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ISOLATION OF NEW CERAMIDES FROM THE GORGONIAN *ACABARIA UNDULATA*

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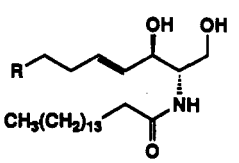
ABSTRACT.—Four ceramides, including three novel compounds, have been isolated from the gorgonian *Acabaria undulata*. The structures of these compounds were determined by a combination of spectroscopic methods and chemical and enzymatic degradation methods. In addition, the absolute stereochemistry of a previously reported ceramide has been revised.

Sphingolipids have been isolated from various marine organisms including algae, coelenterates, echinoderms, sponges, and tunicates (1,2). Some of these metabolites have been recognized as possessing antimicrobial and cytotoxic activities (3,4). In our search for novel secondary metabolites from Korean aquatic organisms, specimens of the bright red gorgonian *Acabaria undulata* Kukenthal (Melithaeidae) were collected along the shore of Keomun Island, Korea. Several ceramides, as *N*-acyl-sphingosines, were isolated by vacuum flash chromatography followed by C_{18} reversed-phase hplc of the CH_2Cl_2 extract. We report herein the structures of four ceramides, including three novel compounds.

Compound **1** was isolated as a white solid which analyzed for $C_{34}H_{65}NO_3$ by a combination of eims and ^{13}C-nmr data. A carbonyl carbon signal at δ 174.11 in the ^{13}C-nmr spectrum, a downfield proton signal at δ 6.29 (br d, J=7.3 Hz) in the ^1H-nmr spectrum, and a strong absorption band at 1625 cm^{-1} in the ir spectrum, indicated the presence of a secondary amide group. A very strong signal at δ 1.24 in the ^1H-nmr spectrum and lack of upfield methine signals in the ^{13}C-nmr spectrum revealed that **1** must be derived from a long-chain fatty acid precursor.



1 R=$CH_3(CH_2)_8CH\overset{E}{=}CH$-

2 R=$CH_3(CH_2)_6CH\overset{E}{=}CH$-$CH\overset{E}{=}CH$-

3 R=$(CH_3)_2CH(CH_2)_7CH\overset{E}{=}CH$-

4 R=$CH_3(CH_2)_{10}$-

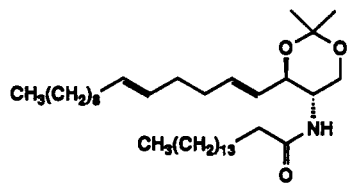
Key functionalities and their connectivities were determined by a combination of ^1H decoupling, ^1H-^1H COSY, and HMQC nmr experiments. The amide proton at δ 6.29 was coupled to the methine proton at δ 3.90 which in turn was coupled to three protons at δ 4.32, 3.95, and 3.69. HMQC data showed that the proton at δ 4.32 was connected to the carbon at δ 74.26, while the protons at δ 3.95 and 3.69 were connected to the carbon at δ 62.29. Couplings of these protons with D_2O exchangeable protons at δ 2.98 and 2.92 revealed the presence of secondary and primary hydroxyl groups. The proton at δ 4.32 was coupled to the olefinic protons at δ 5.77 and 5.53. This double bond was connected to another double bond (δ 5.43 and 5.36) via an ethylene group (δ 2.11 and 2.07, 2H each). The large couplings between the olefinic protons ($J_{4,5}$=15.4 Hz and $J_{8,9}$=15.4 Hz) revealed that both of the double bonds had *E* configurations. Thus, compound **1** was determined as a ceramide of the *N*-acyl-sphinga-4(*E*),8(*E*)-dienine class.

