Cerebrosides and Tocopherol Trimers from the Seeds of *Euryale ferox*

Lie-Ching Row,[†] Jiau-Ching Ho,*[,‡] and Chiu-Ming Chen[§]

Fiber Research Division, Industrial Technology Research Institute, Hsinchu, Taiwan, 310, Republic of China, Department of Chemical and Materials Engineering, Ta Hwa Institute of Technology, Chiung-Lin, Hsin-Chu, Taiwan, 307, Republic of China, and Department of Chemistry, National Tsing Hua University, Hsin-Chu, Taiwan, 300, Republic of China

Received March 5, 2007

Two new cerebrosides, ferocerebrosides A (**1**) [(2*S*,3*R*,4*E*,8*E*,2′*R*)-1-*O*-(*β*-glucopyranosyl)-*N*-(2′-hydroxydocosanoyl)-4,8-sphingadienine] and B (**2**) [(2*S*,3*R*,4*E*,8*E*,2′*R*)-1-*O*-(*β*-glucopyranosyl)-*N*-(2′-hydroxytetracosanoyl)-4,8-sphingadienine], two new tocopherol trimers, ferotocotrimers C (**5**) and D (**6**), and two known tocopherol trimers, IVb (**3**) and IVa (**4**), were isolated from the seeds of *Euryale ferox*. Their structures were determined on the basis of spectroscopic data, especially 1D and 2D NMR experiments. Compounds **1** and **2** showed cytotoxicity in the brine shrimp lethality bioassay, with LC_{50} values of 0.17 and 0.20 mM, respectively.

In a search for bioactive constituents of crude drugs derived from aquatic plants, the constituents of *Euryale ferox* Salisb. (Nymphaeaceae) were investigated. This plant has been widely used in traditional oriental medicine to treat a variety of diseases, such as kidney problems, chronic diarrhea, excessive leucorrhea, and hypofunction of the spleen.[1] Recent studies showed that *E. ferox* could reduce myocardial ischemic reperfusion injury[2] and exhibits immunostimulant activity.[3] The seeds of *E. ferox* contain an extraordinarily high content of tocopherols, which may play a role in the antioxidative activity of this plant.[4,5] Glucosylsterols and cerebrosides were isolated from the rhizomes and adventitious roots of *E. ferox*.[6,7]

This study describes the isolation and structural elucidation of two new cerebrosides (**1** and **2**), two known tocopherol trimers (**3** and **4**), and two new tocopherol trimers (**5** and **6**) from the seeds of *E. ferox*. The cytotoxicity of compounds **1**−**6** determined by the brine shrimp lethality bioassay is also reported.The dried and powdered seeds of *E. ferox* were extracted with MeOH, and removal of solvent from the extract gave a brown residue. Two new cerebrosides, ferocerebrosides A (**1**) and B (**2**), two new tocopherol trimers, **5** and **6**, and two known tocopherol trimers, **3** and **4**,[8,9] were obtained.

The molecular formula of **1** was determined as $C_{44}H_{83}O_9N$ on the basis of high-resolution FABMS. The IR spectrum showed absorption bands at 3300 cm^{-1} (OH), 1640, 1535 cm^{-1} (amide), and 2920 and 1450 cm^{-1} (aliphatic), suggesting the presence of a fatty acid amide.[10] The *β*-glucopyranose moiety was indicated by the anomeric proton at *δ* 4.27 (1H, d, $J = 7.8$ Hz, H-1″) and the chemical shifts in the ^1H and ^{13}C NMR spectra. The HMQC spectrum of **1** showed that protons H-1a (*δ* 4.09), H-1b (*δ* 3.73), H-2 (*δ* 4.00), and H-3 (*δ* 4.13) were bonded to carbons resonating at *δ* 67.9 (C-1), 52.9 (C-2), and 72.0 (C-3), respectively. Other resonances in the ^1H NMR spectrum were a doublet at *δ* 8.33 due to an NH proton, a triplet at *δ* 0.88 (6H, $J = 7.0$ Hz), and four olefinic protons at *δ* 5.48 (1H, dd, $J = 15.5$, 7.2 Hz, H-4), 5.74 (1H, dt, $J = 15.5$, 6.4 Hz, H-5), and 5.41 (2H, m, H-8, 9). The coupling constant (15.6 Hz) indicated an *E*-configuration for the Δ^4 double bond. Chemical shifts of the allylic methylenic carbons in the ^{13}C NMR spectrum at *δ* 32.0 and 32.3 (C-7 and C-10) indicated the presence of an *E*-Δ^8 double bond.[11] Therefore, **1** was defined as a 4*E*,8*E*-sphingadiene-type cerebroside.

