Ophidiacerebrosides: Cytotoxic Glycosphingolipids Containing a Novel Sphingosine from a Sea Star

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Five newly identified glycosphingolipids, ophidiacerebrosides, have been isolated from the sea star *Ophidiaster ophidiamus*. All five compounds showed strong cytotoxicity against L1210 leukemia cells in vitro. On the basis of chemical evidence and ^1H and ^{13}C NMR and mass spectra, the structure of ophidiacerebroside C, the major component, is assigned as (2*S*,3*R*,4*E*,8*E*,10*E*)-1-(β-D-glucopyranosyloxy)-3-hydroxy-2-[((*R*)-2-hydroxydocosanoyl)amino]-9-methyl-4,8,10-octadecatriene. This compound differs from previously isolated marine cerebrosides in that the sphingosine base contains a methyl branch and a conjugated diene. The other four ophidiacerebrosides, A, B, D, and E, were found from their FAB and ^1H NMR spectra and hydrolysis products to be identical to C with respect to the sphingosine and hexose units. However, the α-hydroxy fatty acids for the minor components differed in chain length.

Glycosphingolipid and sphingosine derivatives and analogs have been isolated from a number of marine sources, including sea stars,[1-5] sea anemones,[6] sponges,[7,8] corals,[7] tunicates,[9] and plants.[10] Among the marine invertebrates, the primary sphingolipids include those derived from the doubly unsaturated sphingosine base 4,8-sphingadiene,[6,7] as well as from the saturated phytosphingosine 4-hydroxysphingosine.[1-5] To date, the structures which have been elucidated contain a fatty acid chain and one[1,5,7] or more[2-4,8] hexose units characteristic of cerebrosides and ceramide oligosacharides, respectively. From the sea star *Ophidiaster ophidiamus* we have now purified a group of five cerebrosides not heretofore isolated.[11] These compounds are unusual in that the sphingosine unit contains a methyl branch and a conjugated diene. All five compounds exhibited strong cytotoxicity against L1210 murine leukemia cells in vitro.

Results and Discussion

The toluene-soluble portion of the crude extract from *O. ophidiamus* was cytotoxic against L1210 murine leukemia cells and was separated by column chromatography, preparative TLC, and reversed-phase HPLC into five pure compounds, ophidiacerebrosides A (**2**), B (**3**), C (**1**), D (**4**), and E (**5**), which gave FABMS M + H ions at m/z = 782, 796, 810, 824, and 838, respectively ($C_{45}H_{84}NO_9$, $C_{46}H_{86}NO_9$, $C_{47}H_{88}NO_9$, $C_{48}H_{90}NO_9$, and $C_{49}H_{90}NO_9$ by HRFABMS). All demonstrated cytotoxicity to L1210 cells. The major component, ophidiacerebroside C (**1**), accounted for almost 40% of the mixture.

Structure of 1. Spectral data for **1** (Table 1, Experimental Section, and supplementary material) indicated the presence of a sugar, an amide linkage, and a long chain, strongly suggesting the glycosphingolipid nature of **1**. A FABMS peak at m/z = 630.5885 ($C_{41}H_{76}NO_3$, Δ −3.0 mDa, M + H − $C_6H_{12}O_6$) argued the loss of a hexose from the M + H ion ($C_{47}H_{88}NO_9$), and anomeric signals indicative of the sugar unit were observed at δ 4.24 (d, J = 7.7 Hz, diaxial) and δ 104.1 ppm in ^1H and ^{13}C NMR spectra. In the ^{13}C NMR spectrum of **1**, the signals at 104.1, 74.4, 77.4, 71.0, 77.4, and 62.5 ppm suggested that the sugar in **1** was a β-glucopyranoside.[12] The coupling constant between H-1″ and H-2″, J = 7.7 Hz, also supports the β-configuration of the sugar. Acid hydrolysis of **1** (1 N HCl in 82% MeOH, 10 h) gave methyl glucopyranoside (mixture of anomers) as evidenced by TLC [EtOAc/MeOH/H_2O (5:2:0.5) or $CHCl_3$/MeOH/H_2O (7:3:0.5)] using standard methyl D-glucopyranoside as a reference. The rotation of the methyl glucoside anomeric mixture, $[\alpha]^{25}_D$ +73.0°, determined on the methanolysis product from a mixture of **2**-**5** (cf. below), was close to that of the authentic sample, $[\alpha]^{25}_D$ +77.3°, defining glucose as the D-isomer.