The chain-lengths of the two acyl components of **1** were determined by combination of ms data interpretation and an enzyme-catalyzed hydrolysis. The

mass fragmentation of **1** showed strong peaks at *m/z* 281 (relative intensity 100%) and *m/z* 298 (relative intensity 40%). According to the nomenclature of sphingolipid mass fragmentation patterns, these peaks corresponded to the G and T fragments, respectively (Figure 1) (5). Therefore, **1** must be *N*-palmitoyl-octadecasphinga-4(*E*),8(*E*)-dienine. This interpretation was further supported by an enzyme-catalyzed hydrolysis (6). Treatment of **1** with cholesterol esterase followed by methanolic HCl gave methyl palmitate as the sole product that was confirmed by gc analysis. Thus, the chain-lengths of two components of **1** were unambiguously determined as C_{18} and C_{16}, respectively. A literature survey revealed that a ceramide possessing the same sphingosine and fatty acid moieties was recently isolated from the Indian ocean sea anemone *Paracondylactis indicus* Dave (7).

The remaining problem was determination of the stereochemistry of **1**. Compound **1** possessed asymmetric carbon centers at C-2 and C-3. In previous literature the absolute configurations of these centers were assigned as 2*S*,3*R* on the grounds that all naturally occurring sphingosines possess 2*S*,3*R* configurations, and the chemical shifts of the C-2 and C-3 carbons were very similar to compounds of the same configurations (7–9). However, we felt the need for more conclusive evidence because the sign of optical rotation of **1** was opposite {$[\alpha]^{25}$D $-8.0°$} to the reported value for the ceramide from *P. indicus* {$[\alpha]^{25}$D $+10.6°$}. Moreover, the presence of a sphingosine possessing the enantiomeric 2*R*,3*S* configurations in the sea anemone *Anemonia sulcata* was recently demonstrated by an enantioselective total synthesis (10,11).

Due to the free rotation of **1** in solution, the ^{1}H-nmr coupling constant (J=4.4 Hz) between H-2 and H-3 did not give sufficient information on relative configurations. This problem was solved by a chemical transformation. Treatment of **1** with 2,2-dimethoxyacetone and PPTS gave compound **5**, a cyclic ketal derivative, as the major product. The large coupling between the H-2 and H-3 protons (J=9.3 Hz) revealed the diaxial orientation of these protons. Thus, the relative configurations of **1** were unambiguously determined as erythro-2*S**,3*R**. The absolute stereochemistry was inferred as D-erythro-(2*S*,3*R*) by total synthesis of **4**, as discussed later.



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A closely related ceramide **2** was isolated as a white solid which analyzed for $C_{34}H_{63}NO_3$ by eims and ^{13}C-nmr spectrometry. Spectral data for compound **2** were closely comparable to those obtained for **1**. The only difference in the ^{13}C-nmr spectrum of **2** was the presence of an additional double bond (six olefinic carbons). This new double bond was assigned to C-10 using ^{1}H-nmr and ^{1}H COSY spectra. This interpretation was supported by the uv spectrum in which an absorption maximum of the conju-

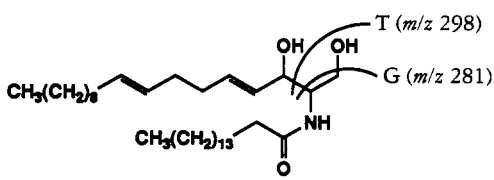

FIGURE 1

gated olefin was found at 223 nm (ε 9500). The configurations of the three double bonds were determined as 4(*E*), 8 (*E*), and 10(*E*) by proton decoupling experiments ($J_{4,5}$=15.4 Hz, $J_{8,9}$=14.7 Hz, and $J_{10,11}$=14.2 Hz). Thus, compound **2** was determined as *N*-palmitoyl-D-*erythro*-(2*S*,3*R*)-octadecasphinga-4(*E*),8(*E*),10(*E*)-trienine.

