		1.29 m			
16′	33.2 t		1.29 m			
17′	23.9 t		1.29 m			
18′	14.6 q		0.90 t (6.0)			
20′				23.9 t		1.29 m
21′				14.6 q		0.90 t (6.5)
22′						
	α-D-glucose unit			β-D-glucose unit		
1″	103.6 d		4.87 d (4.5)	104.9 d		4.27 d (8.0)
2″	79.1 d		3.96 dd (7.5, 4.5)	75.1 d		3.19 dd (9.5, 8.0)
3″	76.3 d		4.10 t (7.5)	78.1 d		3.34 m
4″	74.4 d		3.63 m	71.7 d		3.30 m
5″	83.6 d		3.69 m	78.1 d		3.28 m
6″	64.3 t	a	3.63 m	62.8 t	a	3.87 dd (12.0, 1.5)
		b	3.56 m		b	3.67 dd (12.0, 5.0)

[a] Spectra were measured in CD_3OD (^{1}H, 500 MHz; ^{13}C, 125 MHz). [b] Multiplicities were deduced by HSQC and DEPT experiments. [c] *J* values (in Hz) are in parentheses.

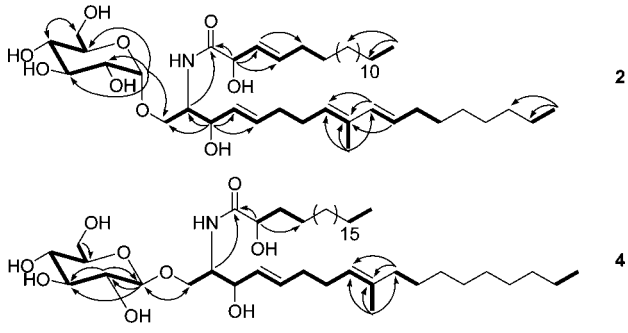

Figure 1. Key $^{1}H-^{1}H$ COSY (—) and HMBC (→) correlations of **2** and **4**.

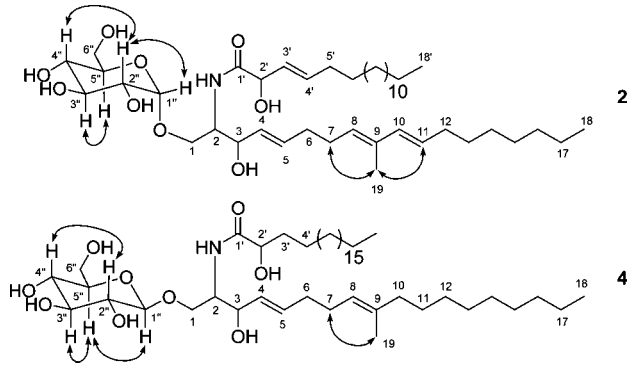

Figure 2. Selected NOESY correlations of **2** and **4**.

authentic sample ($[\alpha]^{25}_{D}$ +77.3).[1f] Therefore, the absolute configuration of the α-glucose moiety was deduced to be the D-isomer.

The large vicinal coupling constants ($J = 15.5$ Hz) between H-4 and H-5, H-10 and H-11, and H-3′ and H-4′ clearly indicated *E* geometry for $\Delta^{4,10,3'}$ in **2**. The key HMBC correlations from H-3 to C-4, from H-2′ to C-3′, and from H_3-19 to C-8, C-9, C-10, and C-11 helped locate the positions of the double bonds (Figure 1). The UV absorption maximum at 224 nm indicated the presence of a conjugated diene moiety.[1f] Furthermore, the stereochemistry of the conjugated diene moiety was assigned as *s-trans* from NOE correlations (Figure 2) from Me-19 to H-7 and H-11. The $^{1}H-^{1}H$ COSY correlations observed between H-2 and both H-1 and H-3 were used to define the 2-amino-1,3-dioxygenated fragment. The relative configurations of C-2 (δ 54.6) and C-3 (δ 72.9) were predicted to be the D-*erythro* stereochemistry at C-2 and C-3, which