An amide N*H* doublet, observed at 7.31 ppm in the ^1H NMR spectrum recorded in acetone-d_6 (see Table 1), collapsed to a singlet on irradiation of H-2 at 3.98 ppm. The signal for the amide carbonyl carbon appeared at 176.7 ppm in the ^{13}C NMR spectrum. The intense signal at 1.27 ppm and the triplet at 0.88 ppm in the ^1H NMR spectrum argued the presence of one or more long aliphatic chains.

Acid hydrolysis of **1** produced methyl 2-hydroxydocosanoate, identified by β- and γ-cleavage ions at m/z 90 and 103, respectively, in the EI mass spectrum, along with fragment ions from the unbranched aliphatic chain of **1**. The rotation of the ester, $[\alpha]^{25}_D$ −4.76°, identified it as the *R*-isomer.[3]

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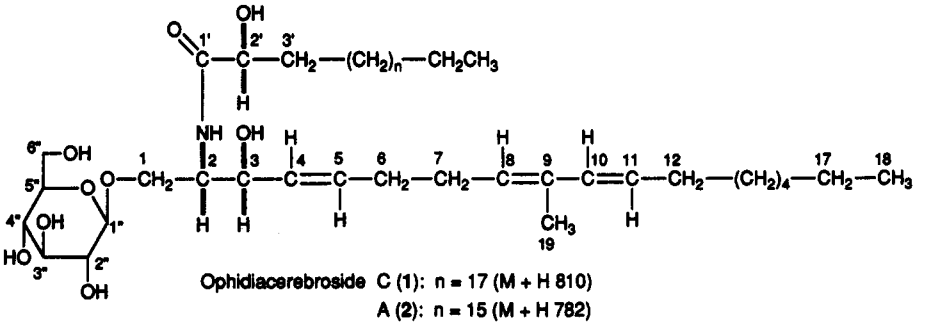
Chart 1



Ophidiacerebroside C (1): n = 17 (M + H 810)
A (2): n = 15 (M + H 782)
B (3): n = 16 (M + H 796)
D (4): n = 18 (M + H 824)
E (5): n = 19 (M + H 838)

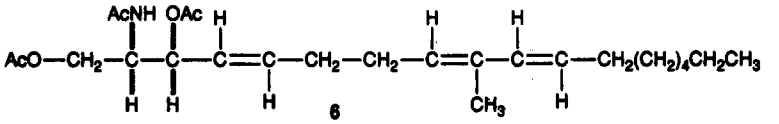


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Table 1. ^1H, ^{13}C, and COSY NMR Data for 1[a]

