New Bioactive Cerebrosides from *Arisaema amurense*

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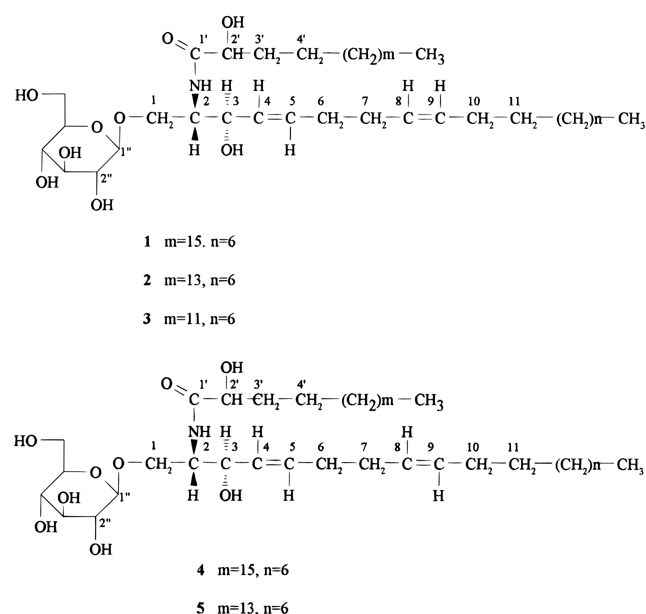
From *Arisaema amurense*, four new cerebrosides were isolated along with a known cerebroside. The new cerebrosides were characterized as 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2(*R*)-hydroxyicosanoyl)amido]-4,8-octadecadiene-1,3-diol (**1**), 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2-hydroxyoctadecanoyl)amido]-4,8-octadecadiene-1,3-diol (**2**), 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*)-2-[(2-hydroxyicosanoyl)amido]-4,8-octadecadiene-1,3-diol (**4**), and 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*)-2-[(2-hydroxyoctadecanoyl)amido]-4,8-octadecadiene-1,3-diol (**5**), respectively. These cerebrosides displayed significant antihepatotoxic activity.

Arisaema amurense Maxim. (Araceae), a perennial herb, is known as a toxic plant in folklore. In this report, we describe the isolation and structure determination of four new cerebrosides (**1**, **2**, **4**, and **5**) along with a known cerebroside (**3**).[1−4]

Dried roots of *A. amurense* were cut into small pieces and extracted with acetone. The acetone extract was concentrated *in vacuo*, and the resulting residue was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 phase was concentrated and further partitioned between 90% aqueous MeOH and *n*-hexane. The alcoholic phase was subjected to successive C-18 reversed-phase chromatography to yield cerebrosides **1**−**5**.



^{1}H and ^{13}C NMR data of **1** (Table 1) indicated the presence of a sugar residue, an amide linkage, and aliphatic long chain(s), strongly suggesting the glycosphingolipid nature of **1**. In the ^{13}C NMR spectrum of **1**, the signals at δ 104.7, 75.0, 77.9 (two carbons), 71.5, and 62.7 suggested that the sugar in **1** was a *β*-glucopyranoside. The coupling constant between H-1″ [δ 4.26 (d, $J = 8.0$ Hz)] and H-2″ [δ 3.19 (dd, $J = 8.0$, 9.0 Hz) also supports the *β*-configuration of the sugar. Methanolysis of **1** gave a mixture of α- and *β*-methyl glucopy-

Table 1. [1]H (500 MHz) and ^{13}C (50 MHz) NMR Data of **1** (CD_3OD)

position	^{1}H NMR δ (m, *J* in Hz)	^{13}C NMR, δ	COSY correlation
1a	4.11 (dd, 10.1, 5.8)	69.7	1b, 2
1b	3.71 (dd, 10.1, 3.7)	69.7	1a, 2
2	3.99 (ddd, 5.8, 3.7, 1.9)	54.6	1a, 1b, 3
3	4.14 (td, 7.5, 1.9)	72.9	2, 4
4	5.49 (dd, 15.1, 7.5)	129.9	3, 5
5	5.73 (dtd, 15.1, 6.2, 1.5)	134.3	4, 6
6	2.07 (m)	33.7	5, 7
7	2.12 (m)	28.0	6, 8
8, 9	5.37 (t, 5.1)	131.3	7, 10
10	2.05 (m)	28.3	9, 11
11	1.35 (m)	33.1, 31.0, 30.8, 30.5, 30.3, 30.2, 26.2, 23.7	
12−17	1.30 (m)		
1″	4.26 (d, 8.0)	104.7	2″
2″	3.19 (dd, 9.0, 8.0)	75.0	1″, 3″
3″	3.35 (t, 9.0)	77.9	2″, 4″
4″	3.28[a] (m)	71.5	3″, 5″
5″	3.27[a] (m)	77.9	6″a, 6″b
6″a	3.86 (dd, 12.0, 2.8)	62.7	6″b, 5″
6″b	3.67 (dd, 12.0, 5.8)	62.7	6″a, 5″
1′		177.1	
2′	3.98 (dd, 8.0, 4.2)	73.0	3′a, 4′b
3′a	1.72 (m)	35.8	2′, 4′, 3′b
3′b	1.54 (m)	35.8	2′, 4′, 3′a
4′	1.40 (m)	33.1, 31.0, 30.8, 30.5, 30.3, 30.2, 26.2, 23.7	5′, 3′a, 3′b
5′−19′	1.30 (m)		
CH_3	0.90 (6H, t, 7.0)	14.5	