The ^{13}C NMR spectrum of **1** had resonances at *δ* 176.3 (C-1′) and 72.0 (C-2′) attributable to an acyl moiety. A proton resonance at *δ* 4.02 (1H, dd, $J = 7.5$, 3.8 Hz, H-2′) was coupled to a methylene group at *δ* 1.70 (1H, m, H-3′a) and 1.45 (1H, m, H-3′b). Methanolysis of **1** in aqueous MeOH−1 N HCl yielded methyl 2-hydroxydocosanoate (**1a**), which was identified by EIMS, HREIMS, and IR analyses. The specific rotation value of **1a** ($[\alpha]_D^{25}$ +18.5) indicated a 2′*R* absolute configuration in **1**.[12,13]

In the HMBC spectrum, the carbon resonance at *δ* 176.3 (C-1′) correlated with proton resonances at *δ* 4.00 (H-2) and 4.02 (H-2′), suggesting that the acyl moiety was connected to the NH of the sphingosine base. The anomeric proton of the glucose unit (*δ* 4.27, H-1″) exhibited a ^3J correlation with C-1 at *δ* 67.9, demonstrating attachment of the glucose moiety at C-1. The relative configuration at C-2 and C-3 was taken as 2*S*,3*R* (erythro) based on ^{13}C NMR chemical shifts and specific rotation.[14,15] Thus, compound **1** was determined to be (2*S*,3*R*,4*E*,8*E*,2′*R*)-1-*O*-(*β*-glucopyranosyl)-*N*-(2′-hydroxydocosanoyl)-4,8-sphingadienine and named ferocerebroside A.

The molecular formula of **2** was determined to be $C_{46}H_{87}O_9N$ on the basis of high-resolution FABMS, ^1H NMR, and ^{13}C NMR data. The NMR results were similar to those of **1**, confirming that **2** was also a glycosphingolipid that differed from **1** only in the length of the lipid base unit. Compound **2** was degraded by aqueous MeOH−1 N HCl to yield methyl 2*R*-hydroxytetracosanoate (**2a**), as revealed by EIMS, HREIMS, and IR analyses. The absolute configuration at C-2 and C-3 in **2** was the same as (+)-(2*S*,3*R*)-sphingosine, evidenced by ^{13}C NMR data and optical rotation.[14,15] Thus, compound **2** was identified as (2*S*,3*R*,4*E*,8*E*,2′*R*)-1-*O*-(*β*-glucopyranosyl)-*N*-(2′-hydroxytetracosanoyl)-4,8-sphingadienine and was named ferocerebroside B.

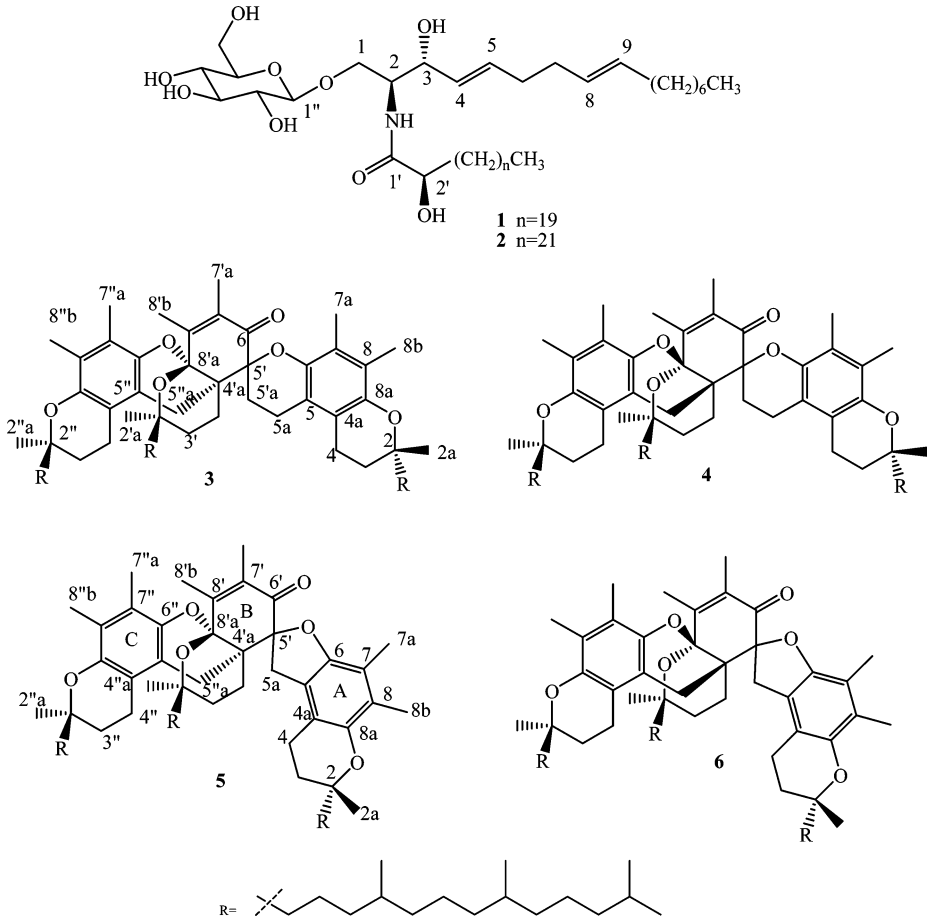
Although tocopherol trimers IVb (**3**) and IVa (**4**) were reported earlier,[8,9] this study examined the specific rotation and Cotton effect in CD measurements for compounds **3** and **4**. Additionally, this study assigned ^1H NMR and ^{13}C NMR data on the basis of ^1H−^1H COSY, HMQC, and HMBC spectra and revised some chemical shift assignments reported earlier for **3** and **4** (Tables 1 and 2). The HMBC measurements revealed correlations between H_2-5a and C-5, H_2-5″a and C-5″, H_3-8b and C-8, and H_3-8″b and C-8″. Through HMBC results, the previously accepted ^{13}C NMR assignment of *δ* 121.9−123.6 (C-5, 5″) and *δ* 115.1−115.8 (8 and 8″) should be revised as *δ* 121.9−123.6 (8 and 8″) and *δ* 115.1−115.8 (C-5, 5″) in **3** and **4**.[9]