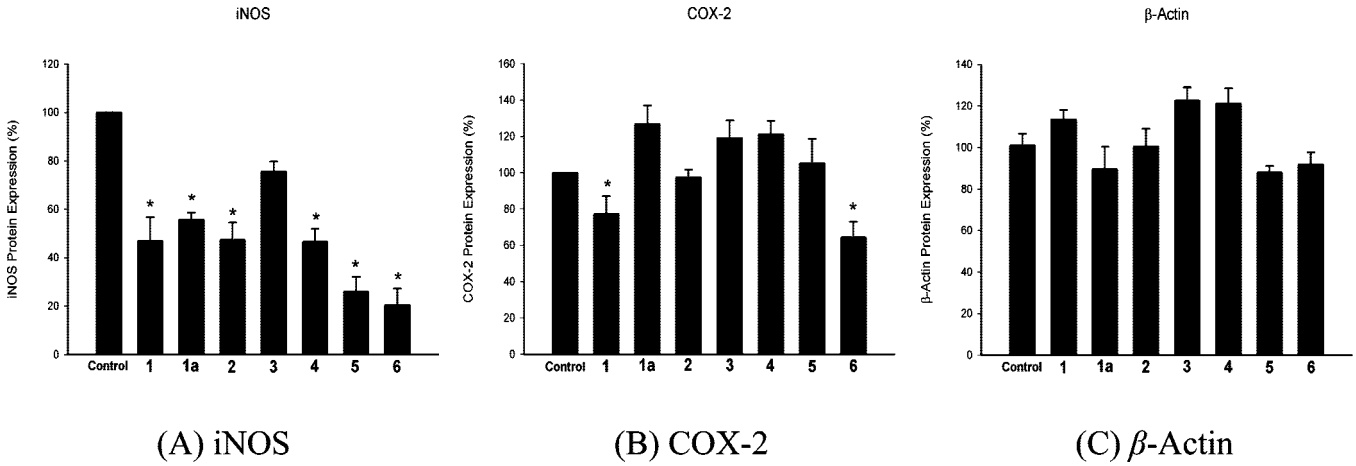

Figure 3. Effect of compounds **1**−**6** at 10 μM on the LPS-induced pro-inflammatory iNOS and on COX-2 protein expression of RAW 264.7 macrophage cells by immunoblot analysis. (A) Immunoblot of iNOS. (B) Immunoblot of COX-2. (C) Immunoblot of β-actin. A and B values are mean ± SEM ($n = 5$). The relative intensity of the LPS alone stimulated group was taken as 100%. *Significantly different from LPS-stimulated (control) group (*$P < 0.05$).

was consistent with those reported for other (2*S*,3*R*,2′*R*) sphingosine moieties.[12,13] The stereochemistry of **2** (2*S*,3*R*), as shown in its structure, was confirmed after determination of the stereochemistry of **1** on the basis of biosynthetic reasoning. Methanolysis of **2** yielded methyl (2′*R*,3′*E*)-2′-hydroxyoctadec-3′-enoate (**2a**), for which the molecular formula $C_{19}H_{36}O_3$ was determined by ESIMS-MS (*m*/*z* 335.0 $[M + Na]^+$) as $C_{19}H_{36}O_3$. On the basis of the aforementioned observations, sarcoehrenoside A (**2**) was characterized unambiguously as 1-*O*-(α-D-glucopyranosyl)-(2*S*,3*R*,4*E*,8*E*,10*E*)-2-[(2′*R*,3′*E*)-2′-hydroxyoctadec-3′-enoylamino]-9-methyloctadeca-4,8,10-triene-1,3-diol.