[a] Assignments may be reversed.

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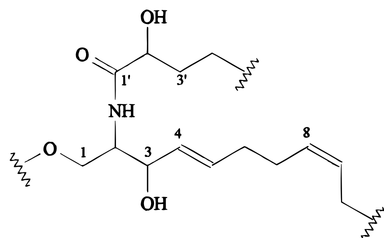

Figure 1. Partial structure of **1**.

ranoside. The optical rotation of the methyl glucoside mixture $[\alpha]^{25}_{D}$ +75.6° (c = 0.18, MeOH) was close to that of authentic sample, $[\alpha]^{25}_{D}$ +77.3°,[5] defining glucose as the D-isomer. A signal of a carbon attached to nitrogen was observed at δ 54.6, and an amide carbonyl signal appeared at δ 177.1 in the ^{13}C NMR spectrum. An intense signal at δ 1.30 and the triplet at δ 0.90 (6H, t, J = 7.0 Hz) in the ^{1}H NMR spectrum suggested the presence of one or more long aliphatic chains. COSY and homonuclear J-resolved 2D experiments on **1** established the partial structure shown in Figure 1. The 4,5 alkene bond was found to be *trans*, as evidenced by the large vicinal coupling constants ($J_{4,5}$ = 15.1 Hz). The *trans* geometry of this double bond was also supported by the chemical shift of C-6 (δ 33.7). Usually, the signals of carbons next to a *trans* double bond appear at δ 32−33, while those of a *cis* double bond appear at δ 27−28.[6] The olefinic protons at 8, 9 were magnetically equivalent, but as evidenced by the carbon signals of C-7 (δ 28.0) and C-10 (δ 28.3), the geometry of the 8,9 alkene bond was determined to be *cis*. The positive FABMS of **1** gave peaks at m/z 792 [M + Na]$^+$, 770 [M + H]$^+$, and 752 [(M + H) − H_2O]$^+$. A peak at m/z 590 [[(M + H) − H_2O] − 162]$^+$ indicated the loss of a hexose unit from the [M + H]$^+$ ion. In the ^{13}C NMR spectrum of **1**, another CH signal appeared at δ 73.0 (C-2′ in acyl moiety), and the corresponding proton at δ 3.98 (1H, dd, J = 8.0, 4.2 Hz) showed coupling with methylene protons (H-3′). The acyl moiety is hence an α-hydroxyl linear acyl chain. The relative stereochemistry of **1** at C-2 and C-3 was predicted to be the same as that of D-sphingosine (D-*erythro*), on the basis of the ^{13}C NMR spectral data, since the chemical shifts of C-2 (δ 54.6) and C-3 (δ 72.9) were in agreement with those of synthetic *N*-octadecanoyl-D-*erythro*-sphingosine (δ 54.7, 73.1)[7] and glucosyl-*erythro*-ceramide (δ 53.8, 72.6).[8] Methanolysis of **1** afforded methyl 2-hydroxyicosanoate. Its optical rotation ($[\alpha]^{25}_{D}$ −3.5°, c = 0.09, $CHCl_3$)[9] identified it as the *R* isomer. Thus, the structure of **1** was assigned as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2(*R*)-hydroxyicosanoyl)amido]-4,8-octadecadiene-1,3-diol.

The ^{1}H NMR spectrum of **2** was almost identical with that of **1**, indicating only subtle differences in the chain length of the α-hydroxy fatty acid moiety and/or long-chain base. In the positive FABMS of **2**, peaks at m/z 764 [M + Na]$^+$, 742 [M + H]$^+$, 724 [(M + H) − H_2O]$^+$, and 562 [[(M + H) − H_2O] − 162]$^+$ were observed. Methanolysis of **2** afforded methyl 2-hydroxyoctadecanoate. Accordingly, the structure of **2** was assigned as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2-hydroxyoctadecanoyl)amido]-4,8-octadecadiene-1,3-diol.