Ferotocotrimer C (**5**), $[\alpha]_D^{25}$ −4.2 (*c* 1.0, $CHCl_3$), was obtained as a pale yellow oil. The molecular formula of **5** was determined as $C_{86}H_{142}O_6$ on the basis of HRFABMS data. The IR spectrum of

* Author to whom correspondence should be addressed. Tel: 886-3-5927700-2878. Fax: 886-3-5927310. E-mail: hochiao@yahoo.com.tw.
† Industrial Technology Research Institute.
‡ Ta Hwa Institute of Technology.
§ National Tsing Hua University.

10.1021/np070095j CCC: $37.00
Published on Web 06/13/2007

Chart 1



5 showed α,*β*-unsaturated carbonyl absorption (1695 cm^{-1}). The UV spectrum indicated aromatic rings (in *n*-hexane, λ_{max} 290 nm). The ^1H NMR spectrum showed 12 side-chain methyl groups (δ 0.84−0.87), three methyl groups on oxygenated carbons (δ 1.17, 1.25, 1.39), two olefinic methyl groups (δ 1.73, 1.96), and four aromatic methyl groups (δ 2.02 (× 2), 2.15, 2.20; Table 1). The ^{13}C NMR and DEPT data for **5** exhibited signals for three oxygenated quaternary sp^3 carbons [δ 74.8, 75.0 (C-2, 2″), 77.0 (C-2′)], one α,*β*-unsaturated carbonyl group [δ 130.3, 151.6, 196.6], and 12 aromatic carbons (Table 2). The 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC) and a fragment ion at *m*/*z* 428 in the EIMS spectrum suggested three characteristic moieties (A, B, and C) exist in **5** (Figure 1).[8,9]

The chemical shifts of a doubly oxygenated quaternary carbon at δc 99.4 (C-8′a) and methylene protons at δ_H 2.50 and 2.70 (H_2-5″a) suggested that the B and C units were linked from C-8′a to 6″ through oxygen and from C-4′a to C-5″ through a methylene group (CH_2-5″a). This conclusion was supported by the HMBC correlations.

The chemical shifts of an oxygenated quaternary carbon at δ_C 93.8 (C-5′a) and methylene protons at δ_H 2.42 and 2.83 (H_2-5a) suggested that the A and B units were linked from C-6 to C-5′ through an oxygen and from C-5 to C-5′ through a methylene group (CH_2-5a). The HMBC data showed that H_2-5a was correlated with C-5′, C-4a, C-4′a, C-5, and C-6 (Figure 2). This observation indicated that the A and B units were spiro-linked, which was further confirmed by the downfield shift of C-5′ by 8.3 ppm in the ^{13}C NMR spectrum.[16,17] Furthermore, the deshielding effect on the resonance of the 2′a-methyl group (δ_H 1.39) indicated that the 2′a-methyl group should be located in a deshielding environment above