Sarcoehrenoside B (**4**) was found to have a molecular formula of $C_{46}H_{87}NO_9$, as indicated by HRESIMS. In addition to the pseudomolecular ion peak at *m*/*z* 820.6 $[M + Na]^+$, the ESIMS-MS of **4** also exhibited an intense fragment peak at *m*/*z* 496.7, produced by elimination of the *N*-acyl unit from the molecular ion. The spectroscopic data for **4** (Table 1) exhibited the presence of an amide linkage, a long chain, and a sugar, consistent with the C-9 methyl cerebroside nature of **4**. In the ^{1}H and ^{13}C NMR spectra of **4**, an anomeric proton appeared at δ 4.27 (1H, d, $J = 8.0$ Hz, H-1″) and ^{13}C NMR signals resonated at δ 104.9 (H-1″), 75.1 (H-2″), 78.1 (H-3″), 71.7 (H-4″), 78.1 (H-5″), and 62.8 (H-6″), supporting the presence of a β-glucopyranose moiety.[3b] Methanolysis of **4** also yielded an α- and β-glucopyranosyl mixture, the specific rotation ($[\alpha]^{24}_D$ +71.4, MeOH) of which was used to identify β-glucose as a D-isomer. The glycerol ether linkage between the anomeric proton (δ 4.27, H-1″) and C-1 was confirmed by HMBC correlations (Figure 1) of H-1″ with C-1 and H-1 with C-1″. The coupling constant (15.5 Hz) indicated an *E* geometry for Δ^4. A NOE correlation from Me-19 to H-7 supported the presence of an (*E*)-8,9 double bond. Therefore, **3** was defined as a C-9 methyl 4*E*,8*E*-sphingadiene-type cerebroside. The connectivity between the acyl moiety and the NH of the sphingosine base was confirmed by HMBC correlations of H-2 with C-1′ and H-2′ with C-1′. Methanolysis of **4** yielded methyl (2′*R*)-2′-hydroxyhenicosanoate (**4a**), for which the molecular formula, $C_{22}H_{44}O_3$, was identified by ESIMS-MS analysis (*m*/*z* 379.3 $[M + Na]^+$). The specific rotation value ($[\alpha]^{24}_D$ −3.8) of **4a** indicated a 2′*R* absolute configuration in **4**.[1f] The relative configuration at C-2 and C-3 was determined as 2*S*,3*R* (erythro) based on their ^{13}C NMR chemical shifts and the specific rotation of this compound.[1f] Consequently, sarcoehrenoside B (**4**) was fully assigned as 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*,4*E*,8*E*)-2-[(2′*R*)-2′-hydroxyhenicosanoylamino]-9-methyl-4,8-octadecadiene-1,3-diol.

Compounds **1**−**6** were tested against five bacterial strains, comprising *Enterobacter aerogenes* (ATCC13048), *Serratia marcescens* (ATCC25419), *Salmonella enteritidis* (ATCC13076), *Yersinia enterocolitica* (ATCC23715), and *Shigella sonnei* (ATCC11060). None of these compounds exhibited any antibacterial activity at a concentration of 100 μg/disk.

A previous study has reported that glycosphingolipids and sphingolipids possess cyclooxygenase-2 inhibition,[14] which prompted us to evaluate the anti-inflammatory effect of our isolated metabolites. As shown in Figure 3, the in vitro anti-inflammatory activity of compounds **1**−**6** was tested using LPS-stimulated cells. Stimulation of RAW 264.7 cells with LPS resulted in up-regulation of the pro-inflammatory iNOS and COX-2 proteins. Both compounds **1** and **6** reduced the levels of iNOS to 46.9 ± 9.7% and 20.3 ± 6.8%, respectively, and of COX-2 to 77.2 ± 9.9% and 64.3 ± 8.6%, respectively, in comparison with those of the control groups. Compounds **1a**, **2**, **4**, and **5** reduced iNOS protein expression (55.6 ± 2.9%, 47.3 ± 7.1%, 46.5 ± 5.3%, and 25.8 ± 6.2%, respectively), but did not inhibit COX-2 protein expression. All compounds did not affect β-actin protein expression at a 10 μM concentration. Under the same experimental conditions, 10 μM CAPE (caffeic acid phenylthyl ester) reduced the levels of iNOS and COX-2 protein to 1.5 ± 2.1% and 70.2 ± 11.5%, respectively, relative to the control cells stimulated with LPS.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P1020 polarimeter. UV spectra were obtained on a Hitachi U-3210 spectrophotometer, and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. The NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C, or on a Varian MR 400 NMR spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C, or on a Varian Unity INOVA 500 FT-NMR spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are expressed in δ (ppm) referring to the solvent peaks δ_H 3.30 and δ_C 49.0 for CD_3OD and δ_H 7.265 and δ_C 77.0 for $CDCl_3$, and coupling constants are expressed in Hz. Electrospray ionization MS/MS analysis was recorded using a positive-mode API 4000 tandem mass spectrometer, with a capillary voltage of 5500 V applied, and the spectra were acquired at *m*/*z* 400−1000 for parent ion spectra and *m*/*z* 50−1000 for fragment ion spectra. ESIMS 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; 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-7420 UV detector L-7100 pump apparatus equipped with a Merck Hibar RP-18e column (250 × 10 mm, 5 μm).