Another ceramide, **3**, was isolated as a white solid that analyzed for $C_{35}H_{67}NO_3$ using eims and ^{13}C-nmr spectroscopic methods. The nmr spectra of **3** and **1** differed in the appearance of peaks corresponding to an additional methyl group. The splitting pattern of the upfield methyl protons [δ 0.88 (3H, t, J=7.3 Hz), 0.86 (6H, d, J=6.8 Hz)] in the ^{1}H-nmr spectrum of **3** revealed that the additional methyl group was attached to the terminus of either sphingosine or palmitic acid. Hydrolysis of **3** by MeOH/H_2SO_4 gave methyl palmitate as the major product, confirmed by gc and mass spectral analysis. Therefore, the additional methyl group must be attached to the C-17 carbon of sphingosine. Thus, compound **3** was determined as *N*-palmitoyl-D-*erythro*-(2*S*,3*R*)-17-methyloctadecasphinga-4(*E*),8(*E*)-dienine.

Finally, ceramide **4** was isolated as a white solid. The molecular formula of $C_{34}H_{67}NO_3$ was deduced using a combination of eims and ^{13}C-nmr data. The structure of this compound was determined as *N*-palmitoyloctadecasphinga-4(*E*)-ene by comparison of spectral data with compounds **1–3**. A literature survey revealed that **4** has been isolated from beef spleen and synthesized by various methods (12–16). To determine the absolute stereochemistry of the asymmetric carbon centers at C-2 and C-3, **4** was synthesized following the methods described by Shibuya *et al.* (15–17). Esterification of palmitic acid with *p*-nitrophenol gave *p*-nitrophenyl palmitate as the sole product. Treatment of D-sphingosine (Sigma) with this palmitate yielded *N*-palmitoyl-D-*erythro*-(2*S*,3*R*)-octadecasphinga-4(*E*)-ene as the major product. Spectral data for the synthetic compounds were identical with those of compound **4**. The optical rotation of the synthetic compound was −4.6° while the natural compound exhibited a value of −5.8°. Therefore, the absolute configurations of the asymmetric centers of **4** must be D-(−)-erythro-(2*S*,3*R*). Since compounds **1–3** possessed the same functionalities as **4** and exhibited very similar optical rotations {[α]^{25}D −8.1°, −4.1°, and −6.0°, respectively}, these compounds probably possess the same absolute stereochemistry as **4**. Thus, the structures of compounds **1–4** were determined as ceramides possessing the D-(−)-erythro-(2*S*,3*R*)-configuration.

As mentioned earlier, a structurally similar ceramide from the sea anemone *Paracondylactis indicus* was proposed to possess the D-(+)-erythro-(2*S*,3*R*)-configuration (7). Because the signals of optical rotation of this compound and **1** were opposite to each other, however, the absolute configurations of the ceramide from *P. indicus* must be revised to L-(+)-erythro-(2*R*,3*S*). It has been generally accepted that the absolute stereochemistries of sphingosine-type amino alcohols are D-erythro-(2*S*,3*R*)(7,8). However, our results and previous synthetic work clearly demonstrated the presence of L-erythro-(2*R*,3*S*)-sphingosines in marine organisms (11). Interestingly, both sphingosines possessing the L-erythro-(2*R*,3*S*)-configurations have been isolated from sea anemones. However, it is not clear whether the presence of enantiomeric sphingosines in coelenterates, and exclusively in sea anemones, is a general phenomenon or not. More biochemical data are needed to clarify this situation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded in $CDCl_3$ solution on a Varian Unity-500 spectrometer. ^{1}H- and ^{13}C-nmr spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal TMS. Ir spectra

were recorded on a Mattson Galaxy spectrophotometer. Uv spectra were obtained in MeOH using a Milton-Roy spectrophotometer. Mass spectral measurements were supplied by the Chemical Analysis Department, Daelim Industrial Co., Ltd. The optical rotations were measured on a Jasco digital polarimeter with a 5-cm microcell. Gc spectra were recorded on a Hewlett-Packard 5890 analytical gas chromatograph using an SP 380 column and N_2 as carrier gas at 50 ml/min. Temperatures of the injector, oven, and fid were 250°, 195°, and 260°, respectively. Mps were measured on a Fisher-Jones apparatus and are uncorrected. All solvents used were spectral grade or were distilled from glass prior to use.