The ^{1}H NMR spectrum of **3** was also almost identical with those of **1** and **2**, indicating only subtle differences in the chain length of the α-hydroxy fatty acid moiety and/or long-chain base. In a positive FABMS of **3**, peaks at m/z 736 [M + Na]$^+$, 714 [M + H]$^+$, and 696 [(M + H) − H_2O]$^+$ were observed. Methanolysis of **3** afforded methyl 2-hydroxyhexadecanoate. Accordingly, the structure of **3** was identified as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2-hydroxyhexadecanoyl)amido]-4,8-octadecadiene-1,3-diol, which has been previously isolated from various plant sources.[1−4]

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **4** (CD_3OD)

position	^{1}H NMR δ (m, J in Hz)	^{13}C NMR, δ	COSY correlation
1a	4.10 (dd, 10.1, 5.2)	69.7	1b, 2
1b	3.70 (dd, 10.1, 3.8)	69.7	1a, 2
2	3.98 (ddd, 5.8, 3.7, 2.0)	54.6	1a, 1b, 3
3	4.13 (td, 8.3, 1.5)	72.9	2, 4
4	5.47 (dd, 15.5, 7.6)	130.7	3, 5
5	5.72 (br. d, 15.5)	134.4	4, 6
6, 7	2.07 (m)	33.71, 33.67	5, 8
8, 9	5.42 (t, 4.9)	132.0, 131.2	7, 10
10	1.98 (m)	33.3	9, 11
11	1.35 (m)	33.1, 30.8, 30.7, 30.5, 30.3, 26.2, 23.7	
12−17	1.30 (m)		
1″	4.26 (d, 7.9)	104.7	2″
2″	3.18 (dd, 9.0, 7.9)	75.0	1″, 3″
3″	3.34 (t, 9.0)	78.0[b]	2″, 4″
4″	3.26[a] (m)	71.6	3″, 5″
5″	3.27[a] (m)	77.9[b]	4″, 6″a, 6″b
6″a	3.86 (dd, 12.1, 1.7)	62.7	5″, 6″b
6″b	3.66 (dd, 12.1, 5.6)	62.7	5″, 6″a
1′		177.2	
2′	3.97 (dd, 8.0, 3.8)	73.1	3′a, 3′b
3′a	1.70 (m)	35.9	2′, 3′b, 4′
3′b	1.55 (m)	35.9	2′, 3′a, 4′
4′	1.40 (m)	33.1, 30.8, 30.7, 30.5 30.3, 26.2, 23.7	3′a, 3′b, 5
5′−19′	1.30 (m)		
CH_3	0.90 (6H, t, 6.9)	14.4	

[a,b] Assignments with the same superscript may be reversed.

The ^{1}H NMR spectral data of **4** exhibited certain differences from those of **1**−**3**. The signal of the olefinic protons at δ 5.37 (H-8, -9, t, J = 5.1 Hz) in **1**−**3** was shifted downfield (δ 5.42, t, J = 4.9 Hz), and the signal of olefinic protons at δ 5.72 (H-5, J = 15.5 Hz) appeared as a broad doublet instead of a doublet of triplet of doublet (δ 5.73, J = 15.1, 6.2, 1.5 Hz) of **1**−**3**. The signals of H-7 and H-19 ranged from δ 2.12 to δ 2.07 and δ 2.05 to δ 1.98, respectively, indicating a possible change in the geometry of the double bond at C-8. A *trans* configuration of 8,9 alkene bond was supported by the downfield shifts of C-7, C-10 carbon signals from δ 28.0, 28.3 to around δ 33.7, 33.3, respectively. The broadening of the H-5, -6, -7, -8, -9, and -10 signals was assumed to be due to greater free rotation along these single bonds in the long-chain base moiety. The positive FABMS of **4** contains peaks at m/z 792 [M + Na]$^+$, 770 [M + H]$^+$, and 752 [(M + H) − H_2O]$^+$ indicating the same molecular weight as **1**. Methanolysis of **4** afforded methyl 2-hydroxyicosanoate. Accordingly, the structure of **4** was assigned as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*)-2-[(2-hydroxyicosanoyl)amido]-4,8-octadecadiene-1,3-diol.