Table 1. ^1H NMR Data for Compounds **3**−**6** (δ, ppm, in $CDCl_3$)

position[a]	**3**	**4**	**5**	**6**
H_2-5″a	2.85, d (17.9)	2.85, d (17.9)	2.70, d (17.8)	2.74, d (16.7)
	2.59, d (17.9)	2.64, d (17.9)	2.50, d (17.8)	2.56, d (16.7)
H_2-5a	2.53, m	2.56, m	2.83, d (16.5)	2.80, d (17.0)
	2.40, m	2.33, m	2.42, d (16.5)	2.47, d (17.0)
H_2-4,4″	2.48, m	2.54, m	2.40, m	2.40, m
	2.42, m	2.41, m	2.50, m	2.50, m
H_3-7″a	2.18, s	2.19, s	2.15, s	2.16, s
H_3-7a,8b,8″b	2.23, s	2.23, s	2.20, s	2.16, s
	2.13, s	2.13, s	2.09, s	2.06, s
	2.09, s	2.09, s	2.09, s	2.05, s
H_3-8′b	1.96, s	1.99, s	1.96, s	2.00, s
H_3-7′a	1.68, s	1.67, s	1.73, s	1.75, s
H_3-2′a	1.43, s	1.26, s	1.39, s	1.27, s
H_3-2a,2″a	1.26, s	1.22, s	1.25, s	1.23, s
	1.19, s	1.19, s	1.17, s	1.18, s

[a] The ^1H NMR spectrum showed 12 side-chain methyl groups between δ 0.84 and 0.87.

Table 2. ^{13}C NMR Data for Compounds **3**−**6** (δ, ppm, in $CDCl_3$)

position[a]	3	4	5	6
C-6′	198.7	198.8	196.6	194.0
C-8′	150.6	150.2	151.6	151.1
C-6,8a,8″a	144.3	144.8	145.6	145.8
	145.6	145.5	145.8	145.8
	145.6	145.7	151.3	151.1
C-6″	142.1	142.5	142.0	142.4
C-7′	129.0	129.7	130.3	130.5
C-7,8,7″,8″	121.9	122.0	119.0	119.1
	122.3	122.2	122.3	122.3
	123.5	123.5	123.7	123.2
	123.6	123.5	124.2	124.2
C-4a,4″a,5,5″	115.1	115.2	114.1	114.5
	115.2	115.5	114.8	115.4
	115.6	115.5	115.9	115.8
	115.8	115.8	116.4	116.4
C-8′a	99.4	100.2	99.4	100.1
C-5′	85.5	85.6	93.8	93.8
C-2′	78.0	77.2	77.0	77.1
C-2,2″	74.5	74.3	74.8	74.8
	74.7	74.8	75.0	75.0
C-4′a	41.6	42.6	40.6	42.1
C-8′b	14.5	14.6	14.8	14.9
C-7a,8b,7″a,8″b	11.5−12.0	11.7−13.0	11.6−12.0	11.9−13.0
C-7′a	11.5	11.6	11.7	11.8

[a] The ^{13}C NMR spectrum showed 12 side-chain methyl groups between δ 18.2 and 24.1

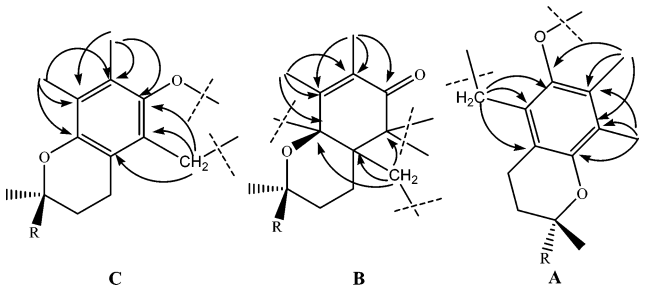


Figure 1. Correlations observed in the HMBC of A, B, and C partial structures of compound **5**.

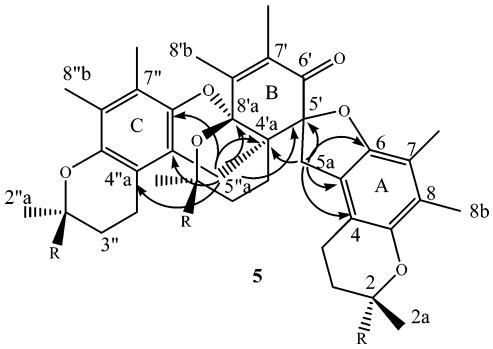


Figure 2. Significant HMBC correlations of compound **5**.

the enone system plane.[9,18] The absolute stereochemistry of 8′a as *R* and for 4′a as *S* was established by comparing the Cotton effect at $\Delta\epsilon_{240}$ −16.8, $\Delta\epsilon_{262}$ +0.78, $\Delta\epsilon_{292}$ −0.43, $\Delta\epsilon_{323}$ +2.90 with the CD spectral pattern of the tocopherol trimer IVb (**3**). On the basis of this evidence, **5** was identified as a new compound, named ferotocotrimer C.