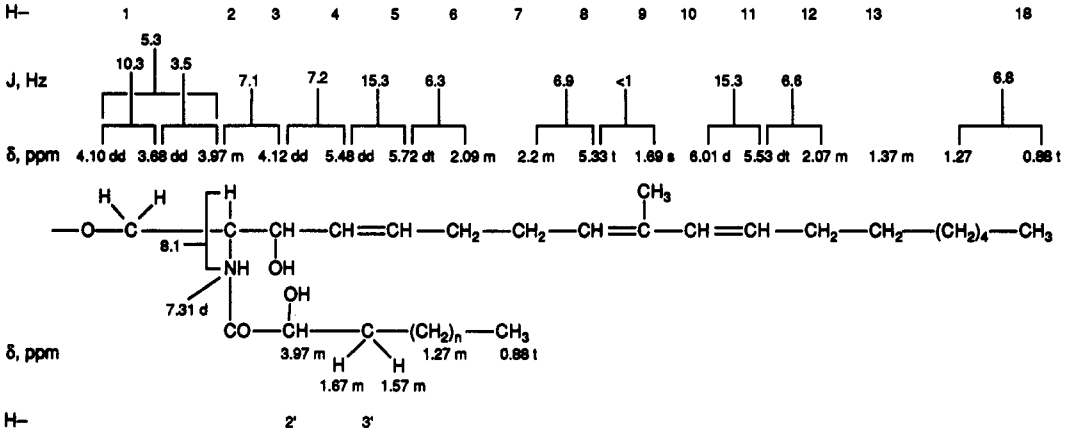
H or C	δ ^1H, ppm (m, J in Hz)[b]	δ ^1H, ppm (m, J in Hz)[h]	δ ^{13}C (ppm)[f,g]	COSY correlation[c]
1a	3.68 (dd, J = 10.3, 3.5)	3.78 (dd, J = 11.0, 3.4)	69.3	1b, 2
1b	4.10 (dd, J = 10.3, 5.3)	3.95 (m)[e]		1a,b
2	3.97 (m)[e]	3.98 (m)[e]	54.1	1a, 1b, 3
3	4.12 (dd, J = 7.2, 7.1)	4.15 (t, J = 7.2)	72.8*	2, 4
4	5.48 (dd, J = 15.3, 7.2)	5.47 (dd, J = 15.6, 6.8)	129.8	3, 5, 6
5	5.72 (dt, J = 15.3, 6.3)	5.65 (dt, J = 15.6, 6.3)	134.2	4, 6
6	2.09 (m)	*i*	33.7#	5, 7
7	2.20 (m)	*i*	35.4	6, 8, 19
8	5.33 (t, J = 6.9)	5.28 (t, J = 6.3)	128.4	7, 19
9			134.8	
10	6.01 (d, J = 15.3)	5.95 (d, J = 15.6)	135.6	11, 12
11	5.53 (dt, J = 15.3, 6.6)	5.49 (dt, J = 15.6, 6.9)	130.6	10, 12
12	2.07 (m)	*i*	33.2#	11, 13
(13)	1.37 (m)			12
14–15	1.27	1.21		
16	1.27	1.21	32.7†	
17	1.27	1.21	23.4	
18	0.88 (t, J = 6.8)	0.88 (t, J = 6.5)	14.4	
19	1.69 (s)	1.65 (s)	12.8	6, 7, 8
1′			176.7	
2′	3.97 (m)[e]	3.99 (m)[e]	72.5*	
3′b	1.67 (m)			2′, 3′a
3′a	1.57 (m)			2′, 3′b
4′–19′	1.27	1.21		
20′	1.27	1.21	32.67†	
21′	1.27	1.21	23.4	
22′	0.88 (t, J = 6.8)	0.88 (t, J = 6.5)	14.4	
1″	4.24 (d, J = 7.7)	4.27 (d, J = 7.8)	104.1	2″
2″	3.15 (dd, J = 7.7, 8.9)	3.19 (dd, J = 7.8, 8.7)	74.4	1″, (3″)
(3″)	*d*	3.38 (dd, J = 8.7, ca. 8)	77.4	2″, 4″
4″	*d*	3.30 (m)	71.0	
(5″)	*d*	3.30 (m)	77.4	6″a, 6″b
6″a	3.64 (dd, J = 11.8, 3.3)	3.66 (dd, J = 11.8, 5.3)	62.2	5″, 6″b
6″b	3.84 (br d, J = 11.8)	3.81 (dd, J = 11.8, 2.6)		5″, 6″a
N*H*		7.31 (d, J = 8.1)		

[a] 300 MHz, MeOH-d_4, except as noted. [b] Signals in parentheses can only be resolved in spectra run at 500 MHz. [c] Protons decoupled by irradiation of H-*n* are underlined. [d] Superimposed with MeOH. [e] Superimposed. [f] 125 MHz, $CDCl_3$/MeOH-d_4 (1:2). For each compound, signals having the same superscript are interchangeable. [g] Assignments are consistent with the attached proton test (APT). [h] 300 MHz, acetone-d_6. [i] Superimposed with acetone.

Subtracting the hexose and hydroxy acid units leaves a $C_{19}H_{35}NO_2$ unit still unaccounted for, and this was established as a highly unusual sphingosine analog. Tandem mass spectrometry (FABMS/MS) on *m/z* 630 ($C_{41}H_{76}NO_3$) confirmed fragmentation to give *m/z* 292 (292.2639, corresponding to $C_{19}H_{34}NO$ by HRFABMS) due to loss of the acyl group ($C_{22}H_{42}O_2$) from *m/z* 630. COSY and spin-decoupling experiments on **1** (Table 1) established the sequence of the sphingosine unit shown in Scheme 1 for C-1 through C-9 and C-10 through C-12. The splitting pattern of H-10 (6.01 ppm, d, J = 15.3 Hz) and the UV spectrum of **1** (λ_{max} 225 nm in MeOH, conjugated diene) indicated that C-10 could only be connected to C-9. These results also indicated that the 19-CH_3 had to be attached to C-9 in a branched chain arrangement.