Animal Material. The octocoral *S. ehrenbergi* was collected by hand using scuba at Dongsha Islands, Taiwan, in April 2007, at a depth of 10 m, and was stored in a freezer for 5 weeks until extraction. This soft coral was identified by one of the authors (C.-F.D.). A voucher specimen (TS-07) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation. Soft coral extracts were prepared by percolating aliquots of 50 g of freeze-dried *S. ehrenbergi* with acetone for 24 h at room temperature. The combined acetone extracts were concentrated to a brown gum, which was partitioned between H_2O and EtOAc. The dried EtOAc-soluble (20.0 g) partition was chromatographed over a silica column using *n*-hexane, *n*-hexane−EtOAc, and EtOAc−MeOH mixtures of increasing polarity to obtain fractions 1−40. Fraction 28 (0.5 g), eluted with EtOAc−MeOH (1:1), 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. Altogether, six fractions were obtained, of which fraction 1 (60 mg) was purified further by RP-18 HPLC column chromatography (5% CH_3CN in MeOH, flow rate 5.0 mL/min) to afford **1** (3 mg), **2** (2 mg), **3** (2 mg), **4** (3 mg), **5** (2 mg), and **6** (6 mg). The retention time for each metabolite was as follows: **1** (26.5 min), **2** (35.6 min), **3** (48.6 min), **4** (44.2 min), **5** (41.0 min), and **6** (33.5 min).

Sarcoehrenoside A (2): white, amorphous powder; $[\alpha]^{23}_D$ +77.0 (*c* 0.2, MeOH); IR (KBr) 3387, 2951, 2859, 1638, 1547, 1456, 1387, 1241, 1131, 1035, 737 cm^{-1}; UV λ_{max} (MeOH) (log ε) 224 (3.97) nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 774.5490 $[M + Na]^+$ (calcd for $C_{43}H_{77}NO_9Na$, 744.5496).

Sarcoehrenoside B (4): white, amorphous powder; $[\alpha]^{23}_D$ +51.3 (*c* 0.3, MeOH); IR (KBr) 3396, 2951, 2857, 1638, 1546, 1457, 1385, 1242, 1178, 1131, 1036, 736 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 820.6274 $[M + Na]^+$ (calcd for $C_{46}H_{87}NO_9Na$, 820.6278).

Methanolysis of 2 and 4. An aliquot (1 mg) of **2** and **4** in a mixture of 1 N aqueous HCl (0.1 mL) and methanol (0.9 mL) was refluxed for 18 h on a magnetic stirrer. The reaction mixture was neutralized with $NaHCO_3$ and diluted with H_2O (1.5 mL). The aqueous solution was extracted with *n*-hexane three times, and the organic phase was dried with anhydrous $MgSO_4$. After removal of solvent and purification by silica gel column chromatography, methyl (2′*R*,3′*E*)-2′-hydroxyoctadec-3′-enoate (**2a**, 0.3 mg) and methyl (2′*R*)-2′-hydroxyhenicosanoate (**4a**, 0.4 mg) were obtained and identified by ESIMS-MS peaks at *m/z* 335.0 and 379.3 $[M + Na]^+$, respectively. In addition, the aqueous layer was removed and purified on a C_{18} reversed-phase column, eluted with MeOH−H_2O (4:1), to give **2b** [0.2 mg, $[\alpha]^{24}_D$ +74.3 (*c* 0.2, MeOH)] and **4b** [0.2 mg, $[\alpha]^{24}_D$ + 72.5 (*c* 0.2, MeOH)].

Methyl (2′*R*,3′*E*)-2′-hydroxyoctadec-3′-enoate (2a): white, amorphous powder; $[\alpha]^{24}_D$ −17.5 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.90 (1H, dt, J = 15.6, 6.4 Hz, H-4′), 5.50 (1H, dd, J = 15.6, 6.4 Hz, H-3′), 4.61 (1H, d, J = 6.4 Hz, H-2′), 3.80 (3H, s, $COOCH_3$), 2.07 (2H, td, J = 7.6, 6.4 Hz, H-5′), 1.39 (2H, m, H-6′), 1.26 (22H, brs, H-7′-H-17′), 0.88 (3H, t, J = 6.4 Hz, H-18′); ESIMS-MS *m/z* 335.0 $[M + Na]^+$.