Ferotocotrimer D (**6**), $[\alpha]_D^{25}$ 11.4 (*c* 0.8, $CHCl_3$), was obtained as a pale yellow oil. The molecular formula of **6** was determined to be $C_{86}H_{142}O_6$ on the basis of HRFABMS. This compound had IR, UV, and NMR spectra similar to those of **5**. However, the shielding effect on the resonance of the 2′a-methyl group (δ_H 1.27) indicated that the 2′a-methyl group must be in a shielded environment below the enone system plane.[9,18] The absolute stereochemistry of 8′a as *S* and 4′a as *R* was assumed by comparing the Cotton effect at $\Delta\epsilon_{239}$ +12.5, $\Delta\epsilon_{262}$ −1.55, $\Delta\epsilon_{291}$ +1.40, $\Delta\epsilon_{320}$ −3.56 (hexane, *c* 0.001) with the CD pattern of tocopherol trimer IVa (**4**). Therefore, the structure of **6** was determined to be as shown, and it was named ferotocotrimer D.

The toxicity of compounds **1**−**6** was evaluated using the brine shrimp lethality bioassay.[19,20] After 24 h, compounds **1** and **2** showed marginal toxicity against brine shrimp, with LC_{50} values of 0.17 and 0.20 mM, respectively. The other compounds were not toxic.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanaco micro-melting point apparatus. Optical rotations were measured on a JASCO DIP-360 digital polarimeter, and CD spectra were obtained with a JASCO J-720 spectropolarimeter. EIMS were recorded with a JMS-HX-100 instrument and FABMS with a JEOL LMS-SX 102 system. IR spectra were taken on a JASCO FT-IR-110 infrared spectrophotometer. UV spectra were recorded on a Perkin-Elmer Lambda 5 UV/vis spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-500 NMR spectrometer. Column chromatography was performed using silica gel (230−400 mesh, Merck). TLC was conducted on precoated Kiesel gel 60 F_{254} plates (0.25 mm, Merck), and spots were located by UV illumination and by spraying the $FeCl_3$ reagent or 10% H_2SO_4 followed by heating. MPLC was carried out on a Buchi MPLC system (pump, Buchi 688; detector, KAUER).

Plant Material. The seeds of *E. ferox* were collected in Gwong-Dung, China. The voucher specimen (FE1988) was deposited at the Ta Hwa Institute of Technology.

Extraction and Isolation. The dried and powdered seeds (16.1 kg) were extracted with methanol under reflux for 4−6 h (six times, each time 120 L) and concentrated to give a deep brown syrup (230 g), which was partitioned between 1:1 EtOAc−H_2O. The EtOAc layer was concentrated to give a brown residue (105.5 g) and then applied to a silica gel column eluted with 30:1 *n*-hexane−EtOAc to furnish five fractions. The second fraction was chromatographed by MPLC (silica gel, *n*-hexane−EtOAc gradient) to yield **3** (48 mg), **4** (55 mg), **5** (28 mg), and **6** (25 mg). The fourth fraction was further separated by a combination of Sephadex LH-20 CC (H_2O−MeOH), MPLC (C-18, 25% H_2O−MeOH → MeOH), HPLC (C-18, 25% H_2O−MeOH → MeOH), and PTLC (C-18, MeOH) to give **1** (68 mg) and **2** (75 mg).