The 4,5 and 10,11 alkene bonds were found to be *trans*, as evidenced by the large coupling constants (15.3 Hz). When H_2-7 was irradiated in an NOE difference exper-

Scheme 1



iment, an NOE enhancement of the 19-H_3 signal was observed, arguing that the 8,9 double bond was also *trans*. In addition, the ^{13}C NMR chemical shift of C-19 (12.8 ppm) supported the assignment of *trans* geometry.[13] The relative stereochemistry at C-2 and C-3 was predicted to be the same as that of D-sphingosine (D-*erythro*), based initially on the ^{13}C NMR spectrum, since the chemical shifts of C-2 (δ 54.1–54.4) and C-3 (δ 72.8–73.1) for the long-chain base present in **1**, **4**, and **5** (Table 1 and supplementary material) were in agreement with those (δ 54.7, 73.1) of synthetic *N*-octadecanoyl-D-*erythro*-sphingosine.[14]

Although the long-chain base was not isolated from **1**, it was isolated from a mixture of **2–5** (cf. below and Experimental Section) and acetylated. The H–H coupling constants $J_{1,2}$, $J_{2,3}$ and $J_{2,NH}$ for the 2-acetamido-1,3-diacetoxy-9-methyl-4,8,10-octadecatriene (**6**) (5.9 and 3.5, 5.9, 9.5 Hz, respectively) are very close to those of triacetyl D-*erythro*-sphingosine [(6.0 and 3.9, 6.0, 9.3 Hz, respectively) prepared by acetylation of D-sphingosine using conventional methods] and (2*S*,3*R*,4*E*,10*E*)-2-acetamido-1,3-diacetoxy-4,10-octadecadiene.[5,15] Moreover, the specific rotation of **6**, $[\alpha]^{25}_D$ −12.5° (*c* = 0.1, 1-PrOH), is very close to that of *N*,*O*,*O*-triacetyl-D-*erythro*-sphingosine, $[\alpha]^{25}_D$ −11.8°, in agreement with the absolute stereochemistry assigned[14,16] to the sphingosine unit of **1**, (2*S*,3*R*,4*E*,8*E*,10*E*)-2-amino-1,3-dihydroxy-9-methyl-4,8,-10-octadecatriene; **1** is then its 1-(β-D-glucopyranosyl)-2-(2-hydroxydocosanoyl) derivative. The latter compound differs from the sphingosine derivatives which have been isolated from other marine invertebrates or from terrestrial sources in that the octadecatriene contains both a methyl branch and a conjugated diene.[11]

Structures of 2–5. The ^1H NMR spectra (CD_3OD, 300 MHz) of **2** (M + H 782.6135, $C_{45}H_{84}NO_9$), **3** (M + H 796.6308, $C_{46}H_{86}NO_9$), **1** (M + H 810), **4** (M + H 824.6559, $C_{48}H_{90}NO_9$), and **5** (M + H 838; M + H − H_2O 810.6666, $C_{49}H_{90}NO_9$) were very similar (see supplementary material) and their chemical shifts agreed within 0.03 ppm for all protons (supplementary material). When the ophidiacerebrosides A, B, D, and E were subjected to FABMS (positive ion), a molecule of glucose was lost and ceramide fragment ions were found at *m*/*z* 602, 616, 644, and 658, respectively. In addition, FABMS/MS on the latter four ions revealed the same intense fragment ion at *m*/*z* 292 ($C_{19}H_{34}NO$), originating from the sphingosine unit, as from **1** (*m*/*z* 630). Thus, the other four ophidiacerebrosides appeared to have similar hexose, hydroxy acid, and sphingosine units.

The ^1H NMR spectra of the hexaacetyl derivatives of **3–5** (supplementary material) were almost superimposable. With the exception of the long-chain polymethylene group, the splitting patterns and the coupling constants of all key protons were clearly observed and like those of the hexaacetyl derivative of **1** (supplementary material). In the ^{13}C NMR spectra of **1**, **4**, and **5**, the chemical shifts were within 0.3 ppm for all three compounds (supplementary material), again indicating common hexose and sphingosine units.