ANIMAL MATERIAL.—*Acabaria undulata* (sample number 91K-2) was collected by hand using scuba at 20–25 m depth in November 1991, along the shore of Geomun Island, Korea. The collected samples were briefly dried in the shade and kept frozen until chemically investigated.

EXTRACTION AND ISOLATION.—The animals (3.9 kg) were defrosted and repeatedly extracted with CH_2Cl_2. The crude extracts (6.5 g) were separated by silica vacuum flash chromatography using sequential mixtures of *n*-hexane and EtOAc. Fractions eluted with moderately polar solvents (50–80% EtOAc in hexane) were combined and separated by semi-prep. C_{18} reversed-phase hplc (YMC ODS column, 1 cm×25 cm, 100% MeOH) to obtain compounds **1**–**4** in the order, **2**, **1**, **3**, and **4**.

CERAMIDE **1**.—White solid (54.6 mg, 0.83% of crude extract); mp 82–83°; $[\alpha]^{25}D$ −8.0° (c=0.5, $CHCl_3$); eims *m/z* 535 (1), 517 (5), 504 (3), 486 (3), 366 (3), 352 (4), 320 (5), 298 (40), 281 (100), 250 (14), 85 (8), 60 (36); ir ν max (KBr) 3400, 3300, 2960, 2920, 2854, 1625, 1470, 1050, 960 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.29 (1H, br d, J=7.3 Hz, NH), 5.77 (1H, dt, J=15.4 and 6.1 Hz, H-5), 5.53 (1H, dd, J=15.4 and 6.8 Hz, H-4), 5.43 (1H, dt, J=15.4 and 6.4 Hz, H-9), 5.36 (1H, dt, J=15.4 and 6.4 Hz, H-8), 4.32 (1H, br dd, J=6.8 and 4.4 Hz, H-3), 3.95 (1H, br dd, J=11.2 and 3.4 Hz, H-1), 3.90 (1H, m, H-2), 3.69 (1H, br dd, J=11.2 and 3.2 Hz, H-1), 2.98 (1H, br s, OH), 2.92 (1H, br s, OH), 2.22 (2H, t, J=7.6 Hz, H-2′), 2.11 (2H, m, H-6), 2.07 (2H, m, H-7), 1.96 (2H, m, H-10), 1.61 (2H, m, H-3′), 1.24 (38H, m), 0.87 (6H, t, J=6.6 Hz, H-18, H-16′); ^{13}C nmr ($CDCl_3$) δ 174.11 (C, C-1′), 133.38 (CH, C-5), 131.26 (CH, C-8), 129.09 (CH, C-4), 128.95 (CH, C-9), 74.26 (CH, C-3), 62.29 (CH_2, C-1), 54.54 (CH, C-2), 36.80 (CH_2, C-2′), 32.58 (CH_2, C-10), 32.34 (CH_2, C-6 or C-7), 32.14 (CH_2, C-6 or C-7), 31.90 (CH_2), 31.88 (CH_2), 29.68 (CH_2×3), 29.66 (CH_2), 29.64 (CH_2×2), 29.58 (CH_2×2), 29.51 (CH_2×2), 29.37 (CH_2), 29.34 (CH_2), 29.32 (CH_2), 29.29 (CH_2), 29.20 (CH_2), 25.75 (CH_2, C-3′), 22.66 (CH_2, C-17, C-15′×2), 14.09 (CH_3, C-18, C-16′×2).