Ferocerebroside A (1): amorphous powder; mp 185−187 °C; $[\alpha]_D^{25}$ +11.2 (*c* 0.08, MeOH); IR (neat) ν_{max} 3300, 2920, 2850, 1740, 1640, 1535, 1450, 1380, 1080 cm^{-1}; ^1H NMR ($CDCl_3$−CD_3OD, 1:1, 500 MHz) δ sphingosine moiety, 8.33 (d, J = 8.6 Hz, N-H), 4.09 (1H, dd, J = 10.2, 5.5 Hz, H-1a), 3.73 (1H, m, H-1b), 4.00 (1H, m, H-2), 4.13 (1H, t, J = 7.2 Hz, H-3), 5.48 (1H, dd, J = 15.5, 7.2 Hz, H-4), 5.74 (1H, dt, J = 15.5, 6.4 Hz, H-5), 2.08 (4H, m, H_2-6, 7), 5.41 (2H, m, H-8, 9), 1.95 (2H, m, H_2-10), 0.88 (3H, t, J = 7.0 Hz, Me), β-D-glucosyl moiety, 4.27 (1H, d, J = 7.8 Hz, H-1″), 3.24 (1H, dd, J = 8.9, 7.8 Hz, H-2″), 3.40 (1H, t, J = 8.9 Hz, H-3″), 3.38 (1H, t, J = 8.9 Hz, H-4″), 3.28 (1H, m, H-5″), 3.71 (1H, m, H-6a″), 3.87 (1H, dd, J = 12.1, 2.5 Hz, H-6b″), *N*-acyl moiety, 4.02 (1H, dd, J = 7.5, 3.8 Hz, H-2′), 1.70 (1H, m, H-3′a), 1.45 (1H, m, H-3′b), 0.88 (3H, t, J = 7.0 Hz, Me); ^{13}C NMR ($CDCl_3$−CD_3OD, 1:1, 125 MHz) δ sphingosine moiety, 67.9 (C-1), 52.9 (C-2), 72.0 (C-3), 130.1 (C-4), 133.2 (C-5), 33.0 (C-6), 32.0, 32.3 (C-7, 10), 129.7 (C-8, 9), 14.3 (C-18), β-glucosyl moiety, 103.0 (C-1″), 73.1 (C-2″), 75.9, 76.0 (C-3″, 5″), 69.6 (C-4″), 61.0 (C-6″), *N*-acyl moiety, 176.3 (C-1′), 72.9 (C-2′), 35.0 (C-3′), 14.3 (C-18′); FABMS (positive) *m*/*z* 792 $[M + Na]^+$ (100), 678 (10), 590 (12), 482 (26); HRFABMS *m*/*z* 792.6027 $[M + Na]^+$ (calcd for $C_{44}H_{83}O_9Na$, 792.5965).

Methanolysis of 1. Compound **1** (4 mg) was heated in 1 N HCl−MeOH (5 mL) under reflux for 3 h. After addition of H_2O (5 mL), the whole solution was extracted with *n*-hexane three times. Removing the solvent from the combined *n*-hexane solution furnished methyl 2*R*-hydroxydocosanoate (**1a**).

2*R*-Hydroxydocosanoate (1a): $[\alpha]_D^{25}$ +18.5 (*c* 0.08, $CHCl_3$); IR (neat) ν_{max} 3400, 1725, 1460, 1100 cm^{-1}; EIMS *m*/*z* 370 $[M]^+$ (10),

339 [M − OMe]$^+$ (10), 311 [M − COOMe]$^+$ (9), 149 (100), 83 (43), 57 (76), 43 (78); HREIMS *m/z* 370.3448 [M]$^+$ (calcd for $C_{23}H_{46}O_3$, 370.3447).

Ferocerebroside B (2): amorphous powder; mp 187−189 °C; $[\alpha]_D^{25}$ +8.7 (*c* 0.08, MeOH); IR (neat) ν_{max} 3350, 2900, 2860, 1735, 1640, 1530, 1460, 1385, 1100 cm^{-1}; ^{1}H NMR ($CDCl_3$−CD_3OD, 1:1, 500 MHz) δ sphingosine moiety, 8.35 (d, J = 8.5 Hz, N-H), 4.10 (1H, dd, J = 10.2, 5.5 Hz, H-1a), 3.75 (1H, m, H-1b), 4.02 (1H, m, H-2), 4.15 (1H, t, J = 7.4 Hz, H-3), 5.50 (1H, dd, J = 15.6, 7.4 Hz, H-4), 5.78 (1H, dt, J = 15.6, 6.4 Hz, H-5), 2.05 (4H, m, H_2-6, 7), 5.40 (2H, m, H-8, 9), 1.95 (2H, m, H_2-10), 0.87 (3H, t, J = 6.5 Hz, Me), β-glucosyl moiety, 4.29 (1H, d, J = 8.0 Hz, H-1″), 3.34 (1H, dd, J = 9.0, 8.0 Hz, H-2″), 3.45 (1H, t, J = 9.0 Hz, H-3″), 3.39 (1H, t, J = 9.0 Hz, H-4″), 3.30 (1H, m, H-5″), 3.75 (1H, J = 12.2, 5.2 Hz, H-6a″), 3.87(1H, dd, J = 12.2, 2.5 Hz, H-6b″), *N*-acyl moiety, 4.05 (1H, dd, J = 7.8, 3.8 Hz, H-2′), 1.70 (1H, m, H-3′a), 1.45 (1H, m, H-3′b), 0.87 (3H, t, J = 6.5 Hz, Me); ^{13}C NMR ($CDCl_3$−CD_3OD, 1:1, 125 MHz) δ sphingosine moiety, 68.2 (C-1), 53.4 (C-2), 72.2 (C-3), 130.4 (C-4), 133.5 (C-5), 33.4 (C-6), 32.1, 32.3 (C-7, 10), 129.8 (C-8, 9), 14.6 (C-18); β-glucosyl moiety, 102.0 (C-1″), 73.1 (C-2″), 75.7, 76.2 (C-3″, 5″), 69.3 (C-4″), 62.0 (C-6″), *N*-acyl moiety, 176.8 (C-1′), 73.2 (C-2′), 35.4 (C-3′), 14.6 (C-18′); FABMS (positive) *m/z* 820 [M + Na]$^+$ (100), 590 (18), 482 (40), 376 (20); HRFABMS *m/z* 820.6303 [M + Na]$^+$ (calcd for $C_{46}H_{87}O_9Na$, 820.6278).