Since samples of the individual homologs **2–5** were limited, a mixture of those compounds was methanolyzed to give a mixture of methyl glucopyranosides (α and β) whose rotation, $[\alpha]^{25}_D$ +73.0°, established the sugar as the D-isomer. (An authentic mixture of anomers of methyl D-glucopyranosides had $[\alpha]^{25}_D$ +77.3°.) Methanolysis of the mixture of **2–5** also gave (after acetylation) the triacetylophidiasphingosine, $[\alpha]^{25}_D$ −12.5° (*c* 0.1, 1-PrOH), whose rotation agreed with that of the D-*erythro* isomer, as well as a mixture of the α-hydroxy acid methyl esters, $[\alpha]^{25}_D$ −5.0° (*c* 0.2, $CHCl_3$), with rotation very close to that of methyl (2*R*)-2-hydroxydodecanoate from **1** (see above).

Acid hydrolysis (1 N HCl in 82% MeOH) of **2–5** individually gave methyl D-glucoside (mixture of anomers) and methyl 2-hydroxyeicosanoate, methyl 2-hydroxyheneicosanoate, methyl 2-hydroxytricosanoate, and methyl 2-hydroxytetracosanoate from **2**, **3**, **4**, and **5**, respectively. The specific rotations of these pure methyl esters have not been measured due to the very limited samples available, but the mixture of the methyl esters isolated from hydrolysis of mixed **2–5** has a specific rotation very similar to that of methyl 2-hydroxydocosanoate isolated from **1**, as noted above, so the stereochemistry at C-2 of the fatty acids should be the same, i.e., *R*, and with common sugar and sphingosine units **1–5** should have the same stereochemistry and differ only in the chain length of their α-hydroxy fatty acid constituents.

Cytotoxicities of 1–5. At a concentration of 2 μg/mL, **1–5** exhibited 96, 92, 70, 90, and 84% inhibition of L1210 murine leukemic cell growth, respectively; thus, all are quite active cytotoxic agents.

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Experimental Section

L1210 Cytotoxicity. Individual samples were dissolved in MeOH and dried. For each sample, 1 mL of Eagle's medium containing 1000 L1210 cells in exponential growth was added to each individual well of a tissue culture plate containing 2, 5, 10, and 20 μg of dried sample. The resulting solutions were incubated at 37 °C for 3 d. Inhibition of cell growth was estimated by counting the number of viable cells per well and expressing the result as a percentage of the control wells containing no cytotoxin.

Isolation and Purification. A specimen of the large sea star *O. ophidiamus* (7-X-88-1-24, 986 g) collected at Islote Dragonera, Balearic Islands, Spain, was ground three times with methanol, and the extract was filtered and concentrated by rotary evaporation to give a crude extract. Three g of this residue was triturated four times with toluene to give a cytotoxic solution. The toluene extract was chromatographed on a silica gel column (1.5 × 24 cm, solvent 10% MeOH in $CHCl_3$), and the cytotoxic fractions (vs L1210 leukemia cells) were concentrated and further separated by preparative TLC (silica gel, 12% MeOH in $CHCl_3$, R_f = 0.25), yielding a 10-mg, active mixture of ophidiacerebrosides. Final purification was achieved by reversed-phase HPLC [C-18 (10 μm), 10-mm × 25-cm column, mobile-phase MeOH, UV detector 235 nm, flow rate 4 mL/min], which gave 2 mg of ophidiacerebroside C, 1.1 mg of D, ca. 0.5 mg each of B and E, and less than 0.5 mg of A.

Ophidiacerebroside C (1): colorless; UV λ_{max}^{MeOH} (log e) 225 (4.16); positive FABMS *m/z* 810, 792 (M + H − H_2O, $C_{47}H_{86}NO_8$, HRFABMS 792.6337, Δ −1.6 mmu), 630 (M + H − glucose, $C_{41}H_{76}NO_3$, HRFABMS 630.5885, Δ −3.0 mmu); FABMS/CID/MS *m/z* 630 → 452, 356, 292, 274, 257; ^1H NMR and COSY (MeOH-d_4, 300 MHz), ^1H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR [MeOH-d_4/$CDCl_3$ (2:1)], see Table 1; HRFABMS calcd for $C_{47}H_{88}NO_9$ M_r 810.6459 (M + H), found M_r 810.6429.