The ^{1}H NMR spectrum of **5** was almost identical with those of **4**, indicating only subtle differences in the chain length of the α-hydroxy fatty acid moiety and/or long-chain base. The positive FABMS of **5** had peaks at m/z 764 [M + Na]$^+$, 742 [M + H]$^+$, and 724 [(M + H) − H_2O]$^+$ indicating the same molecular weight as **2**.

Table 3. Antihepatotoxic Activities of Compounds **1**−**5**

compd	GPT assay[c] (%)	SDH assay[c] (%)
ctrl[a]	100 ± 0.1	100 ± 0.1
ctrl[b]	0 ± 0.1	0 ± 0.1
silybin[d]	48.7 ± 0.5	42.3 ± 0.8
1	53.8 ± 0.2[e]	51.2 ± 0.9[e]
2	35.1 ± 0.6	36.9 ± 3.9
3	54.4 ± 0.9[e]	71.2 ± 0.9[f]
4	22.5 ± 0.4	7.2 ± 0.0
5	51.6 ± 0.4[e]	46.1 ± 0.8

[a] Control. [b] Control treated with CCl_4. [c] The percent of activity is calculated as 100 (GPT and SDH activity of ctrl[b]−GPT and SDH activity of sample)/(GPT and SDH activity of ctrl[b] − GPT and SDH activity of ctrl[a]). [d] Silybin was used as a positive control. Each sample was tested at 5 μM concentration. [e] $P < 0.05$. [f] $P < 0.01$.

Methanolysis of **5** afforded methyl 2-hydroxyoctadecanoate. Accordingly, the structure of **5** was assigned as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*)-2-[(2-hydroxyoctadecanoyl)amido]-4,8-octadecadiene-1,3-diol.

Sphingosine derivatives and several cerebrosides have been reported to possess cytotoxicity and other bioactivities.[10−12] We tested cerebrosides **1**−**5** for cytotoxicity against human tumor cells such as A549 (lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-2 (melanoma), XF498 (CNS cancer), and HCT15 (colon cancer), but these cerebrosides did not show significant cytotoxicity. However, cerebrosides **1**, **3**, and **5** showed significant antihepatotoxic activity comparable to silybin against CCl_4-induced toxicity in primary cultured rat hepatocytes (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-360 polarimeter. Solutions in CD_3OD were used for all the NMR studies. Chemical shifts were reported relative to the residual solvent peaks (CD_3OD: δ 3.3, δ 49). ^{1}H NMR, COSY, TOCSY, and homonuclear *J*-resolved 2D experiments were recorded at 500 MHz with a Varian Unity 500 instrument using Varian standard pulse programs. ^{13}C NMR and HETCOR spectra were recorded at 125 MHz with a Varian Unity 500 or at 50 MHz with a Varian VXR-200S instrument. FABMS were measured on JMS-SX 102 mass spectrometer with a direct inlet system using glycerol as a matrix. Europrep 60-60 (Knauer) was used for reversed-phase flash column chromatography. YMC-pack ODS-A (7 μm, 250 × 10 mm) column was used with Alltech guard catridge column for HPLC. RP-18 F_{254} S (Merck) was used for TLC.

Plant Material. *A. amurense* was collected in Chirisan, Korea, in May 1993, and identified by Dr. Hyung Joon Chi, Natural Products Research Institute, Seoul National University. A voucher specimen was deposited at the herbarium of the Natural Products Research Institute (NAPRI-94-04-13). Field-collected material (210 g) was used for the pilot experiment. For the main separation procedure, dried roots of *A. amurense* were purchased from a commercial supplier.

Extraction and Isolation. Dried roots of *A. amurense* (3.6 kg) were cut into small pieces and extracted with acetone at room temperature. The acetone extract was concentrated under vacuum, and the resulting residue was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 partition was concentrated and further partitioned between 90% aqueous MeOH and *n*-hexane. The 90% aqueous MeOH partition was subjected to C-18 reversed-phase flash column chromatography. The column was eluted in the sequence of MeOH:CH_3CN (10:1) → MeOH:CH_3CN (10:3) → MeOH:CH_3CN (10:5) → MeOH:CH_3CN (10:10) → CH_3CN → CH_3CN:CH_2Cl_2 (1:1) → CH_2Cl_2 → CH_2Cl_2:*n*-hexane (1:1) → *n*-hexane. A total of nine fractions were obtained. Fraction 2 (1.31 g) was further subjected to C-18 reversed-phase gravity column chromatography, eluted with MeOH:CH_3CN (10:1), MeOH:CH_3CN (2:1), and CH_3CN. A total of eight fractions were obtained from which fractions 4 (420 mg) and 5 (360 mg) were further subjected to C-18 reversed-phase HPLC [mobile phase MeOH:CH_3CN (5:3), flow rate 2.4 mL/min] equipped with refractive index monitor to afford **1** (53.6 mg), **2** (46.5 mg), **3** (0.8 mg), **4** (10 mg), and **5** (3 mg). The retention time for each compound was as follows: **1** (74.6 min), **2** (47 min), **3** (32.4 min), **4** (76.8 min), and **5** (49.4 min).