Methyl (2′*R*)-2′-hydroxyhenicosanoate (4a): white, amorphous powder; $[\alpha]^{24}_D$ −3.8 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.20 (1H, dd, J = 7.6, 4.4 Hz, H-2′), 3.79 (3H, s, $COOCH_3$), 1.76 (1H, m, H-3a′), 1.58 (1H, m, H-3b′), 1.45 (2H, m, H-4′), 1.26 (32H, brs, H-5′-H-20′), 0.88 (3H, t, J = 6.4 Hz, H-21′); ESIMS-MS *m/z* 379.3 $[M + Na]^+$.

In Vitro Antimicrobial Activity. Bacterial strains were grown in LB (Luria−Bertani) broth medium for 24 h at 37 °C. Then, 17 mL of LB hard agar (1.5% agar) was poured into sterile Petri dishes (9 cm) and allowed to set. Next, 2.7 mL of molten LB soft agar (0.7% agar, 45 °C) was inoculated with 0.3 mL of broth culture of the test organism and poured over the base hard agar plates, forming a homogeneous top layer. Sterile paper disks (Advantec, 8 mm) were placed onto the top layer of the LB agar plates. Ten microliters (2 μg/μL) of the tested compounds was applied onto each filter paper disk. Ampicillin (5 μg/μL) and the same solvents served as positive and negative controls. All plates were incubated at 37 °C, 24 h prior to antibacterial activity evaluation. The antimicrobial activity of compounds **1**−**6** was tested up to 100 μg/mL against *E. aerogenes* (ATCC13048), *S. marcescens* (ATCC25419), *S. enteritidis* (ATCC13076), *Y. enterocolitica* (ATCC23715), and *S. sonnei* (ATCC11060). All bacterial strains were obtained from the American Type Culture Collection. The antibiotic activity evaluation method was conducted on the basis of previous reports.[15]

In Vitro Anti-inflammatory Assay. The anti-inflammatory assay was modified from Ho et al.[16] and Park et al.[17] Murine RAW 264.7 macrophages were obtained from the American Type Culture Collection (ATCC, No. TIB-71). The cells were activated by incubation in medium containing *Escherichia coli* LPS (0.01 μg/mL; Sigma) for 16 h in the presence or absence of various compounds. Then, cells were washed with ice-cold PBS, lysed in ice cold lysis buffer, and centrifuged at 20000*g* for 30 min at 4 °C. The supernatant was decanted from the pellet and retained for Western blot analysis. Protein concentrations were determined by the DC protein assay kit (Bio-Rad) modified by the method of Lowry et al.[18] Samples containing equal quantities of protein were subjected to SDS-polyacrylamide gel electrophoresis, and the separated proteins were electrophoretically transferred to polyvinylidene difluoride membranes (PVDF; Immobilon-P, Millipore, 0.45 μm pore size). The resultant PVDF membranes were incubated with blocking solution and incubated for 180 min with antibody against inducible nitric oxide synthase (iNOS; 1:1000 dilution; Transduction Laboratories) and cyclooxygenase-2 (COX-2; 1:1000 dilution; Cayman Chemical) protein. The blots were detected using ECL detection reagents (Perkin-Elmer, Western Blot Chemiluminescence Reagent Plus) according to the manufacturer's instructions.

Acknowledgment. Financial support was provided by Ministry of Education (96C031703) and National Science Council of Taiwan (NSC96-2320-B-110-003-MY3) awarded to C.Y.D.

References and Notes

(1) (a) Kawano, Y.; Higuchi, R.; Isobe, R.; Komori, T. *Liebigs Ann. Chem.* **1988**, *1*, 19–24. (b) Kawano, Y.; Higuchi, R.; Isobe, R.; Komori, T. *Liebigs Ann. Chem.* **1988**, *12*, 1181–1183. (c) Kawano, Y.; Higuchi, R.; Komori, T. *Liebigs Ann. Chem.* **1990**, *1*, 43–50. (d) Higuchi, R.; Natori, T.; Komori, T. *Liebigs Ann. Chem.* **1990**, *1*, 51–55. (e) Higuchi, R.; Kagoshima, M.; Komori, T. *Liebigs Ann. Chem.* **1990**, *7*, 659–663. (f) Jin, W.; Rinehart, K. L.; Jares-Erijman, E. A. *J. Org. Chem.* **1994**, *59*, 144–147.