Ophidiacerebroside A (2): colorless; UV λ_{max}^{MeOH} 225 nm; positive FABMS *m/z* 782, 764 (M + H − H_2O, $C_{45}H_{82}NO_8$, HRFABMS 764.6041, Δ −0.1 mmu), 602 (M + H − glucose, $C_{39}H_{72}NO_3$, HRFABMS 602.5496, Δ 1.6 mmu); FABMS/CID/MS *m/z* 602 → 424, 328, 292, 274, 257; ^1H NMR (MeOH-d_4, 300 MHz), see supplementary material (Table II); HRFABMS calcd for $C_{45}H_{84}NO_9$ M_r 782.6147 (M + H), found M_r 782.6135.

Ophidiacerebroside B (3): colorless; UV λ_{max}^{MeOH} 225 nm; positive FABMS *m/z* 796, 778 (M + H − H_2O, $C_{46}H_{84}NO_8$, HRFABMS 778.6200, Δ −0.3 mmu), 616 (M + H − glucose, $C_{40}H_{74}NO_3$, HRFABMS 616.5653, Δ 1.6 mmu); FABMS/CID/MS *m/z* 616 → 438, 342, 292, 274, 257; ^1H NMR (MeOH-d_4, 300 MHz), see supplementary material (Table II); HRFABMS calcd for $C_{46}H_{86}NO_9$ M_r 796.6303 (M + H), found M_r 796.6308.

Ophidiacerebroside D (4): colorless; UV λ_{max}^{MeOH} 225 nm; positive FABMS *m/z* 824, 806 (M + H − H_2O, $C_{48}H_{88}NO_8$, HRFABMS 806.6495, Δ 1.5 mmu), 644 (M + H − glucose, $C_{42}H_{78}NO_3$, HRFABMS 644.5991, Δ −0.9 mmu); FABMS/MS *m/z* 644 → 466, 370, 292, 274, 257; ^1H and ^{13}C NMR (MeOH-d_4, 300 MHz, 75 MHz) and ^{13}C NMR ($CDCl_3$/MeOH-d_4, 75 MHz), see supplementary material (Tables II and III); HRFABMS calcd for $C_{48}H_{90}NO_9$ M_r 824.6615 (M + H), found M_r 824.6559.

Ophidiacerebroside E (5): colorless; UV λ_{max}^{MeOH} 225 nm; positive FABMS *m/z* 838 (M + H), 820, 658 (M + H − glucose, $C_{43}H_{80}NO_3$, HRFABMS 658.6138, Δ 0.0 mmu); FABMS/CID/MS *m/z* 658 → 480, 384, 292, 274, 257; ^1H NMR (MeOH-d_4, 300 MHz) and ^{13}C NMR ($CDCl_3$/MeOH-d_4, 75 MHz), see supplementary material (Tables II and III); HRFABMS calcd for $C_{49}H_{90}NO_9$ M_r 820.6636 (M + H − H_2O), found M_r 820.6666.

Acetylation of 1. A mixture of 2.5 mg of 1, Ac_2O (0.15 mL), and pyridine (0.15 mL) stood overnight at rt and then was diluted with 2 mL of H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried, and evaporated with a stream of N_2. The residue was further purified by TLC (hexane:EtOAc = 6:4) to give the hexaacetyl derivative of 1: ^1H NMR ($CDCl_3$, 300 MHz), see supplementary material (Table IV).

Acetylation of 3, 4, 5, and D-*erythro*-Sphingosine. Each sample (1 mg) was treated as for 1.

Hexaacetyl-3, hexaacetyl-4, hexaacetyl-5, and triacetyl-D-*erythro*-sphingosine: ^1H NMR ($CDCl_3$, 300 MHz), see supplementary material (Table IV).