CERAMIDE **2**.—White solid (7.7 mg, 0.12%); mp 85–86°; $[\alpha]^{25}D$ −4.1° (c=0.5, $CHCl_3$); eims *m/z* 533 (3), 515 (19), 352 (23), 320 (19), 298 (32), 281 (61), 280 (70), 256 (32), 179 (100), 164 (48), 82 (30), 60 (70); uv λ max (CH_3CN) 223 nm (ε 9500); ir ν max (KBr) 3400, 3300, 2960, 2920, 2850, 1625, 1570, 1470, 1050, 980, 720 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.24 (1H, br d, J=7.3 Hz, NH), 5.99 (2H, m, H-9, H-10), 5.77 (1H, dt, J=15.4 and 6.1 Hz, H-5), 5.59 (1H, m, H-11), 5.53 (1H, dd, J=15.4 and 6.1 Hz, H-4), 5.52 (1H, m, H-8), 4.32 (1H, br dd, J=6.8 and 4.4 Hz, H-3), 3.93 (1H, br dd, J=11.2 and 3.4 Hz, H-1), 3.90 (1H, m, H-2), 3.69 (1H, br dd, J=11.2 and 3.2 Hz, H-1), 2.78 (1H, br s, OH), 2.65 (1H, br s, OH), 2.22 (2H, t, J=7.6 Hz, H-2′), 2.16 (4H, m, H-6, H-7), 2.04 (2H, m, H-12), 1.63 (2H, m, H-3′), 1.25 (34H, m), 0.87 (6H, t, J=6.8 Hz, H-18, H-16′); ^{13}C nmr ($CDCl_3$) δ 173.92 (C), 133.26 (CH), 133.10 (CH), 131.16 (CH), 130.74 (CH), 129.96 (CH), 129.50 (CH), 74.58 (CH), 62.50 (CH_2), 54.38 (CH), 36.80 (CH_2), 32.61 (CH_2), 32.05 (CH_2), 32.01 (CH_2), 31.92 (CH_2), 31.82 (CH_2), 29.70 (CH_2×3), 29.66 (CH_2×2), 29.63 (CH_2), 29.50 (CH_2), 29.39 (CH_2), 29.36 (CH_2×2), 29.29 (CH_2), 29.19 (CH_2), 29.18 (CH_2), 25.75 (CH_2), 22.69 (CH_2), 22.66 (CH_2), 14.12 (CH_3), 14.10 (CH_3).

CERAMIDE **3**.—White solid (12.3 mg, 0.19%); mp 76–77°; $[\alpha]^{25}D$ −6.0° (c=0.5, $CHCl_3$); eims *m/z* 549 (0.3), 531 (4), 500 (4), 332 (8), 320 (12), 298 (33), 281 (100), 250 (33), 154 (8), 60 (45); ir ν max (KBr) 3400, 3300, 2960, 2920, 2850, 1625, 1460, 1040, 965 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.27 (1H, br d, J=7.3 Hz, NH), 5.79 (1H, dt, J=15.1 and 6.6 Hz, H-5), 5.55 (1H, dd, J=15.4 and 6.8 Hz, H-4), 5.43 (1H, dt, J=15.4 and 6.0 Hz, H-9), 5.36 (1H, dt, J=15.4 and 5.4 Hz, H-8), 4.32 (1H, br dd, J=6.8 and 4.4 Hz, H-3), 3.95 (1H, br dd, J=11.2 and 3.4 Hz, H-1), 3.91 (1H, m, H-2), 3.70 (1H, br dd, J=11.2 and 3.2 Hz, H-1), 2.82 (2H, br s, OH), 2.23 (2H, t, J=7.6 Hz, H-2′), 2.13 (2H, m, H-6), 2.06 (2H, m, H-7), 1.98 (2H, m, H-10), 1.64 (2H, m, H-3′), 1.51 (1H, m, H-17), 1.25 (36H, m), 0.88 (3H, t, J=7.3 Hz, H-16′), 0.86 (6H, d, J=6.8 Hz, H-18, H-19); ^{13}C nmr ($CDCl_3$) δ 173.94 (C), 133.49 (CH), 131.31 (CH), 129.11 (CH), 128.93 (CH), 74.61 (CH), 62.44 (CH_2), 54.41 (CH), 39.04 (CH_2), 36.84 (CH_2), 32.60 (CH_2), 32.33 (CH_2), 32.13 (CH_2), 31.93 (CH_2), 29.90 (CH_2), 29.70 (CH_2×3), 29.67 (CH_2×2), 29.65 (CH_2×2), 29.60 (CH_2), 29.57 (CH_2), 29.52 (CH_2), 29.37 (CH_2×2), 29.29 (CH_2), 29.22 (CH_2), 27.97 (CH), 27.41 (CH_2), 25.77 (CH_2), 22.70 (CH_3), 22.67 (CH_3), 14.14 (CH_3).