Cerebroside 1: colorless amorphous powder; positive FABMS *m/z* 792 $[M + Na]^+$, 770 $[M + H]^+$, 752 $[(M + H) - H_2O]^+$, 590 $[[(M + H) - H_2O] - 162]^+$; $[\alpha]^{25}_D$ +17° (c = 0.085, MeOH); ^{1}H and ^{13}C NMR, see Table 1.

Methanolysis of 1. A solution of **1** (15.5 mg) in 5% HCl−MeOH (5 mL) was refluxed for 7 h. The reaction mixture was extracted with *n*-hexane. The *n*-hexane layer was concentrated under reduced pressure to yield fatty acid methyl ester (methyl 2-hydroxyicosanoate) (6.3 mg); $[\alpha]^{25}_D$ −3.5° (c = 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.89 (t, J = 7.0 Hz, CH_3), 1.26 (long chain $-CH_2-$), 2.69 (d, J = 5.9 Hz, OH), 3.79 (s, $COOCH_3$), 4.20 (ddd, J = 4.3, 5.9, and 7.5 Hz, H-2), 1.78 (m, H-3), 1.63 (m, H-4); EIMS *m/z* (rel int) 342 (M^+, 88), 283 ($M^+ - COOCH_3$, 81). The aqueous MeOH layer of the hydrolysate was evaporated under reduced pressure to remove residual HCl. The resulting residue was partitioned between H_2O and EtOAc. The H_2O layer was concentrated (6.3 mg) and purified on a C-18 reversed-phase column to afford methyl glucopyranoside (mixtures of anomers, 3.5 mg): $[\alpha]^{25}_D$ +75.6° (c = 0.18, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 4.66 (H-1, α-anomer, d, J = 3.8 Hz), 4.16 (H-1, β-anomer, d, J = 7.8 Hz), 3.52 (OCH_3, β-anomer), 3.39 (OCH_3, α-anomer).

Cerebroside 2: colorless amorphous powder, positive FABMS *m/z* 764 $[M + Na]^+$, 742 $[M + H]^+$, 724 $[(M + H) - H_2O]^+$, 562 $[[(M + H) - H_2O] - 162]^+$. Methanolysis of **2** (0.4 mg) afforded methyl 2-hydroxyoctadecanoate: EIMS *m/z* (rel int) 314 (M^+, 73), 255 ($M^+ - COOCH_3$, 100).

Cerebroside 3: colorless amorphous powder; positive FABMS *m/z* 737 $[M + Na]^+$, 714 $[M + H]^+$, 696 $[(M + H) - H_2O]^+$. Methanolysis of **3** (0.4 mg) afforded methyl 2-hydroxyhexadecanoate: EIMS *m/z* (rel int) 286 (M^+, 35), 227 ($M^+ - COOCH_3$, 81).

Cerebroside 4: colorless amorphous powder; positive FABMS *m/z* 792 $[M + Na]^+$, 770 $[M + H]^+$, 752 $[(M + H) - H_2O]^+$; ^{1}H and ^{13}C NMR, see Table 2. Methanolysis of **4** (0.5 mg) afforded methyl 2-hydroxyicosanoate: EIMS *m/z* (rel int) 342 (M^+, 100), 283 ($M^+ - COOCH_3$, 94).

Cerebroside 5: colorless amorphous powder; positive FABMS *m/z* 764 $[M + Na]^+$, 742 $[M + H]^+$, 724 $[(M + H) - H_2O]^+$, 562 $[[(M + H) - H_2O] - 162]^+$. Methanolysis of **5** (0.5 mg) afforded methyl 2-hydroxyoctadecanoate: EIMS *m/z* (rel int) 314 (M^+, 83), 255 ($M^+ - COOCH_3$, 100).

Biological Evaluations. The isolated hepatocytes were plated and then were cultured for 1 day. The isolate was exposed to 10 mM CCl_4 for 1.5 h to induce cytotoxicity. The activity of GPT (glutamic pyruvic transaminase) in the culture medium was determined by the method of Reitman−Frankel[13] using an assay kit. The activity of SDH (sorbitol dehydrogenase) in the culture medium was determined by the method of Gerlach[14] with minor modifications.

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