Methanolysis of 2. Compound **2** (4 mg) was heated in 1 N HCl−MeOH (5 mL) under reflux for 3 h. After addition of H_2O (5 mL), the whole solution was extracted with *n*-hexane three times. Removing the solvent from the combined *n*-hexane solution furnished methyl 2*R*-hydroxytetracosanoate (**2a**).

2*R*-Hydroxytetracosanoate (2a): $[\alpha]_D^{25}$ +20.7 (*c* 0.08, $CHCl_3$); IR (neat) ν_{max} 3350, 1730, 1460, 1100 cm^{-1}; EIMS *m/z* 398 [M]$^+$ (15), 339 [M − COOMe]$^+$ (7), 149 (100), 83 (43), 57 (76), 43 (78); HREIMS *m/z* 398.3766 [M]$^+$ (calcd for $C_{25}H_{50}O_3$, 398.3760).

Tocopherol Trimer IVb (3): pale yellow wax; $[\alpha]_D^{25}$ −6.6 (*c* 1.2, $CHCl_3$); IR (neat) ν_{max} 2910, 2850, 1687, 1462, 1375, 1250, 1100 cm^{-1}; UV (hexane) λ_{max} (log ϵ) 292 (3.84) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; CD $\Delta\epsilon_{238}$ −17.5, $\Delta\epsilon_{256}$ +0.78, $\Delta\epsilon_{286}$ −0.21, $\Delta\epsilon_{307}$ +2.80 (hexane, *c* 0.001); EIMS *m/z* 430 (100), 428 (93), 165 (82), 164 (27); FABMS (positive) *m/z* 1285 [M + H]$^+$ (8), 857 (100), 855 (21), 632 (14).

Tocopherol Trimer IVa (4): pale yellow wax; $[\alpha]_D^{25}$ +27.1 (*c* 2.6, $CHCl_3$); IR (neat) ν_{max} 2920, 2850, 1695, 1460, 1380, 1260, 1080 cm^{-1}; UV (hexane) λ_{max} (log ϵ) 293 (3.79) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; CD $\Delta\epsilon_{238}$ +19.3, $\Delta\epsilon_{258}$ −2.01, $\Delta\epsilon_{285}$ + 2.06, $\Delta\epsilon_{307}$ −3.37 (hexane, *c* 0.001); EIMS *m/z* 631 (24), 428 (100), 203 (32), 165 (57); FABMS (positive) *m/z* 1285 [M + H]$^+$ (8), 857 (100), 165 (62).

Ferotocotrimer C (5): pale yellow oil; $[\alpha]_D^{25}$ −4.2 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 2920, 2850, 1695, 1460, 1380, 1260, 1100 cm^{-1}; UV (hexane) λ_{max} (log ϵ) 290 (3.83) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; CD $\Delta\epsilon_{240}$ −16.8, $\Delta\epsilon_{262}$ +0.78, $\Delta\epsilon_{292}$ −0.43, $\Delta\epsilon_{323}$ +2.90 (hexane, *c* 0.001); EIMS *m/z* 690 (36), 428 (100), 416 (16), 203 (24), 165 (45); FABMS (positive) *m/z* 1271 [M + H]$^+$ (14), 1046 (2), 855 (7), 844 (69), 826 (100), 428 (32); HRFABMS *m/z* 1272.0829 [M + H]$^+$ (calcd for $C_{86}H_{143}O_6$; 1272.0885), *m/z* 1271.0897 (calcd for $C_{86}H_{142}O_6$, 1271.0806).