Hydrolysis of 1. A 2-mg sample of 1 was heated in 2.5 mL of refluxing 1 N HCl in 82% MeOH for 15 h. The reaction mixture was extracted with *n*-hexane, and the hexane layer was washed with water, dried, and evaporated to give methyl 2-hydroxydocosanoate: $[\alpha]^{25}_D$ −4.76° (*c* 0.1, $CHCl_3$) [lit.[3] $[\alpha]^{25}_D$ −2.2° (*c* 2.0, $CHCl_3$)]; EIMS *m/z* 370 (M^+, $C_{23}H_{46}O_3$, HREIMS 370.3441, Δ 0.5 mmu), 311 ($M^+ - COOCH_3$), 103 $[CH_2CH(OH)COOCH_3]^+$, 90 $[CH_2(OH)COOCH_3]^+$; ^1H NMR ($CDCl_3$, 300 MHz) 0.86 (t, *J* = 6.3 Hz, terminal CH_3), 1.23 (long chain $-CH_2-$), 2.67 (d, *J* = 5.7 Hz, OH), 3.77 (s, $COOCH_3$), 4.18 (m, H-2). The aqueous MeOH layer of the hydrolysate was evaporated to dryness and redissolved in MeOH for a total of three times to ensure removal of residual HCl. The residue was separated by TLC [silica gel, $CHCl_3$/MeOH/H_2O (7:3:0.5)], and the presence of methyl glucopyranoside was confirmed by reference, using TLC [silica gel; EtOAc/MeOH/H_2O (5:2:0.5), R_f = 0.49; $CHCl_3$/MeOH/H_2O (7:3:0.5), R_f = 0.32], to pure methyl D-glucopyranoside (mixture of α- and β-anomers) prepared from D-glucose under the same conditions employed for obtaining methyl glucopyranoside from hydrolysis of the ophidiacerebrosides.

Hydrolysis of 2–5. Each sample (2.3 mg of 4, 1 mg of 2, 3, and 5) was treated the same as 1, affording methyl glucoside, confirmed by TLC compared to pure methyl D-glucoside. In addition, methyl 2-hydroxyeicosanoate [EIMS *m/z* 342 (M^+, $C_{21}H_{42}O_3$, HREIMS 342.3129, Δ 0.4 mmu), 283, 103, 90], methyl 2-hydroxyheneicosanoate [EIMS *m/z* 356 (M^+, $C_{22}H_{44}O_3$, HREIMS 356.3286, Δ 0.4 mmu), 297, 103, 90], methyl 2-hydroxytricosanoate [EIMS *m/z* 384 (M^+, HREIMS 384.3610, Δ −0.7 mmu), 325, 103, 90], and methyl 2-hydroxytetracosanoate [EIMS *m/z* 398 (M^+, HREIMS 398.3767, Δ −0.7 mmu), 339, 103, 90] were isolated from hydrolysates of 2, 3, 4, and 5, respectively.

Hydrolysis of a Mixture of Ophidiacerebrosides 2–5. Isolation of (2*S*,3*R*,4*E*,8*E*,10*E*)-2-Acetamido-1,3-diacetoxy-9-methyl-4,8,10-octadecatriene (6). A mixture of 2–5 (10 mg) was hydrolyzed under the same conditions as 1. The hydrolyzate was extracted with hexane and the hexane layer concentrated to afford a mixture of the methyl esters of the hydroxy fatty acids [2.7 mg, $[\alpha]^{25}_D$ −5.0° (*c* 0.2, $CHCl_3$)]. The aqueous methanolic layer was neutralized with Amberlite CG-400 (OH^- form) and concentrated, and the residue was dissolved in water and extracted with EtOAc. The aqueous phase afforded methyl D-glucoside, which was purified by TLC (silica gel, $CHCl_3$:MeOH:H_2O = 7:3:0.5), $[\alpha]^{25}_D$ 73.0° (*c* 0.1, MeOH) [authentic sample, $[\alpha]^{25}_D$ 77.3° (*c* 0.1, MeOH)].

The EtOAc phase afforded the crude long-chain base, which was acetylated in the usual way. The acetylated product was purified by TLC (silica gel, EtOAc:hexane = 6:4, developed twice) to afford 0.8 mg of 2-acetamido-1,3-diacetoxy-9-methyl-4,8,10-octadecatriene: $[\alpha]^{25}_D$ −12.5° (*c* 0.1, 1-PrOH); ^1H NMR ($CDCl_3$, 300 MHz), see supplementary material (Table IV).

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Supplementary Material Available: Table II with ^1H NMR data for 2–5, Table III with ^{13}C NMR data for 4 and 5, Table IV with ^1H NMR data for hexaacetyl-1, 3, 4, and 5, *N,O,O* triacetyl-2-amino-9-methyl-4,8,10-octadecatrien-1,3-diol (6), and triacetyl-D-*erythro*-sphingosine, ^1H NMR spectra for 1–6 and the hexaacetyl derivatives of 1–5, and ^{13}C NMR spectra for 1, 4, and 5 (17 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.