CERAMIDE **4**.—White solid (11.4 mg,

0.17%); mp 96–98°; $[\alpha]^{25}D$ −5.8° (c=0.5, $CHCl_3$); eims m/z 537 (0.2), 519 (2), 506 (4), 500 (2), 312 (10), 298 (33), 281 (100), 250 (27), 154 (8), 60 (57); ir ν max (KBr) 3300, 2960, 2920, 2850, 1640, 1550, 1460, 1070, 970 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.24 (1H, br d, J=7.3 Hz, NH), 5.78 (1H, dt, J=15.5 and 6.6 Hz, H-5), 5.53 (1H, dd, J=15.5 and 6.8 Hz, H-4), 4.31 (1H, br dd, J=6.8 and 4.4 Hz, H-3), 3.95 (1H, br dd, J=11.2 and 3.9 Hz, H-1), 3.90 (1H, m, H-2), 3.70 (1H, br dd, J=11.2 and 3.2 Hz, H-1), 2.68 (2H, br s, OH), 2.22 (2H, t, J=7.8 Hz, H-2′), 2.05 (2H, m, H-6), 1.63 (2H, m, H-3′), 1.25 (46H, m), 0.87 (6H, t, J=6.6 Hz, H-18, H-16′); ^{13}C nmr ($CDCl_3$) δ 173.91 (C), 134.30 (CH), 128.77 (CH), 74.68 (CH), 62.51 (CH_2), 54.46 (CH), 36.84 (CH_2), 32.28 (CH_2), 31.92 (CH_2×2), 29.70 (CH_2×3), 29.69 (CH_2×3), 29.66 (CH_2×3), 29.64 (CH_2×2), 29.51 (CH_2), 29.49 (CH_2), 29.36 (CH_2×2), 29.29 (CH_2), 29.22 (CH_2), 29.12 (CH_2), 25.76 (CH_2×2), 22.69 (CH_2×2), 14.12 (CH_3×2).

ENZYME-CATALYZED HYDROLYSIS OF **1**.—To a stirred solution of **1** (1 mg) in 0.1 ml of MeOH were added 1 unit of cholesterol esterase and 0.5 ml of Tris buffer (50 mmol, pH 7.5). The mixture was stirred overnight at 37° and was extracted with hexane (1 ml×2). After removing the solvent under vacuum, 0.5 ml of 5% methanolic HCl was added. The mixture was stirred for 1 h at room temperature and was extracted with hexane (1 ml×2). After removing hexane under vacuum, the residue was subjected to gc using various fatty acid methyl esters as standards. Methyl palmitate was detected as the sole product (R_t 3.5 min under standard conditions).