Ferotocotrimer D (6): pale yellow oil; $[\alpha]_D^{25}$ +11.4 (*c* 0.8, $CHCl_3$); IR (neat) ν_{max} cm^{-1} 2920, 2850, 1690, 1460, 1373, 1250, 1100. UV (hexane) λ_{max} (log ϵ) 292 (3.78) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) Table 2; CD $\Delta\epsilon_{239}$ +12.5, $\Delta\epsilon_{262}$ −1.55, $\Delta\epsilon_{291}$ +1.40, $\Delta\epsilon_{320}$ −3.56 (hexane, *c* 0.001); EIMS *m/z* 444 (25), 428 (100), 410 (36), 165 (63); FABMS (positive) *m/z* 1271 [M + H]$^+$ (4), 826 (11), 824 (3), 428 (10); HRFABMS *m/z* 1272.0820 [M + H]$^+$ (calcd for $C_{86}H_{143}O_6$, 1272.0884), *m/z* 1271.0885 [M]$^+$ (calcd for $C_{86}H_{142}O_6$, 1271.0806).

Brine Shrimp Lethality Bioassay. The toxic effect of compounds **1**−**6** was evaluated by the brine shrimp lethality test. Compounds were dissolved in DMSO, and five doses of each compound, 62.5, 125, 250, 500, and 1000 μg/mL, respectively, were used in 5 mL of seawater containing 10 brine shrimp in each group. The number of survivors was counted after 24 h, and the LC_{50} was determined by probit analysis described by Meyer.[19] The experiments were carried out in quadruplicate, and mean LD_{50} values were calculated. Pure compounds with LD_{50} values > 200 μg/mL were considered inactive.[20]

References and Notes

(1) Hou, D. *Flora of Taiwan*, 2nd ed.; Editorial Committee of the Flora of Taiwan: Taiwan 1996, Vol. 2, p 547.
(2) Das, S.; Der, P.; Raychaudhuri, U.; Maulik, N.; Das, D. K. *Mol. Cell Biochem.* **2006**, *289*, 55−63.
(3) Puri, A.; Sahai, R.; Singh, K. L.; Saxena, R. P.; Tandon, J. S.; Saxena, K. C. *J. Ethnopharmacol.* **2000**, *71*, 89−92.
(4) Su, J. D.; Osawa, T.; Namiki, M. *Agric. Biol. Chem.* **1986**, *50*, 199−203.
(5) Lee, S. E.; Ju, E. M.; Kim, J. H. *Exp. Mol. Med.* **2002**, *34*, 100−106.
(6) Zhao, H.; Zhao, S.; Sun, C.; Guillaume, D. *J. Lipid Res.* **1989**, *30*, 1633−1637.
(7) Zhao, H.; Zhao, S.; Guillaume, D.; Sun, C. *J. Nat. Prod.* **1994**, *57*, 138−141.
(8) Nilsson, J. L. G.; Daves, G. D.; Folkers, K. *Acta Chem. Scand.* **1968**, *22*, 200−206.
(9) Yamauchi, R.; Kato, K.; Ueno, Y. *Lipids* **1988**, *23*, 779−783.
(10) Noda, N.; Tanaka, R.; Miyahara, K. *Chem. Pharm. Bull.* **1994**, *42*, 1247−1251.
(11) Shibuya, H.; Kawashima, K.; Sakagami, M.; Kawanishi, H.; Miho, S.; Ohashi, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1990**, *38*, 2933−2938.
(12) Su, B. N.; Hwang, B. Y.; Chai, H.; Carcache-Blanco, E. J.; Kardono, L. B.; Afriastini, J. J.; Riswan, S.; Wild, R.; Laing, N.; Farnsworth, N. R.; Cordell, G. A.; Swanson, S. M.; Kinghorn, A. D. *J. Nat. Prod.* **2004**, *67*, 1911−1914.
(13) Shibuya, H.; Kurosu, M.; Minagawa, K.; Katayama, S. *Chem. Pharm. Bull.* **1993**, *41*, 1534−1537.
(14) Pettit, G. R.; Tang, Y.; Knight, J. C. *J. Nat. Prod.* **200**5, *68*, 974−978.
(15) Sarmientos, F.; Schwarzmann, G.; Sandhoff, K. *Eur. J. Biochem.* **1985**, *146*, 59−62.
(16) Chatterjee, A.; Vetrivel, R.; Sreekumar, R.; Murthy, Y. V. S. N.; Pillai, C. N. *J. Mol. Catal. A: Chem.* **1997**, *127*, 153−162.
(17) Snider, B. B.; Han, L.; Xie, C. *J. Org. Chem.* **1997**, *62*, 6978−6984.
(18) Grams, G. W.; Eskins, K.; Inglett, G. E. *J. Am. Chem. Soc.* **1972**, *94*, 866−868.
(19) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31−34.
(20) Othman, R.; Ibrahim, H.; Mohd, M. A.; Mustafa, M. R.; Awang, K. *Phytomedicine* **2006**, *13*, 61−66.

NP070095J