(2) (a) Hirsch, S.; Kashman, Y. *Tetrahedron* **1989**, *45*, 3897–3906. (b) Muralidhar, P.; Kumar, M. M.; Krishna, N.; Rao, C. B.; Rao, D. V. *Chem. Pharm. Bull.* **2005**, *53*, 168–171.

(3) (a) Zhang, G.-W.; Ma, X.-Q.; Zhang, C.-X.; Su, J.-Y.; Ye, W.-C.; Zhang, X.-Q.; Yao, X.-S.; Zeng, L.-M. *Helv. Chim. Acta* **2005**, *88*, 885–890. (b) Mansoor, T. A.; Shinde, P. B.; Luo, X.; Hong, J.; Lee, C.-O.; Sim, C. J.; Son, B. W.; Jung, J. H. *J. Nat. Prod.* **2007**, *70*, 1481–1486.

(4) Chebaane, K.; Guyot, M. *Tetrahedron Lett.* **1986**, *27*, 1495–1496.

(5) (a) Carter, G. T.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 7441–7442. (b) Durán, R.; Zubia, E.; Ortega, M. J.; Naranjo, S.; Salva, J. *Tetrahedron* **1998**, *54*, 14597–14602.

(6) (a) Hannun, Y. A.; Bell, R. M. *Science* **1989**, *243*, 500–507. (b) Springer, T. A.; Lasky, L. A. *Nature (London)* **1991**, *349*, 196–197. (c) Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 1532–1568.

(7) (a) Duh, C.-Y.; Hou, R.-S. *J. Nat. Prod.* **1996**, *59*, 595–598. (b) Duh, C.-Y.; Wang, S.-K.; Chung, S.-G.; Chou, G.-C.; Dai, C.-F. *J. Nat. Prod.* **2000**, *63*, 1634–1637.

(8) Su, J.; Li, Y.; Yu, X. *Zhongshan Daxue Xuebao, Ziran Kexue Ban* **1989**, *28*, 33–38.

(9) Batrakov, S. G.; Konova, I. V.; Sheichenko, V. I.; Esipov, S. E.; Galanina, L. A.; Istratova, L. N.; Sergeeva, Y. E. *Phytochemistry* **2004**, *65*, 1239–1246.

(10) Tan, J.-W.; Dong, Z.-J.; Liu, J.-K. *Lipids* **2003**, *38*, 81–84.

(11) Bonar-Law, R. P.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 259–271.

(12) Chen, X.; Wu, Y.-L.; Chen, D. *Tetrahedron Lett.* **2002**, *43*, 3529–3532.

(13) (a) Azuma, H.; Takao, R.; Niiro, H.; Shikata, K.; Tamagaki, S.; Tachibana, T.; Ogino, K. *J. Org. Chem.* **2003**, *68*, 2790–2797. (b) Tsukasa, T.; Tokoro, H.; Kato, D.; Nishiyama, S.; Sugai, T. *Tetrahedron Lett.* **2005**, *46*, 3291–3295.

(14) Kang, S. S.; Kim, J. S.; Son, K. H.; Kim, H. P.; Chang, H. W. *Chem. Pharm. Bull.* **2001**, *49*, 321–323.

(15) (a) Arias, M. E.; Gomez, J. D.; Cudmani, N. M.; Vattuone, M. A.; Isla, M. I. *Life Sci.* **2004**, *75*, 191–202. (b) Hou, L.; Shi, Y.; Zhai, P.; Le, G. *J. Ethnopharmacol.* **2007**, *111*, 227–231.

(16) Ho, F. M.; Lai, C. C.; Huang, L. J.; Kuo, T. C.; Chao, C. M.; Lin, W. W. *Br. J. Pharmacol.* **2004**, *141*, 1037–1047.

(17) Park, E. K.; Shin, Y. W.; Lee, H. U.; Kim, S. S.; Lee, Y. C.; Lee, B. Y.; Kim, D. H. *Biol. Pharm. Bull.* **2005**, *28*, 652–656.

(18) Lowry, D. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265–275.

NP800362G