KETAL FORMATION OF **1**.—To a stirred solution of **1** (3.4 mg) in Me_2CO (20 ml) were added PPTS (3.2 mg) and 2,2-dimethoxypropane (0.5 ml) and the mixture was refluxed for 8 h under N_2. Triethylamine (0.3 ml) was added to the mixture and was refluxed for an additional 1 h. After removing the solvent and excess reagents under vacuum, the residue was extracted with 30% EtOAc/hexane (1 ml×3). Final purification by silica hplc (25% EtOAc/hexane) gave 2.1 mg of **5** (61% yield); ^1H nmr ($CDCl_3$) δ 5.76 (1H, dt, J=15.5 and 6.4 Hz, H-5), 5.45 (1H, dd, J=15.5 and 7.3 Hz, H-4), 5.40 (1H, m, H-9), 5.37 (1H, m, H-8), 5.13 (1H, br d, J=8.3 Hz, NH), 4.08 (1H, dd, J=9.4 and 7.3 Hz, H-3), 4.00 (1H, dd, J=11.2 and 4.9 Hz, H-1), 3.83 (1H, m, H-2), 3.65 (1H, dd, J=11.2 and 9.4 Hz, H-1), 2.12 (2H, t, J=7.8 Hz, H-2′), 2.11 (2H, m, H-6), 2.06 (2H, m, H-7), 1.96 (2H, m, H-10), 1.56 (2H, m, H-3), 1.49 (3H, s, ketal-CH_3), 1.42 (3H, s, ketal-CH_3), 1.26 (38H, m), 0.88 (6H, t, J=6.8 Hz, H-18, H-16′).

ACID-CATALYZED HYDROLYSIS OF **3**.—To a solution of **3** (2.8 mg) in MeOH (8 ml) was added 2 M H_2SO_4 (2 ml) and the mixture was refluxed for 6 h. Saturated NaCl solution was added to the mixture and extracted with hexane (5 ml×3). After drying with $MgSO_4$ and removing hexane under vacuum, final purification by silica hplc (15% EtOAc/hexane) gave 1.0 mg of a nonpolar compound as the major product. This compound was subjected to gc under standard conditions and was detected as pure methyl palmitate; eims m/z 270 (72), 239 (26), 227 (39), 213 (8), 199 (13), 185 (15), 171 (14), 149 (33), 143 (37), 87 (89), 74 (100).

p-NITROPHENYL ESTERIFICATION OF PALMITIC ACID.—To palmitic acid (0.85 g, 3.4 mmol) was added $SOCl_2$ (4 ml) and refluxed for 1 h under N_2. After removing the excess $SOCl_2$ under vacuum, the residue, dissolved in dry CH_2Cl_2 (10 ml), was added dropwise to a stirred solution of *p*-nitrophenol (0.473 g) and pyridine (0.3 ml) in dry CH_2Cl_2 (20 ml). The mixture was stirred at room temperature overnight. After removing pyridine and CH_2Cl_2 under vacuum, separation by silica cc (10% EtOAc/*n*-hexane) gave *p*-nitrophenyl palmitate (1.09 g, 85% yield); ^1H nmr ($CDCl_3$) δ 8.27 (2H, dt, J=6.8 and 2.0 Hz), 7.27 (2H, dt, J=6.8 and 2.0 Hz), 2.60 (2H, t, J=7.3 Hz), 1.76 (2H, m), 1.55–1.26 (24H, m), 0.88 (3H, t, J=6.3 Hz).

SYNTHESIS OF *N*-PALMITYL-D-*ERYTHRO*-OCTADECASPHINGA-4(*E*)-ENE [**4**].—To a stirred solution of D-*erythro*-octadecasphinga-4(*E*)-ene (10.0 mg, 0.033 mmol) in dry THF (4 ml) was added *p*-nitrophenyl palmitate (16.6 mg, 0.033 mmol). The mixture was stirred under N_2 overnight at room temperature. After removing THF under vacuum, separation by C_{18} reversed-phase hplc gave 4.8 mg (0.009 mmol) of *N*-palmityl-D-*erythro*-octadecasphinga-4(*E*),8(*E*)-diene [**4**] (27% yield). Nmr data for the synthetic compound were identical with those of compound **4**; $[\alpha]D$ −4.6° (c=0.8, $CHCl_3$).

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